



XXII European Conference on
ANALYTICAL CHEMISTRY

EUROANALYSIS



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ABSTRACT BOOK

WELCOME

Welcome to the next Euroanalysis 2025 in Barcelona, a forum to share knowledge and experience, a think tank and a place to promote Analytical Chemistry networking.

The motto of this conference is **Analytics 5.0: answering societal challenges**. As we all know, the current analytical knowledge has broken through many technological and methodological barriers and it is now the responsibility of our analytical community to put all this progress at the service of the sustainable development goals that society demands. By using green methodologies and technologies, ranging from the smallest smart sensors to multimodal and high throughput techniques, the Analytics 5.0 framework will contribute to the improvement of health and well-being, the responsible production based on industry innovation and the care for the environment and the protection of ecosystems. Achieving these goals in the near future could not be done without the committed engagement of young researchers and it is also the intention of Euroanalysis 2025 to give an active role to the newest members of our scientific field.

In this venture, we are very grateful for the support provided by the **Catalan Chemical Society (SCQ)**, a scientific institution actively involved in many events promoted by the EuChemS. We also thank a large number of research centres, societies, companies and industrial consortia, who have seen the possibility to host Euroanalysis in Barcelona as an excellent scientific initiative for their communities. We are also very pleased to announce that the **Spanish Society of Chromatography and Related Techniques (SECYTA)** incorporates the XXIV Edition of its annual Meeting within Euroanalysis 2025.

We trust that Euroanalysis 2025 will be the starting point of fruitful scientific collaborations and a great event to promote cutting-edge analytical knowledge and interdisciplinary scientific challenges and opportunities.

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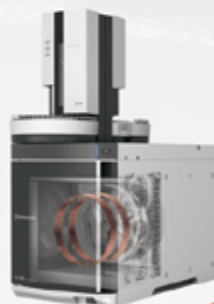
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PLENARY LECTURES



History lecture

ANALYTICAL CHEMISTRY IN SPAIN: AN OVERVIEW OF THE LAST 50 YEARS

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In this History Lecture, an overview of the Analytical Chemistry in Spain along the last 50 years is presented. This period covers the full academic life of the author, from his undergraduate years to the present, and offers a personal and direct perspective of the evolution of the Analytical Chemistry and the University in Spain in that period.

The teaching of Chemistry in Spain began in the 18th century with unofficial initiatives of the Economic Societies of Friends of the Country. The teaching of Chemistry in universities was established during the 19th century, and the creation of Analytical Chemistry chairs was done later inside Faculties of Sciences or Faculties of Pharmacy, emphasizing its relevance to pharmaceutical studies.

The situation and evolution in the 1970's and 80's are presented, with the inflection point that represented the Spain's integration into the EEC on January 1, 1986. Further evolution is outlined.

The State of the Art of the Higher Education in Spain is summarized, with special attention to Chemistry and Chemical-related studies.

A summary of the Scientific Societies related to Analytical Chemistry is presented.

An overview of the Research Institutes and Structures dealing with investigations related to the objectives and tools of Analytical Chemistry is also summarized.

This presentation is dedicated to the memory of Prof. Enric Casassas i Simó (1920-2000) mentor of several generations of academics and researchers.

PL-01**TEMPERATURE RESPONSIVE AND PER-AQUEOUS LIQUID CHROMATOGRAPHY: NEW SOLUTIONS FOR MORE SUSTAINABLE MULTIDIMENSIONAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY****Lynen, Frederic.***Ghent University, Belgium, Gent, Belgium.*

High-performance liquid chromatography has become a pivotal enabling technique for molecular separations in a multitude of application fields including e.g. pharmaceutical, environmental, food, art-historical, natural product analyses, or in life sciences in general. The bulk of such HPLC implementations is based on the well-established separation modes such as reversed phase (RPLC), hydrophilic interaction (HILIC) or e.g. normal phase liquid chromatography (NPLC). However, a drawback of these modes is their non-negligible consumption of organic solvents, inevitably leading to sustainability issues. Additionally their necessity for compositional solvent gradients often limits the choice in detector compatibility. Another drawback of conventional (1-D) HPLC is that it is fundamentally limited in terms of the achievable resolving power. Therefore, two-dimensional LC approaches are increasingly studied and implemented. While more challenging from an instrumental point of view, the separation benefits obtained in this way also often come at the cost of various compromises, including a decline in method sensitivity, enhanced solvent consumption and sub-optimal separations. In the light of such drawbacks, the emergence of purely aqueous separation modes in liquid chromatography attracts increasing attention beyond the inherent sustainability benefits they offer.

Temperature Responsive Liquid Chromatography (TRLC) is such an emerging green HPLC mode similar to reversed phase separations, requiring only water as the mobile phase. Because the retention and separation is thereby solely based on the temperature-tunable hydrophobicity of stimuli-responsive polymers immobilized in the column, this approach forfeits the need for organic solvents in HPLC. Alternatively, Per-Aqueous Liquid Chromatography (PALC) is also a recent HPLC mode, which also allows for the use of (mainly) aqueous mobile phases. PALC offers some similar benefits as TRLC albeit on commercial columns, and has already proven particularly advantageous for analytes depicting a high polarity or limited solubility in organic solvents.

In this contribution, the benefits of TRLC and PALC are explored in the context of high-end 1-D and 2-D HPLC separation strategies. The temperature-tunable stationary phases therein e.g. prove to be ideally suitable for cheap purely aqueous separations of many molecular classes. Additionally the approach effectively foregoes with detector issues related to compositional solvent gradients. The advantages of such aqueous separation methods in HPLC are first illustrated through 1-D HPLC based pharmaceutical and natural product analyses. The unique benefits purely aqueous separations can offer in comprehensive two-dimensional liquid chromatography (LCxLC) are also introduced. When paired with reversed phase LC in the second dimension, complete and generic peak refocusing occurs at the start of the second column. This provides several benefits, effectively addressing modulation issues common in LCxLC, while much simplifying the method development. This makes LCxLC more accessible to non-expert users. Additionally, these refocusing mechanisms enhance detection sensitivity and allow for direct, split-free coupling of TRLCxRPLC to mass spectrometry, unlike standard LCxLC practices that often result in sensitivity losses due to modulation challenges. Such multidimensional platforms also hold promise for enhanced chiral screening of complex natural or synthetic mixtures. The benefits of TRLCxRPLC will be demonstrated through various applications, including the analysis of flavonoids in natural products, pharmaceutical impurities in therapeutic formulations, or the study of the degradation cascades in appearing in oxidatively treated wastewater samples.

PL-02**ULTRASENSITIVE AND MULTIPLEXED NANOPHOTONIC SENSORS FOR NEXT-GENERATION POINT-OF-CARE ANALYTICAL APPLICATIONS****Lechuga, Laura M.***ICN2, CSIC, BIST & CIBER-BBN, Barcelona, Spain.*

We are living in an era marked by unprecedented global challenges—climate change, environmental crises, and rising antibiotic resistance—all contributing to an increase in complex and difficult-to-treat diseases. The COVID-19 pandemic exposed critical gaps in our healthcare infrastructure, revealing both our vulnerability and the urgent need for rapid, decentralised diagnostic solutions. At the same time, it demonstrated the transformative power of deploying point-of-care (POC) diagnostics for real-time detection, monitoring, and containment of disease spread.

Nanophotonic biosensors are powerful tools capable of meeting these demands by enabling ultrasensitive, rapid, and reliable diagnostics directly at the point of need. We have developed cutting-edge nanophotonic biosensors based on silicon photonics and nanoplasmonics that allow for label-free detection of disease biomarkers within minutes. Through tailored surface biochemistry, our biosensor chips can sensitively and selectively detect proteins, genetic markers, or pathogens in under 15 minutes—achieving detection limits in the picomolar range. A central challenge in clinical biosensing is biofouling—non-specific adsorption of proteins and cells—which can impair sensor performance in complex biological fluids. To address this, we develop tailored antifouling strategies using hydrophilic and zwitterionic polymers, enabling robust biochip surface chemistry adapted to each application.

Our technology has been clinically validated across a range of applications, including therapeutic drug monitoring (e.g., antibiotics), allergy testing from plasma, early-stage cancer diagnostics (colorectal and lung cancer), and detection of bacterial, viral, and antimicrobial-resistant infections. Recently, we have further advanced our platform to enable multiplexed detection—capable of simultaneously analysing up to seven protein or genetic biomarkers from a single patient sample.

Beyond clinical diagnostics, we have integrated crystalline porous metal–organic frameworks (MOFs) and molecular metal–organic polyhedra (MOPs) into our sensor platforms. These materials offer versatile functionalization capabilities, enabling the selective and real-time detection of small pollutant molecules (<500 Da) in water. This advancement broadens the scope of our technology to include rapid, field-deployable environmental sensing.

Our nanophotonic biosensor platform holds significant promise for the future of decentralised diagnostics. Its compact, user-friendly format enables operation by non-experts on-side or in remote settings, potentially transforming how and where we diagnose disease—and ultimately enabling faster, more informed analytical decision-making.

PL-03

GREENNESS METRICS AND ASSESSMENTS

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Multi-Criteria Decision Analysis (MCDA) is a powerful tool that supports decision-making processes by enabling scientifically grounded choices. When traditional greenness factors are used as criteria, MCDA functions can be applied as a greenness assessment or metric tool. Common criteria include compound toxicity across various organisms and exposure pathways, environmental persistence factors such as hydrolysis half-life and biodegradability, and safety parameters like flash point, flammability, or the formation of toxic degradation byproducts. Incorporating these criteria allows alternatives to be ranked according to their overall greenness. Given the multi-dimensional nature of greenness, a multi-criteria assessment approach is particularly well-suited for this purpose.

MCDA has been applied to assess the greenness of organic solvents, ionic liquids, deep eutectic solvents, mobile phase compositions and derivatization agents. In each case, complete rankings were generated, providing a greenness score for each alternative. This enabled informed recommendations on selecting green solvents and reagents while identifying the best. Additionally, MCDA can be integrated with experimental design strategies, supporting the simultaneous optimization of process efficiency and environmental sustainability.

Metric tools designed to evaluate greenness, whiteness, and related aspects of analytical procedures are becoming increasingly popular. However, the lack of standardization in their application often leads to unintentional misuse.

To obtain comprehensive information on the results of greenness assessment two datasets were generated: one comprising greenness assessments of 27 analytical procedures (for general evaluation) and another with 3 procedures (for reproducibility analysis), using various available metric tools. Multivariate statistical analysis of the first dataset revealed that the tools produce correlated results. The second dataset was used to assess the reproducibility of these tool-based evaluations.

Based on multivariate statistical and reproducibility analyses, practical guidelines for using metric tools are proposed. Following these guidelines a more standardized assessment approach, ensure consistent results, and enable more meaningful interpretations is promoted. Therefore, CLEANS GUIDELINES are formed, which are mnemotechnic way to express recommendations. CLEANS are recommendations for analytical chemists, who develop new procedures, to fully characterize the impacts or threats of the method. This is especially important in terms of amounts of wastes generated, energy demands, time of analysis and full information on the type and amount of every reagent and solvent used.

On the other hand, GUIDELINES provide a practical framework for effectively selecting, applying Green Analytical Chemistry and related metric tools. GUIDELINES put stress on the proper understanding of the metric tool, their proper selection for the given task. The application of the tool should include the comparison of the greenness assessment results of the newly developed procedure with previously published ones. To reduce the bias, the assessments should be performed by a single assessor. The results should be treated critically and proper discussion on the greenness improvement should be supplied in the manuscript.

Another notice is that metric tools that appear in the literature do not bring any new approach. They only show a new way of presenting the results, while the assessment criteria are the same as in previous contributions. LCA based, thus very detailed, assessment of analytical procedures would be very welcome.

Robert Kellner lecture**THE TRANSFORMATIVE JOURNEY FROM GREEN SAMPLE PREPARATION TO SUSTAINABLE ANALYTICAL CHEMISTRY****Psillakis, Elia.***Technical University of Crete, Chania-Crete, Greece.*

The pursuit of green and sustainable practices has become a cornerstone of modern analytical chemistry, urging the scientific community to fundamentally rethink the design, implementation, and assessment of analytical workflows. This presentation highlights recent advancements in sample preparation, including the introduction of the green sample preparation concept [1], the development of AGREeprep [2], and a new solvent selection guide that integrates environmental, health, safety, and life cycle considerations. Building on this foundation, the talk introduces the framework of Circular Analytical Chemistry [3], which aims to decouple analytical performance from resource consumption and support the transition to a resource-efficient, closed-loop, and waste-free analytical sector. The presentation also examines how sustainability is currently perceived within the analytical chemistry community [4], emphasizing the gap between partial compliance with green criteria and the systemic transformation required for true sustainability. It frames sustainability through the three interdependent pillars (environmental, societal, and economic) critiquing reductionist approaches and advocating for systems thinking to avoid the unintentional transfer of burdens across domains.

By identifying key areas for improvement and offering actionable recommendations, this talk calls for a paradigm shift in analytical chemistry. The ultimate goal is to present our latest understanding and perspective on navigating the complexities of sustainability, dispelling common myths while revealing practical pathways for impactful change. Through challenging long-held assumptions and fostering deeper engagement with sustainable practices, this presentation aims to inspire a transformative dialogue on the future of analytical chemistry.

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DAC award**SENSORS, TOOLS FOR BIOINTELLIGENCE?****Günter, Gauglitz.***Eberhard-Karls-University, Tübingen, Germany.*

For some years now, the term biointelligence has been used to combine the concepts of biology, technology, and information sciences. Sensors play a significant role not only in production processes. Biointelligent systems contain a technical and a biological component as well as a feedback loop, and exhibit features of artificial intelligence. It is expected that they will contribute to a sustainable design of products and production in various areas of human needs, whether it be health, nutrition, energy, housing, or consumption. Sensors play a significant role not only in production processes. They can monitor biological processes and transform them into digital data, which can be essential for controlling these processes, ideally in real-time.

For this reason, the lecture will classify sensors first according to transducer, detection method, and recognition element. Thereby, the focus will be on optical biosensors that use reflectometry. Based on the development of this detection method over decades, it should be demonstrated by applications how label-free biosensors can be used in various areas of human needs. Methodical changes and developments over the years will be discussed, particularly with the aim of multi-analyte detection and imaging. Finally, it should be shown how biosensors can make a significant contribution in virus bio-intelligent manufacturing processes as a current project.



KEYNOTE LECTURES



KL-01

MASS SPECTROMETRY: A GAME CHANGER IN ANALYTICAL CHEMISTRY

Moyano, Encarnación

University of Barcelona, Barcelona, Spain.

Mass spectrometry (MS) has become one of the most powerful and transformative tools in modern analytical chemistry. Its ability to detect, identify, and quantify chemical species with high sensitivity, accuracy, and speed has revolutionized the way scientists approach complex analytical problems. Over the past few decades, MS has evolved from a specialized technique into a central pillar of research and diagnostics across disciplines such as environmental science, biomedicine, food safety, materials science, and forensic analysis.

The core strength of MS lies in its capacity to provide molecular-level information, even in highly complex mixtures and at trace concentrations. Advances in ionization techniques—such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), and ambient ionization methods—have expanded the range of analytes that can be studied, from small organic molecules to large biomacromolecules like proteins and nucleic acids. Coupled with high-resolution mass analyzers (e.g., Orbitrap, time-of-flight, and Fourier-transform ion cyclotron resonance), MS now offers unparalleled mass accuracy and resolution, enabling the elucidation of molecular structures and the detection of subtle chemical modifications.

This presentation will explore how recent technological and methodological innovations have positioned MS as a game-changing platform in analytical chemistry. Special attention will be given to the integration of MS with separation techniques such as liquid chromatography (LC-MS) and gas chromatography (GC-MS), which enhance selectivity and reduce matrix effects. These hyphenated techniques are now standard in metabolomics, lipidomics, and proteomics workflows, allowing for comprehensive profiling of biological systems and the discovery of novel biomarkers.

Another key development is the increasing role of computational tools and artificial intelligence in MS data analysis. The complexity and volume of spectral data generated by modern instruments demand advanced algorithms for peak detection, compound identification, and quantification. Machine learning approaches are being applied to automate spectral interpretation, improve deconvolution of overlapping signals, and predict fragmentation patterns, thereby accelerating data processing and improving reproducibility.

Case studies will be presented to illustrate the impact of MS in various domains. In clinical diagnostics, MS is enabling the detection of disease biomarkers with high specificity, supporting early diagnosis and personalized medicine. In environmental monitoring, it is used to trace pollutants and emerging contaminants at ultra-trace levels. In food safety, MS ensures the detection of adulterants, allergens, and residues with regulatory compliance. In forensic science, it provides robust evidence through the analysis of drugs, explosives, and biological samples.

Despite its many advantages, MS also faces challenges, including instrument cost, the need for skilled operators, and issues related to standardization and data sharing. However, ongoing efforts in miniaturization, automation, and open-access databases are addressing these limitations and making MS more accessible to a broader scientific community.

In conclusion, mass spectrometry is not merely a tool but a transformative force in analytical chemistry. Its continuous evolution is reshaping how we understand and interact with the chemical world, offering new possibilities for innovation, discovery, and societal impact.

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KL-02**MASS SPEC IMAGING - A COMPREHENSIVE VIEW OF MOLECULAR INSIGHTS**

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A single modality is very often not good enough to understand all functional, structural, temporal and chemical relations underlying certain biological conditions. Imaging a specimen with two or more complementary modalities creates an informative, composite view of a sample that spans all relevant resolution ranges. Here we shortly introduce the concept of MALDI Mass Spectrometry based imaging (MALDI MSI) and its potential to localize molecules in a tissue without a priori knowledge. Advantages, limitations, and potentials for applications are briefly highlighted.

In this talk our investigations on aging skin are presented to show the potential of MALDI MSI. Our skin is continually subjected to sunlight, high oxygen levels, and environmental pollutants, which are significant stressors that expedite premature aging, inflammation, and skin cancer. The most noticeable clinical effect of skin aging is the alteration in skin appearance. To counteract oxidative stress, cells deploy several defense mechanisms.

Oxidized lipids, such as oxidized phosphatidylcholines (OxPCs), function as danger-associated molecular patterns. In our research, we study the impact of UV light on human skin and compare a 3D skin model with human skin biopsies. The high mass accuracy of Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS), along with the spatial localization and relative abundance of analytes, enabled us to confidently identify various OxPCs and associate them with specific skin compartments. By integrating these findings with high-resolution light microscopy and immunohistology. Our observations in skin models were mirrored in samples from both young and elderly donors. We recently added more general metabolite data after developing an improved sample preparation strategy. Especially glucose distributions are of relevance in aging as previously shown.

We introduce a multimodal imaging workflow to explore spatially resolved, senescence-related alterations in the skin N-glycome, which affects the extracellular matrix and is a key determinant of skin aging. Age-related changes were studied by correlating N-glycan composition with MALDI-IHC, immunofluorescence, autofluorescence, and tissue staining, all conducted on a single tissue section. Additional tissue staining and spatial transcriptomics were performed on an adjacent section to reinforce the N-glycan data by co-registering all datasets at full resolution, and then integrating the data to create a common coordinate system and match readouts that accounted for the differences in spatial resolutions. This allowed for simultaneous viewing and enabled integrated spatial correlation analysis across assays, multi-omics tissue segmentation and differential expression analysis.

KL-03**BIOANALYTICAL STRATEGIES FOR ANTIBODY-BASED THERAPEUTICS IN DRUG DISCOVERY AND DEVELOPMENT****Eichhorn, Peter.***Merck KGaA, Darmstadt, Germany.*

The bioanalysis of therapeutic antibodies and antibody-drug conjugates (ADCs) is crucial in drug discovery and development, given the expanding role of these modalities in targeted therapies for the treatment of cancer, autoimmune diseases, and infectious diseases. Antibody-based drugs represent a rapidly growing market, with global sales exceeding tens of billions of dollars annually. The increasing complexity of antibody structures, including bispecifics and multispecific antibodies as well as next-generation ADCs, poses new challenges in bioanalytical strategies. Their quantification in pharmacokinetic, in vivo efficacy and toxicology studies in preclinical species facilitates the selection of promising drug candidates and ensures the accurate calculation of safety margins. Furthermore, robust exposure measurements inform Modeling&Simulation approaches to support human dose predictions and to establish exposure-response relationships in clinical trials. Ultimately, bioanalytical assessments are fundamental for meeting regulatory requirements. In this lecture the bioanalytical strategies implemented at Merck's NBE-DMPK Department are presented and illustrated with a number of selected case studies from recent projects employing ligand binding assays and hybrid LC-MS methods for ADC and multispecific antibodies.

KL-04**FROM MASS SPECTRUM TO STRUCTURE: ACCELERATING METABOLITE IDENTIFICATION WITH AI AND CLOUD COMPUTING****Yanes, Oscar.***Universitat Rovira i Virgili, Reus - Tarragona, Spain.*

Accurate identification of metabolites from mass spectrometry (MS/MS) data remains a central bottleneck in metabolomics. Traditional approaches rely heavily on matching experimental spectra with reference libraries of known compounds. However, most biologically relevant metabolites lack empirical spectra, and reference databases remain fragmentary. To address this gap, we need scalable *in silico* solutions that can reliably annotate unknown spectra and keep pace with modern, high-throughput MS and MS/MS workflows.

In this keynote, I will present two novel AI-driven tools designed to advance computational metabolite identification:

- *SingleFrag*, a deep learning model that predicts individual fragments of a molecule independently, instead of modeling the entire fragmentation pattern. This flexible architecture overcomes limitations of existing *in silico* fragmentation tools, achieving improved performance in annotating MS/MS spectra of unknown compounds. *SingleFrag* enabled the identification of multiple recurrent, previously unannotated molecules in human biofluids, demonstrating its practical utility in metabolomics research.
- *ChemEmbed*, a neural network framework that learns joint representations of chemical structures and MS/MS spectra using enhanced spectral features—such as merged collision energies and computed neutral losses—from over 38,000 compounds. This richer input enables more accurate candidate ranking in metabolite identification. *ChemEmbed* outperforms leading tools, including SIRIUS 6.0, across several benchmarks (e.g., CASMI challenges, ARUS dataset), and successfully resolved dozens of recurrent unidentified compounds in human samples.

Both tools are seamlessly integrated into DATOMA, our cloud-native platform for scalable metabolomics data analysis. DATOMA provides access to a curated suite of over 20 tools spanning LC-MS, MS/MS, GC-MS, and MS imaging workflows, all accessible via an intuitive web interface or programmatically through a dedicated Python package and API. Among its modular workflows, we highlight *MS2Xplorer*, which enables comprehensive annotation of unknown MS/MS spectra by combining orthogonal molecular formula prediction strategies—SIRIUS (top-down) and MSBuddy (bottom-up)—alongside spectral matching against both experimental (MS2ID) and *in silico* (*SingleFrag*) databases. The workflow also incorporates structural vector representations via *ChemEmbed*, enhancing identification accuracy. As an open and extensible platform, DATOMA welcomes community-driven contributions of new tools, scripts, and workflows to foster innovation in metabolomics.

Together, these advances represent a leap forward in metabolite identification—combining machine learning, signal processing, and cloud infrastructure to accelerate the journey from mass spectrum to molecular structure.

KL-05

MASS SPECTROMETRY APPROACHES TO THE ANALYSIS OF NANO- AND MICROPLASTICS

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The accumulation of plastic waste in the environment has led to a growing prevalence of small plastic particles, commonly known as microplastics and nanoplastics. Unlike larger plastic debris, these minute particles - characterized by a high surface-area-to-volume ratio and strong sorptive properties - exhibit unique behaviors that raise concerns about their ecological impacts, potential risks to human health, and broader environmental consequences. Additionally, they can act as vectors for the transport of organic pollutants and serve as sources of toxic metals, either introduced during manufacturing as additives or absorbed from contaminated water sources. Consequently, there is an increasing demand for robust analytical techniques to quantify plastic pollution across various environmental matrices, assess exposure levels, and investigate related health risks.

Mass spectrometry (MS) appears to offer valuable tools for advancing the study of micro- and nanoplastics. In particular, inductively coupled plasma mass spectrometry (ICP-MS), when operated in single-particle mode (SP-ICP-MS), has demonstrated potential due to its sensitivity and detection capabilities. Several analytical strategies have been explored, including monitoring natural constituents of plastic particles - such as the ^{13}C isotope or fluorine in PTFE - as well as the application of metal-based tagging approaches. ICP-MS may also be used to analyze inorganic substances associated with plastics, such as metal-based pigments, catalysts, or adsorbed heavy metals. In parallel, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) have been employed to detect degradation products, and organic contaminants either present in or adsorbed onto plastic particles. Together, these methods contribute to a better understanding of the role of microplastics as potential carriers of environmental pollutants.

Recent advancements in mass spectrometric methods for analyzing nano- and microplastic contamination will be illustrated through case studies conducted at the Institute of Analytical and Physical Chemistry for the Environment and Materials (IPREM) in Pau, France. The presentation will emphasize how integrating multiple analytical approaches provides a comprehensive understanding of the risks associated with micro- and nanoplastics.

KL-06**WHY DO WE NEED A THEORY OF SAMPLING (TOS) FOR ANALYSIS?**

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The 'before analysis' domain is prone to sampling errors resulting in uncertainties influencing the quality of both analysis and subsequent data analysis/data modelling. Non-representative sampling of heterogeneous materials, batches, lots and process streams 'before analysis' contribute significantly to the total Measurement Uncertainty, $MU_{total} = MU_{sampling} + MU_{analysis}$. The total sampling error (TSE) can dominate over the total analytical error (TAE) by factors ranging 5, 10 or *higher*, depending on the *degree* of material heterogeneity encountered and the specific sampling procedure employed to produce the final analytical aliquot, which is the only material actually analysed. The analytical aliquot is the physical manifestation of transgressing the boundary from the before analysis (sampling) domain to the domain of analysis. It is only possible to guarantee representativity of the analytical aliquot, and thus of the analytical results *with respect to the original target batch/lot/process stream*, by invoking the necessary sampling domain competence stipulated by Theory of Sampling (TOS). TOS offers ways and means to deal actively with a potential sampling bias (which is fundamentally different from the analytical bias). Overlooking, or deliberately ignoring dealing appropriately with sampling effects constitutes a lack of due diligence, which has critical bearings on the QC/QA demands on both analysis and subsequent data analysis/modelling.

KL-07

EXCELLENCE IN ANALYTICAL CHEMISTRY: A JOINT MASTER'S PROGRAMME

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EACH - Excellence in Analytical Chemistry (www.analyticalchemistry.eu)¹ is an **international two-year Erasmus Mundus master's programme** that educates specialists in analytical chemistry qualifying them to work in various industries (food, pharmaceutical, materials, energy, etc) and chemical analysis laboratories (environment, food, health, etc) worldwide.

EACH is taught in collaboration with **four partner universities** – University of Tartu (UT, coordinator), Estonia; Uppsala University (UU), Sweden; University Claude Bernard Lyon 1 (UCBL), France; and Åbo Akademi University (AAU), Finland – and a number of **associated partners** – industries (Bruker, Axel'One, Arkema, IFP, etc), laboratories and academic institutions.

Student mobility is a fundamental property of the programme. Students spend the first study year (**Scheme 1**) at UT learning the fundamental concepts and skills of analytical chemistry, metrology in chemistry, and the quality and socio-economic aspects of analytical chemistry. The second year is specialization oriented and is spent either at UCBL, UU or AAU. The master's thesis is done at one of the second-year universities or at associated partners.

EACH provides knowledge and skills in both fundamental and applied aspects of modern analytical chemistry, aiming to produce entrepreneurial and innovation-prone graduates. Teaching in the programme involves a significant amount of **learning by doing**, as opposed to the typical lecture-based teaching. The **active involvement of industry** via teaching courses, providing internship placements and supervising master's theses ensures that EACH responds to the needs of industry and laboratories, including applications in the interdisciplinary areas (sustainable energy, trade, climate, security, “omics”, industrial processes, etc).

The presentation will focus on the following:

1. Highlights of some of the more interesting and innovative **teaching approaches** used in the programme.
2. **Employability** of the EACH graduates – what measures the programme has taken to achieve it and what are the results.
3. Developing EACH into a **pan-European Analytical Chemistry Hub** – involving universities from different European University Alliances with the aim of developing a shared European competence framework, aligning curricula and creating resources with a European dimension.

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KL-08

ADDING COLOUR TO ION-SELECTIVE SENSORS

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The composition of classical ion-selective potentiometric sensors receptors requires the presence of ionophore and ion-exchanger within plasticized polymeric matrix. Typically, none of these compounds is active in optical mode, thus typically electrochemical, potentiometric, ion-selective sensors are colorless.

Adding dyes to ion-selective membranes is attractive challenge that can extend application of highly selective sensors beyond the current paradigm, benefiting from absorption (colorimetry) or emission (fluorimetry) modes. Even more challenging it is to achieve these using dyes that do not perturb electrochemical performance of the sensors, and do not induce receptor sensitivity to pH changes. This can be accomplished using Nile blue or Nile red dyes, that are useful for ion-selective sensing [1].

The presence of electrically neutral dye, such as Nile red, in the ion-selective membrane intended for potentiometric sensing offers the possibility of colorimetric/ fluorometric or even visual monitoring ions and water transport within the phase during pretreatment – to optimize pretreatment time, to track membrane history [2].

Another possibilities arise from using cationic dyes as cation-exchanger counter ions, necessary additive while preparing neutral ionophores containing receptors. Thus ionophore driven ion-exchange between sample and the membrane during conditioning gives rise to possibility of monitoring this process. It is shown that analytical information can be obtained from the conditioning process. It is also shown that colorimetric, analytically useful signal obtained is especially attractive for analysis of samples where vast majority of interfering ions prevails.

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KL-09**EFFECT OF ALKYL LENGTH IN POLYAMIDE NANOFIBERS ON SAMPLE PREPARATION USING SPE****Svec, Frantisek; Czys, Ewelina; Šatínský, Dalibor.***Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Kralove, Czech Republic.*

Solid phase extraction (SPE) is one of the most popular sample preparation methods because it can significantly reduce the amount of interfering ballast compounds in the sample prior to analysis and matrix effects in MS detection. A large number of advanced sorbents suitable for SPE-HPLC and capable of purifying a variety of matrices includes among many other nanomaterials and, in particular, nanofibers [1,2]. Although various approaches can be used to prepare nanofibers, the relatively simple and inexpensive electrospinning is the most widely used. Electrospun fibers have already been applied in both off-line and on-line sample preparation by solid phase extraction while treating different types of matrices. Their advantages include affordability, the wide variability of chemistries of available polymers, and high extraction efficiency. We tested fibers prepared from fairly common polymers including poly(vinylidene difluoride), polystyrene, polyacrylonitrile, various polyamides and copolyamides, poly(ϵ -caprolactone), poly(lactic acid), poly(ethylene imine), polyethylene, and polypropylene. Fibers made from these polymers encompass a wide range of adsorption mechanisms due to their different chemistries, hydrophobicity/hydrophilicity, acceptor/donor properties, dipole-dipole, and π - π interactions. They also differ in their glass temperature T_g , a property that significantly affects their mechanical properties.

Most of our micro- and nanofibers were prepared by simple DC and AC electrospinning techniques from the polymers alone. Alternatively, we have also introduced modifications allowing us to produce hybrid materials and coated fibers. For example, hybrid polymer fibers were prepared by dispersing particles, such as magnetic nanoparticles, graphene, activated carbon, and carbon black, into the polymer solution prior to electrospinning. Surface modification of polymer nanofiber sorbents or coating is another way to improve their performance by changing their functionalities. This was achieved by dip-coating, where the surface of polymer fibers was covered with polyhydroxy compounds including graphene oxide, tannin, dopamine, hesperidin, and heparin. The sorbents in free fibers format were packed in commercial stainless-steel cartridges and used for on-line SPE-HPLC. Alternatively, small 10 mm diameter nanofiber discs were cut from a compact and mechanically stable 1–2 mm thick micro/nanofiber mat. The best mat was prepared by the AC electrospinning of poly(ϵ -caprolactone) solution containing graphene nanoplates. Extraction was carried out in a stirred sample containing the disk located in a beaker. In another implementation, the disc was pierced with a steel staple wire and placed in the sample. The magnetic stirrer rotated the disc at the bottom of the beaker and simultaneously stirred the liquid thus facilitating the adsorption. The off-line SPE using these discs was demonstrated with the extraction of compounds from local river water and its spiked counterpart. We determined typical contaminants of different polarities, including bisphenols A, C, S, Z, fenoxycarb, kadethrin, and deltamethrin. Compared to previous reports, our method allowed a significant reduction in sample preparation steps, which included only sorbent conditioning, adsorption, and final desorption directly in the HPLC vial. Very small amounts of sorbent and organic solvent were required for the extraction in the HPLC vial making our approach environmentally friendly with a method greenness of 0.61.

KL-10

NON-TARGET CHEMOMETRIC ANALYSIS OF MASS SPECTROMETRY DATA

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While Mass Spectrometry Data-Dependent Acquisition with Targeted Chromatographic Analysis (MS-TA-DDA) focuses on specific ions from known compounds, offering high sensitivity and precise quantification, Mass Spectrometry Data-Independent Acquisition with Non-Targeted Chromatographic Analysis (MS-NTA-DIA) provides a broad, unbiased analysis of all ions within a defined mass range. This makes MS-NTA-DIA ideal for discovery and non-targeted profiling, overcoming the limitations of DDA, which cannot identify compounds outside preselected targets. However, the data generated by DIA is significantly more complex and requires advanced computational tools for accurate interpretation.

The Regions of Interest Multivariate Curve Resolution (ROIMCR) chemometric approach¹ integrates two powerful methods: ROI² and MCR-ALS³. The ROI method efficiently compresses data in the m/z dimension, significantly reducing their size, while preserving spectral resolution. MCR-ALS (Multivariate Curve Resolution–Alternating Least Squares) is a flexible tool for resolving mixtures without prior knowledge of the number or identity of components. It supports the inclusion of constraints and known information for improved results. ROIMCR-generated mass spectra allow improved annotation and identification through comparison with public databases and known standards. To aid this process, the MSident⁴ tool has been developed for annotating and identifying unknown chemical constituents. ROIMCR elution (concentration) profiles—measured via peak areas or heights—can be used for: a) relative quantification and concentration estimation; b) calibration; c) pattern recognition (e.g., PCA, PLS-DA); d) features and biomarker selection; and e) statistical evaluations (e.g., DOE, t-tests, ANOVA, ASCA).

In this presentation, we demonstrate the application of ROIMCR to full-scan NTA-DIA datasets, including simultaneous analysis of MS1 and MS2 in both positive and negative ion modes⁵, mass spectrometry imaging⁶, and ion mobility spectrometry (IMS)⁷. The ability to link diverse MS data acquisition types through the elution and spectral profiles of each ROIMCR-resolved component extends the utility of MS techniques for analyzing complex natural samples.

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KL-11**DIGGING INTO THE MULTIFACETED VARIABILITY OF (AUTO)ANTIBODY MOLECULES USING NOVEL MS-BASED APPROACHES****Domínguez Vega, Elena.***Leiden University Medical Center, Leiden, Netherlands.*

Antibodies recruit immune responses via interaction with different Fcγ receptors (FcRs). These interactions are strongly influenced by structural features of the antibody, including glycosylation. Antibody structures are very diverse, challenging comprehensive characterization. In particular, functional understanding of antibody variants possess a huge endeavor as most binding techniques such as SPR provide an overall affinity response for all different (glyco)forms requiring tedious production or enrichment of specific forms.

In our lab, we have developed multiple MS-based approaches to dissect the structural and functional variability of (auto)antibodies. We established different middle-up strategies using specific hinge-region enzymes permitting to decipher (auto)antibody Fc-proteoforms. The strategy permitted to study IgG-Fc heterogeneity of autoantibodies isolated from rheumatoid arthritis (RA) revealing associations with disease markers. To assess functional changes, we have exploited the capabilities of affinity CE-MS to study the binding of antibodies and FcRs in a proteoform-resolved fashion. We will show that the proposed approach is able to determine the relative binding affinity of different glycoforms based on the shifts on their mobility. As anticipated, Fc glycosylation was key for the binding. Hemiglycosylated antibodies showed strong decrease in the binding affinity towards the FcγRs while non-Fc glycosylated forms showed near no binding. Fc-glycoforms behaved differently between receptors with clear differences for afucosylated and high mannose variants. Furthermore, our results showed an altered binding for the Fab-glycosylated variants. As Fab glycans are far from the binding site, the change in binding properties most likely correlates to a change in protein conformation in the interaction surface.

KL-12**ADVANCING FOOD METABOLOMICS: ANALYTICAL STRATEGIES, MAIN AREAS OF INTEREST AND EXAMPLES OF INNOVATIVE APPLICATIONS**

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The emergence of food metabolomics has opened new frontiers for exploring food composition with unprecedented depth and precision. Through the qualitative and quantitative analysis of the food metabolome, researchers can gain valuable insights into nutritional content, quality attributes, authenticity, safety, and potential health benefits, driving innovation across food science and industry. Recent advances in analytical techniques and chemometric tools—previously viewed as technically challenging—have now made these analyses increasingly accessible. Innovative methodologies enable the simultaneous detection of compounds from diverse chemical classes within a single run, greatly enhancing metabolite coverage while improving efficiency, reducing analysis time, and lowering costs.

Applications of food metabolomics can be broadly categorized into several key areas, though the full scope of potential uses extends beyond these classifications: (a) compositional characterization of foods, (b) assessment of safety, quality, and authenticity (e.g., verifying botanical or geographical origin), (c) agrotechnological and industrial research—focusing on how various practices influence food composition, (d) evaluation of the results of breeding programmes at metabolic level and selection of specific varieties, (e) valorization of by-products, and (f) studies related to health and nutrition.

This contribution focuses on applications related to the determination of metabolites of interest in olive-derived matrices and avocado tissues. Both targeted and non-targeted approaches combined with advanced chromatographic techniques coupled with mass spectrometry (in some cases ion mobility spectrometry), have been applied by our team to assess endogenous metabolites in olive and avocado samples. This presentation will highlight selected applications from the two areas we have prioritized in recent years, reflecting several of the main categories described above. It will feature a range of analytical methodologies and metabolomics strategies, address key analytical challenges, explore emerging trends, and outline current needs within the olive and avocado sectors.

By integrating robust analytical platforms with interdisciplinary collaboration, food metabolomics is set to transform our understanding and use of food systems. The goal of this presentation is to spark new ideas and stimulate dialogue on how to effectively embed metabolomics into a sustainable, health-oriented, innovation-driven food science.

KL-13**ADVANCES IN CHROMATOGRAPHY, MASS SPECTROMETRY AND DATA PROCESSING: THEIR INFLUENCE ON FOOD ANALYSIS**

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Food quality and safety are critically dependent on chromatographic methods coupled with mass spectrometry techniques. Chromatography-MS methods excel in resolving power, sensitivity, and the reliability of the information they provide. This holds both for gas chromatography as well as liquid chromatography. Trace levels of contaminants, taste- or aroma compounds, and micronutrients can be rapidly and reliably identified, even in samples with high concentrations of fats, salt, proteins or other interfering species. This all thanks to recent developments such as comprehensive two-dimensional chromatography methods (GC×GC, LC×LC or even LC×GC), large volume injection in GC, advanced sample preparation methods such as QuEChERS and (Arrow-)Solid-Phase Micro-Extraction, high-resolution Orbitrap GC- or LC-MS(/MS) methods, and last-but-not-least advanced data processing tools. How these developments have revolutionized the way we today perform food analysis will be illustrated with a number of examples ranging from the trace level detection of individual processing contaminants as acrylamide, to multi-analyte methods for the quantification of bitter peptides in sustainable foods, or the detailed characterisation of lipids to the detection of post-translational modifications in proteins using high resolution LC-MS etc.

Despite the massive improvements in the chromatography-MS analysis of foods, many challenges remain. Even if monitoring hundreds of pesticides the risk remains that compounds are missed. Moreover, data processing of such multi-methods requires far too much human involvement. Also, conversion products and structurally related analytes are missed in strictly targeted analytical methods. -Omics technologies are needed to detect 'deviating patterns' in new food sources. Whatever method is used, time-to-results is often too long as samples have to be transported to the laboratory and analysis and data processing is slow. Faster methods, and ideally methods that can be used in the field, are urgently needed. These challenges will be discussed and initial results we have e.g. in taking the lab to the sample rather than vice-versa, in detecting structural relationships between compounds, and in automated data processing in multi-residue methods will be shared.

KL-14**SUSTAINABLE SAMPLE PREPARATION: FROM PLANAR SUBSTRATES TO AFFORDABLE AMBIENT MASS SPECTROMETRY****Cárdenas, Soledad; Lucena, Rafael.***University of Córdoba, Córdoba, Spain.*

Nowadays, the term 'sustainability' is commonly used to emphasise analytical approaches that go beyond the concept of 'greenness', as they also involve reusability, renewable materials, and a commitment to the simplicity and affordability of the analytical process. Sample preparation is probably the most challenging step and the area most concerned with adopting this trend, with different ways to achieve this compromise. Planar substrates are an excellent alternative for thin-film microextraction (TFME) as they generally permit an increase in the sorbent phase used for analytes retention. They are also compatible with a variety of microextraction workflows, as well as with direct combination with mass spectrometric detection under direct infusion and substrate spray techniques. Typical planar substrates used include cellulose paper, adhesive tapes and blades. This communication presents the alternatives developed by the research group in the realm of sample preparation. Each substrate has its own advantages and disadvantages. For example, cellulose paper is sustainable and versatile. Its porosity enhances the superficial area of the extractant phase, and its surface functional groups contribute to the stability of the sorbent thin film. However, its poor mechanical stability is a disadvantage. Adhesive tapes are easily modified with micro- and nano-particulate sorbents, but, like paper, their poor mechanical stability requires them to be combined with a more rigid support. Metallic blades do not have this limitation, but the procedure to immobilise the sorbent is not straightforward due to their smooth surface. The possibility of directly combining these supports with mass spectrometry will also be presented. The affordability of the designed interfaces is the key factor in all developments.

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KL-15

INNOVATION & COLLABORATION FOR PROCESS ANALYTICAL TECHNOLOGY & ADVANCED PHARMACEUTICAL MANUFACTURING

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Process Analytical Technology (PAT) invites chemists to move out of the laboratory and increase their contribution to manufacturing processes. In PAT the chemist is no longer isolated from the manufacturing process and waiting for samples to arrive at the Quality Control laboratory. PAT requires collaboration to design and implement methods capable of providing timely measurements to control a manufacturing process. These timely measurements are typically achieved through non-destructive analytical methods.

Sampling is not eliminated in PAT. Sampling also occurs in PAT, enabling analysis through on-line or in-line methods. In PAT composite sampling and analysis may be performed through spectroscopic methods.

The presentation will show how the Principles of the Theory of Sampling (TOS) have helped to elucidate the effect of sampling errors in PAT measurements[1]. TOS has served as the basis for the development of a powder stream sampler which enables sampling and analysis of flowing powder mixtures in a continuous manufacturing process[2]. Variographic analysis has been applied to continuous manufacturing processes to understand the effect of sampling and analytical errors [3].

In summary, this talk will go over the key ideas that have guided the research of our Analytical & Pharmaceutical group over the past 25 years. The conference will seek to further inspire analytical chemists to approach challenges with an open mind to new ways of thinking and moving beyond current established practices.

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KL-16**FROM INFLUENT TO EFFLUENT: UNDERSTANDING MICROPLASTIC DISTRIBUTION IN WASTEWATER TREATMENT**

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Nowadays, water treatment and efficient water resource management are critical challenges for humanity, especially in arid regions with limited natural supplies. The scarcity of freshwater, combined with increasing demands driven by urban development, tourism and agriculture, among others, requires the advancement of technologies for wastewater treatment, desalination, and water reuse. In this context, Analytical Chemistry plays a fundamental role in ensuring the quality of treated water and sludge through the precise monitoring of emerging contaminants as well as key physicochemical parameters.

In recent years, microplastics (MPs) have been recognized as emerging contaminants of environmental concern as a result of their widespread occurrence, high mobility and persistence in the environment, and their adverse effects on biota, including humans. One of the most important sources and sinks of MPs are currently wastewater treatment plants (WWTPs). On one hand, WWTPs receive large quantities of MPs from domestic, industrial, and stormwater discharges; on the other, and despite they are critical barriers that prevent the release of these particles into the environment, they can disperse MPs when reclaimed water is used or when sludge are applied for fertilization purposes.

This keynote lecture pretends to provide a general vision on how MP concentrations vary within wastewater treatment steps and how they can be dispersed in the environment considering such sources. For this purpose, one year monitoring data regarding the occurrence, distribution and removal efficiency of MPs in a municipal WWTP located in Tenerife (Canary Islands, Spain), which incorporates a membrane bioreactor (MBR) and an electrodialysis reversal (EDR) system as part of its treatment line, will be presented. Additionally, sludge samples with varying moisture contents were analysed through another year to understand the partitioning of MPs in different solid waste fractions. Complementary to this, reclaimed water samples from other WWTPs across the Canary Islands were also examined to provide a broader regional perspective on MPs concentration and distribution.

Analytical methodologies involved a combination of physical and chemical sample preparation steps, including digestion, density separation, and filtration, followed by visual identification under stereomicroscopy and subsequently characterized using micro-Fourier Transform Infrared spectroscopy to determine polymer type.

KL-17**REFLECTANCE HYPERSPECTRAL IMAGING FOR ARTWORKS AND ARCHAEOLOGICAL ASSETS: STATE OF THE ART AND PERSPECTIVES****Cucci, Costanza.***Italian National Research Council (CNR), Sesto Fiorentino, Italy.*

Over the past few decades, Imaging Spectroscopy (IS) has evolved into a significant and structured research domain within the field of Cultural Heritage, demonstrating increasing utility in non-invasive analysis and documentation. Among IS techniques, Vis-NIR-SWIR reflectance Hyperspectral Imaging (HSI) is now acknowledged as one of the most comprehensive methodologies for the non-invasive and extensive investigation of polychrome surfaces and their underlying layers. The HSI data acquired on painted surfaces include high-resolution spatial and spectral information, which can be used not only for the identification and mapping of pigments and artists' materials, but also for the retrieval and inspection of hidden details (e.g., underdrawings, pentimenti, signatures). Thanks to its complementarity with other chemical imaging methods (e.g., MA-XRF), reflectance HSI has become pivotal in the multi-modal analytical protocols adopted by major museums for an in-depth, fully non-invasive analysis and enhanced documentation of important masterpieces.

In addition to the well-established HSI applications on museum objects (paintings, manuscripts, etc.), recent advances in device portability have enabled the extension of HSI to outdoor applications. As a result, a new research branch has emerged, focusing on archaeological applications of HSI, yielding promising results in subfields such as Roman mural paintings, Egyptological studies, and the detection of faded patterns in prehistoric rock-art sites.

The exceptional expansion of HSI applications in Cultural Heritage has also stimulated growth in data-driven research, particularly in the development of tailored algorithms for HSI data acquired on artworks. A prominent area of focus is the integration of HSI data with Artificial Intelligence (AI) methods for automatic data processing and exploitation of large datasets related to paintings, documentary heritage, and more.

The lecture will review the use of reflectance HSI in Cultural Heritage. The specific issues related to the implementation of HSI technique for investigating artworks will be discussed and illustrated through case studies from prestigious museums and cultural contexts (e.g., Uffizi Gallery, Pompeii archaeological site, Museu Picasso, etc.). The lecture will also address current technological challenges, open issues, and future perspectives in the field.

KL-18**SUSPECT AND NON-TARGET ANALYSIS FOR DETERMINATION OF PESTICIDES AND EMERGING CONTAMINANTS IN WATER AND FOOD SAMPLES**

Lambropoulou, Dimitra¹; Alampanos, Vasileios¹; Koronaiou, Lelouda - Athanasia¹; Petromelidou, Styliani¹; Danis, Theocharis²; Evgenidou, Eleni¹.

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The presence of pesticide residues and emerging contaminants in water and food samples poses significant risks to human health. Traditionally, monitoring of such residues has relied on targeted analysis using triple quadrupole mass spectrometry. However, recent advancements have shifted the focus toward suspect screening and non-target analysis (NTA), broadening the scope to include compounds for which limited information exists—substances that often go undetected by conventional targeted methods. To this end, the primary goal of the study was to display an integrated strategy by combining suspect and NTA approaches for screening different groups of micropollutants such as pesticides and emerging contaminants in water (surface waters) and food samples (peaches). To support this effort, a mass spectral library was built, alongside an evaluation of key parameters for peak picking, deconvolution, and compound annotation. Water samples were collected from diverse locations with varying degrees of anthropogenic impact. After solid-phase extraction, samples were analyzed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS), enabling comprehensive contamination profiling. For food analysis, a generic QuEChERS-based sample preparation method was employed, followed by LC-HRMS for detection. NTA results from the Aliakmonas River revealed the presence of various pesticides and emerging contaminants, such as pharmaceuticals, plasticizers, PFAS, and flame retardants, indicating continuous input of new pollutants. In peach samples, commonly detected pesticide residues included acetamiprid, boscalid, pyraclostrobin, fluopyram, tebuconazole. Finally, suspect screening through a stepwise-optimized workflow and a comprehensive in-lab database, more than 15 TPs of pesticides and emerging contaminants were tentatively identified in the Aliakmonas River, and over 5 pesticide TPs were detected in peach samples. These findings underscore the critical value of suspect and non-target screening approaches in uncovering the complex and evolving landscape of chemical contamination in both environmental and food matrices—highlighting the need for expanded monitoring strategies beyond conventional target analysis.

Funding

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KL-19**WHERE SCENT MEETS DIAGNOSIS: DATA-DRIVEN VOLATOLOMICS AND CHEMOMETRICS****Smolinska, Agnieszka.***Department of Pharmacology and Toxicology, NUTRIM, Maastricht University, Maastricht, Netherlands.*

Volatile organic compounds (VOCs), detectable in exhaled breath, urine, and feces, offer a compelling route to non-invasive diagnosis and disease monitoring. As reflections of underlying metabolic activity, VOCs can reveal condition-specific biochemical changes, making them valuable biomarkers across a range of diseases. To harness their diagnostic potential, a comprehensive analytical strategy is required—one that spans thoughtful experimental design, precise analytical workflows, and robust data interpretation.

This presentation introduces a chemometrics-driven volatolomics framework, built on gas chromatography–mass spectrometry (GC-MS), and applied across diverse biomedical studies. Case studies in asthma, colorectal cancer, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD) illustrate how VOC profiles can support differential diagnosis, patient stratification, and therapy monitoring in both clinical and pre-clinical settings.

Essential data processing steps—including baseline correction, peak deconvolution, retention time alignment, normalization, and quality control—will be highlighted as critical foundations for analytical reliability. The role of chemometric techniques, such as unsupervised clustering, supervised classification, and one-class modeling, will be explored in the context of extracting meaningful structure from high-dimensional VOC data.

By combining analytical rigor with biological insight, volatolomics supported by chemometric analysis provides a pathway toward robust, reproducible, and clinically relevant diagnostics. This presentation will offer practical recommendations for integrating data analysis strategies into VOC study design to maximize translational value and diagnostic applicability.

KL-20**THE NEW FRONTIERS IN FOOD AND FEED RISK ASSESSMENT****Paoletti, Claudia.***European Food Safety Authority – EFSA, Parma, Italy.*

The European Union established clear priorities to steer the objectives of food and feed safety assessment in an evolving multidisciplinary frame, facing the challenges of a steady increase of food demand and the need to guarantee large-scale sustainable production at a reasonable price. The objectives are ambitious: the European Green Deal aiming to make EU climate neutral by 2050; the Chemical Strategy boosting innovation for safe and sustainable chemicals; the One Health Approach promoting an integrated approach to balance and optimise the health of people, animals and ecosystems; the Farm to Fork Strategy enforcing focus on all the steps of the chain, from production to consumption. Clearly, food safety assessment is an evolving multidisciplinary science, influenced by the rigor of scientific research and the subjectivity of risk perception.

Sampling plays a self-evident role in food and feed safety assessment as representativity of test materials for the improvement and/or development of methodologies are critical pre-requisites for taking informed decisions regarding public, animals, and environmental health. Indeed, potential health risks for humans and animals can only be estimated accurately when exposure scenarios to a given food or feed are realistic, i.e. based on reliable sampling of food/feed consumption habits. Furthermore, from an analytical perspective, the vast variety of food and feed matrices and commodities, raw or (semi-) processed, pose challenges to develop appropriate sampling strategies that best facilitate correct analytical methods.

Despite the universally recognised right of any citizen to be guaranteed the same level of protection, as of today risk assessment (RA) approaches in food and feed safety are not harmonised, creating trade problems and inequality in safety protection around the world. The future will demand even stronger efforts towards RA harmonization by exploring innovative approaches to resolve the problems that prevent this aspiration today. The development of large databases to share information is strongly needed; the revision RA methodologies to address new food products continuously emerging and imposing new interdisciplinary approaches, is also necessary. To effectively tackle safety problems, we must develop approaches capable of considering the multiple interactions that the complexity of innovative food producing systems will require in the future, supporting interdisciplinary approaches (e.g. one substance - one assessment). In this setting, correct sampling is and will continue to play an important role in ensuring that all the relevant information necessary to take the best possible decisions is collected appropriately. The goal of correct risk management is not the elimination of all risks – an impossibility by itself – rather getting to know which risks can be taken, which must be minimised, and which have enough of an assured negative impact to be avoided. In this context, the Theory of Sampling (TOS) establishes itself as an essential practical tool needed to ensure the best possible estimation of risks to inform safety decision-making across societal sectors at large, including biological sciences, agro-business, technology, industry, trade, commerce, environment. Sampling is about being accountable for the trust that society puts into governmental and inter-governmental regulatory and control systems for the safety of food and feed products of today and of the future. Society has no other choice: we all eat food that we buy at supermarkets, trusting it is safe, trusting that the control system works to protect us, the consumers, the citizens.

KL-21

LOOKING AT WORKS OF ART WITH X-RAY EYES: NON-DESTRUCTIVE SPECTROSCOPIC IMAGING AND ANALYSIS

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Macroscopic X-ray fluorescence (MA-XRF) and X-ray powder diffraction (MA-XRPD) are forms of non-invasive hyperspectral imaging that allow to obtain information on painted works of art, in the form of phase specific or elemental images. By means of these methods the following can be done:

- revisualization of overpainted representations, revealing early stages of the creative process or intentional alterations of the composition done during the lifetime of the artwork;
- (highly specific) identification of the pigment types used and the pigment subtypes present, providing opportunities to study the provenance and authenticity of works of art; and
- identification of the nature and distribution of secondary products, formed on the paint surface by degradation of the original painting materials.

In the past decade we have imaged a variety of (renowned) paintings, mainly in European museums. In a number of cases, macro-scale information was combined with (X-ray based) micro-analysis of paint samples.

After describing the principles behind and instrumentation required for MA-XRF and MA-XRPD scanning, several recent case studies will be discussed, including (a) imaging the spontaneous degradation of the pigments of the 13th vault paintings in the Basilica of St. Francis in Assisi, IT [1]; (b) scanning highlights of 17th c. Dutch created by Rembrandt and Vermeer [2,3] and (c) investigating the exact nature and degradation behavior of pigments in works by 19/20th c. painters such as Vincent Van Gogh, Robert Delaunay and Edvard Munch [4,5].

Acknowledgement: This work was supported by InterReg project SmartLight 2.0.

Keywords: Cultural Heritage, Imaging, Spectroscopy, Instrumental Innovation.

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FULL ORAL



O-001

WHAT'S ROTTING HERE?– VOC PROFILING FOR THE DETECTION OF QUARANTINE PATHOGENS IN AGRICULTURAL COMMODITIES USING TD-GC-MS

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The increasing global trade of agricultural commodities heightens the risk of unintentional introduction of quarantine pathogens into the European Union, such as bacteria, fungi, and other microorganisms, which can severely impact crop production and plant health. [1] Rapid and reliable detection methods are essential for phytosanitary inspections, particularly in import control and storage facilities, to prevent economic and ecological losses. This study explores the use of volatile organic compound (VOC) profiling via thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) for the detection of specific bacterial pathogens affecting key plant matrices.

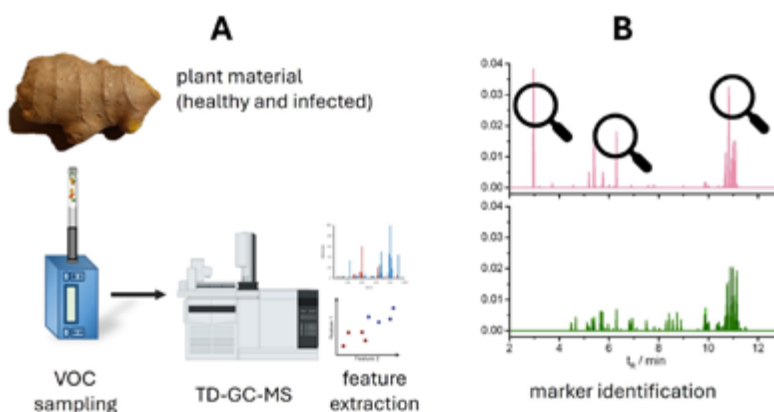
We focus on three major bacterial pathogens of concern: *Ralstonia pseudosolanacearum* in ginger (*Zingiber officinale*) and curcuma (*Curcuma longa*), *Pantoea stewartii* in corn (*Zea mays*) seeds, and *Curtobacterium flaccumfaciens* in bean (*Phaseolus vulgaris*) seeds. These pathogens are listed as quarantine pests in the European Union and are responsible for severe plant diseases, such as wilts and rots, posing significant threats to international trade and domestic agricultural production.

In this study, VOC emissions from both healthy and infected plant material, as well as from *in vitro* bacterial cultures grown under controlled conditions were analyzed. Air samples were collected on Tenax® adsorbents and measured using TD-GC-MS to identify pathogen-specific volatile markers. Multivariate data analysis, feature extraction, and machine learning techniques were employed to differentiate VOC profiles associated with infected and non-infected samples. [2] Preliminary results indicate both VOC generally associated with rot and microbial activity and distinct VOC patterns linked to each pathogen, allowing for their differentiation from healthy plant material and other microbial contaminants. [3-5] The identification of unique marker compounds provides strong evidence that VOC profiling can serve as a non-invasive diagnostic tool for quarantine pathogen detection. This approach has the potential to enhance phytosanitary inspection protocols by enabling rapid, on-site screening of imported agricultural commodities.

Future research will focus on refining VOC marker databases, improving method and model robustness, and developing portable detection systems for field applications. Integrating VOC analysis into quarantine inspection workflows could significantly improve early detection strategies and reduce the risk of pathogen introduction through global trade.

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O-002

RAPID MEASUREMENT OF ^{237}Np AND Pu ISOTOPES IN SEAWATER AND FRESHWATER SAMPLES BY PSEUDO-ISOTOPE DILUTION METHOD AND SF-ICP-MS ANALYSIS

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^{237}Np , owing to its long half-life ($t_{1/2}=2.14\times 10^6$ y) and similar conservatism to ^{137}Cs , has the potential to replace ^{137}Cs for water mass circulation studies on decades and even longer time scales. Due to its high mobility in the environment, monitoring ^{237}Np activity concentration level is important for radiation protection study. A new method for the determination of ^{137}Cs , ^{237}Np , and Pu isotopes in seawater and freshwater samples was proposed to solve the difficulty of ^{237}Np quantitative analysis. The developed method includes the separation technique of ammonium phosphomolybdate (AMP) adsorption for ^{137}Cs and anion exchange chromatography for ^{237}Np and Pu, measurement technique of gamma spectrometry for ^{137}Cs and SF-ICP-MS for ^{237}Np and Pu isotopes. ^{242}Pu as a pseudo isotope dilution tracer for ^{237}Np , the negligible chemical fractionation between ^{237}Np and ^{242}Pu of 1.02 ± 0.06 ($k=2$) was obtained by implementing sophisticated control of the redox system and chromatographic elution optimization. The analytical results for IAEA-443 Certified Reference Materials agreed with the reference values, showing chemical yields of 65–88%, U decontamination factor above 10^6 level, and improved sample throughput (5 days for 12 samples). Meanwhile, the lower method detection limits (MDLs) of ^{237}Np , ^{239}Pu , and ^{240}Pu were 1.3×10^{-3} , 0.065, and $0.15\text{ }\mu\text{Bq L}^{-1}$ for 15 L water sample, respectively. This confirms that our method can efficiently analyze ultra-trace ^{237}Np and Pu isotopes in seawater and freshwater samples. In addition, the ^{237}Np activity concentrations of $0.122\text{--}0.156\text{ }\mu\text{Bq L}^{-1}$ in the Pacific Ocean seawater around the FDNPS were reported for the first time. This new rapid analytical method developed in this study improved the analytical capabilities of ultra-trace ^{237}Np and Pu isotopes and was used for monitoring seawater following the authorized release of contaminated water from the FDNPS to the ocean to evaluate the potential radiological impact on the marine ecosystem. It was also employed for the investigation on activity concentration level of ^{237}Np and Pu isotopes in freshwater samples. Results on the activity concentration level of ^{237}Pu and Pu isotopes in seawater and in freshwater (river and tap water) and their contamination sources will be discussed.

O-003**FUNCTIONALIZED MEMBRANES FOR IN SITU EXTRACTION AND UNTARGETED DETECTION OF HEAVY METAL POLLUTION IN SURFACE WATERS****Fontàs, Clàudia¹; Marguí, Eva¹; Anticó, Enriqueta¹; Puebla, Gonzalo²; Gallardo, Tamara²; López, Silvana²; Fadic, Ximena²; Cereceda-Balic, Francisco³.**

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Functionalized membranes are polymer-based materials including specific extractants designed to selectively extract and retain target analytes from aqueous environments. In this study, we explore their potential for in situ heavy metal extraction in different polluted sites, enabling sample preservation and facilitating subsequent laboratory analysis. These membranes passively accumulate metal contaminants from water sources, offering a simple, low-cost, and portable alternative to traditional water sampling and preconcentration methods. In previous studies, we have demonstrated that after deployed, metals remain stably retained within the membrane, allowing for further analysis in the laboratory [1]. Moreover, these polymeric membranes allow for the use of non-destructive analytical techniques such as Energy Dispersive X-Ray Fluorescence (EDXRF). Although EDXRF is not suitable for analyzing water samples with low metal content, once the metals are retained in the membrane, the simultaneous multielement analysis can be performed on the organic thin layer without the need for matrix corrections [1]. Therefore, the combination of membrane-based extraction and EDXRF detection offers a fast, non-destructive, and comprehensive method for evaluating metallic pollution, enabling the identification of heavy metal pollutant without prior targeting.

In our study, we have developed functionalized membranes designed for the extraction of divalent metal ions from water samples. Field sampling campaigns were conducted at various locations in Chile, where the membranes were deployed for 7 days before being transported to Spain for analysis, with no special handling required. In parallel, stability tests were performed to ensure that the membranes effectively preserve the metal content over time. The analysis of the membranes has enabled the detection of metals such as Fe, Cu, and Zn at different sampling points, providing valuable insights into the heavy metal pollution in the studied sites.

Acknowledgements

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O-004

COMPARISON OF METHODS FOR THE PREPARATION OF SCINTILLATING RESINS FOR RADIONUCLIDE ANALYSIS

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Scintillating resins are an innovative solution for the selective analysis of alpha and beta-emitting radionuclides, as they combine selective separation and measurement in a single material. This integration leads to faster and less contaminant procedures. The development of functional scintillating resins requires the successful execution of two key functions. Firstly, elective separation is achieved by incorporating specific molecules into the polymeric support that exhibit a strong and unique affinity for the radionuclide of interest in various media. Secondly, scintillation relies on an aromatic polymeric structure, typically based on polystyrene or polyvinyltoluene, with encapsulated fluorescent solutes. These solutes must efficiently convert the energy of radioactive particles into detectable photons, which necessitates the absence of quenching phenomena.

Scintillating resins are typically prepared through surface deposition. The plastic scintillator, composed of linear polystyrene, in the form of microspheres, is mixed with an extractant solution in an organic solvent, which is subsequently evaporated. This method can yield good results, provided the extractant is compatible with the plastic scintillator, does not produce quenching effects, and adheres strongly to the polystyrene surface. There are successful examples where these requirements have been met. However, in some cases, issues such as extractant leaching (e.g., TK102 extractant and dimethylglyoxime), low capacity (e.g., Sn in TBP-PS resin), and incompatibility between the extractant and the scintillator (e.g., tropolone or TBP) have been encountered. Additionally, for certain separations, such as with ^{55}Fe , no suitable extractant is available.

This work introduces new strategies for preparing scintillating resins and compares them with classical extractive resins in terms of stability, metal capacity, preparation viability, and scintillation efficiency. The strategies studied include new compositions (e.g., microspheres made of cross-linked polystyrene), novel structures (e.g., porous microspheres), new monomers (acrylic acid) and innovative approaches such as covalently linking the extractant to the polymer surface or ion imprinting.

The results obtained highlight both the advantages and limitations of the new strategies developed. Generally, composition and structural modifications tend to cause quenching effects and reduce detection efficiency. However, resin capacity can be improved by increasing the surface area available to the extractant through the use of porogens during the process. Additionally, tuning the polarity of the polymer surface can enhance chemical compatibility between the extractant and polymer, thereby reducing extractant leaching. These findings pave the way for a new generation of extractive scintillators that could be utilized to address challenges in radioactivity analysis (^{210}Pb , ^{63}Ni , ^{55}Fe), where there is still room for improvement.

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O-005**ANALYTICAL METHODS FOR THE EVALUATION OF PHOTOCATALYTIC DEGRADATION OF PFAS****Kraševac, Ida¹; Žener, Boštjan¹; Jamil, Qasim¹; Matoh, Lev¹; Šuligoj, Andraž¹; Kumar, Praveen¹; Kušić, Hrvoje²; Lavrenčič Štangar, Urška¹.***¹Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia; ²Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia.*

Due to their environmental stability and persistence to degradation, per- and polyfluoroalkyl substances (PFAS) are sometimes labelled as forever chemicals and are recognised as contaminants of emerging concern. Their use has been regulated since 2009, but their widespread use and poor removal in wastewater treatment has led to their detection in matrices such as human blood food, rainwater, and soil.

Photocatalytic degradation based on modified titanium dioxide is a promising technique to enable degradation and mineralization of PFAS. In our study, perfluorooctanoic acid (PFOA) was used as a model compound to study photodegradation of PFAS as a possible quaternary treatment method in water-based samples. To enhance charge separation and reduce electron-hole recombination, sulfur- and nitrogen-doped TiO₂ (N,S-TiO₂) was synthesized and further modified with Pt (N,S-TiO₂+1%Pt). Photocatalytic experiments were conducted in a batch reactor under UV light ($\lambda_{\text{max}} = 365 \text{ nm}$) with an intensity of 80 W/m² up to 48 hours.

Ion chromatography with conductivity detection was used to follow the defluorination via quantification of F⁻ anions in the calibration range 2-380 µg/L. An LC-MS/MS method was developed to track the formation of shorter chained products (C₄-C₇) in the calibration range 5-1000 µg/L, together with an Oasis WAX SPE method to enable their detection in lower concentrations (enrichment factors over 90 for all analytes).

The main analytical challenges we faced in this research include the absorption of F⁻ ions at low concentrations, which hinders their detection, as well as the loss of analytes during sample filtration. Fluoride ions were not detected in solutions even after 48 hours of irradiation of low concentration PFOA water solutions (1-100 µg/L). Experiments with low concentration NaF solutions confirmed that up to 82 ± 3% fluoride ions were adsorbed to the catalyst surface and reactor walls and were incapable of desorption even with pH adjustment.

To successfully follow the degradation pathways, further experiments were conducted at higher PFOA concentrations (40 mg/L) in polyethylene reactors. The choice of the filter material used for pre-injection syringe filtration was also shown to have a large effect on both fluoride ions as well as PFAS products. For F⁻, polyacrylate filters were shown to have the least effect (less than 10 % loss) but had the largest effect on PFAS (over 90 % loss of PFOA). Among the commonly used filter materials (glass, PA, PTFE, PES, PVDF), the loss of PFOA was lowest in PVDF (less than 5 %), these filters were selected for further use.

The photodegradation experiments at high PFOA concentrations yielded defluorination efficiency of 37 ± 3% and degradation of PFOA exceeding 90%, with both processes improved when the test solution was purged with N₂ gas to produce reductive conditions. The decomposition of PFOA was confirmed with the formation of shorter-chained PFAS, mainly perfluoroheptanoic acid, as well as a reduced toxicity of the test solution.

O-006**PACKED IONIC RESINS TO PERFORM SOLID PHASE EXTRACTION (SPE) AND SOLID PHASE SPECTROPHOTOMETRY (SPS) FOR METAL IONS DETERMINATION AND SPECIATION IN A SMART SAMPLING APPROACH****Mesquita, Raquel; Pereira, Mafalda; Melo, Maria; Ribas, Tânia; Rangel, António.***Universidade Católica Portuguesa, Porto, Portugal.*

The adverse effects in the environment of metal ions are well documented, notably for those displaying toxic effects for living organisms according to their concentration. Novel sampling and real-time monitoring methods will have a significant impact on understanding the spatial-temporal distribution of the metal ions and will contribute to act on-time to detect and mitigate sources of contamination of aquatic systems. So, a project, Aqua_Smart, has been implemented to devise an integrated smart sampling and automatic monitoring of toxic metal ions in aquatic systems.

The aim is to attain metal ions assessment by employing on-site smart sampling approaches to tackle the bioavailability of some metallic ions in different water bodies. The idea relies upon the use of solid phase extraction (SPE) for analyte retention as a replacement for transporting high volumes of samples to the laboratory. The SPE process is achieved by using ionic exchange resins to selectively collect the analytes, and, for analysis, we propose to use flow-based methods, like sequential injection (SI) analysis (Mesquita & Rangel, 2009) with miniaturized optical detection. The use of an automated SI method with optical detection in the flow cell also enables to carry out the determination in the solid phase itself in a solid phase spectrophotometry approach. This approach efficiency is demonstrated with the speciation of iron(II) and iron(III) in different types of natural waters.

Additionally, for employing ionic exchange for the metal ion retention, the charge of the ion is highly relevant, and although most metal ions are cations in aquatic systems, it is also known that some metal ions form chloro-complexes with chloride, resulting in negatively charged complexes. So, considering the chloride content of high salinity waters like seawater, some metal ions, namely cobalt, are indeed complexed. Consequently, another approach for smart sampling is the use of polymer inclusion membranes, PIMs. These can be described as a thin, flexible and stable polymeric film that presents the property of selectively separating solute/s of interest depending on the composing extractant. These membranes can be prepared by using an ion-exchanger immobilized in a polymeric matrix. A PIM prepared for anionic species retention may act as exchangers to selectively collect a specific metal ion negatively charged complex from an aquatic system, after being incorporated in miniaturized devices.

After a proof-of-concept of this process, previously demonstrated for the cobalt determination in sea water, several studies of the PIM preparation were carried out. These studies aimed to obtain the best performance in flow methods of the PIM as a sorbent. For this purpose, an electrospinning process was used to produce solid fibres.

O-007**ANALYTICAL CHALLENGES AND WORKFLOWS TO STUDY TRANSFORMATION PRODUCTS OF WATER CONTAMINANTS****Kravos, Aleksander¹; Bajt, Oliver²; Prosen, Helena¹.***¹University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia; ²National Institute of Biology, Marine Biology Station Piran, Ljubljana, Slovenia.*

Water contaminants are widespread in the natural environment, where they dissipate and/or undergo chemical transformations. This leads to the formation of potentially problematic transformation products (TPs). The natural chemical transformations are primarily either part of biodegradation or photodegradation processes. TPs are nowadays a burning topic in environmental chemistry that has been mostly under-researched due to the analytical challenges linked to non-target analysis. Thus, there is a big knowledge gap related to the identity and environmental fate of TPs of water contaminants that will need to be explored in the future.

In this study, we have identified and studied the fate of TPs of five selected parent emergent contaminants, namely, pesticides cycloxydim (CYC) and pirimicarb (PIR), pharmaceuticals moxifloxacin (MOX) and rosuvastatin (ROS), and an industrial compound 2-mercaptobenzothiazole (MBT). Firstly, the TPs were pre-formed from each contaminant in pure water via direct photolysis in the photoreactor using 125 W Hg lamp with a cut-off filter below 300 nm to simulate sunlight in the best possible way. All the contaminants showed photolability but slow degradation kinetics, except for ROS with fast photodegradation.

The aliquots were analyzed with triple-quadrupole UHPLC-MS to search for TPs and nominal m/z values of their respective precursors. Numerous TPs were found but up to 5 most abundant TPs per contaminant were selected for further analyses. For the suspect TPs, high-resolution MS/MS spectra were recorded with UHPLC-QTOF for detailed structural elucidation and annotation of the structures. Diverse structures were found, such as hydroxylated and oxo analogues, while cyclized and/or cleaved TPs were the most interesting and these were typical for ROS and CYC. Knowing their fragmentation pattern, the TPs were screened in actual seawater from the North Adriatic Sea, Slovenia, using solid-phase extraction coupled to UHPLC-MS/MS. Interestingly, ROS TPs were frequently detected showing their high environmental relevance.

Evolution profiles and stabilities during photolysis were investigated for the selected TPs, as well as their dissipation via sorption onto marine sediments and natural minerals, such as calcite, dolomite, quartz, muscovite, etc. These experiments suggested TPs' persistence and mobility due to low tendencies to bind onto sediments. This may also imply that these TPs could be more bioaccessible to marine organisms than their parent compounds, leading to negative ecological impact. In this regard, ROS TPs may be highlighted as they had a high detection frequency in seawater as well as relatively higher predicted ecotoxicity (ECOSAR software) to *Daphnia magna* and green algae.

Overall, this study shows an integrated and expanded methodology to study the critically under-researched TPs in environmental chemistry using high- and low-resolution mass spectrometry.

O-008

TEMPORAL PROFILING OF STRESS-RELATED DEPRESSION SYMPTOMS FROM A SINGLE HAIR BY NANO-PALDI IMAGING MASS SPECTROMETRY

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Introduction. Chronic psychological stress is a major contributing factor to mental health disorders, including depression. However, capturing the biochemical signatures of stress and its temporal progression remains a challenge due to the limitations of conventional diagnostic methods such as interviews, questionnaires, and blood sampling. These methods are often subjective, invasive, and unsuitable for long-term or continuous monitoring.

In this study, we propose a novel, non-invasive strategy to monitor the onset and progression of stress-related depression by analyzing a single strand of human hair using nanoparticle-assisted laser desorption/ionization imaging mass spectrometry (Nano-PALDI IMS) (1-3).

To elucidate the molecular underpinnings of stress-induced depressive symptoms, we employed a two-pronged approach: (1) omics-based biomarker discovery in a mouse model of depression and (2) spatial and temporal visualization of candidate BMs using Nano-PALDI IMS.

Human hair grows at an average rate of approximately 0.4 mm per day and incorporates biological information from the bloodstream via capillaries. Therefore, analyzing the longitudinal sections of hair enables retrospective profiling of physiological changes over time. In this presentation, we introduce Nano-PALDI IMS using longitudinal section of human hair, along with stress-related depression BMs data obtained from the omics analysis.

Results. In our animal model study, we identified 15 metabolites that significantly differed between healthy and stress-exposed (depression model) mice ($p < 0.05$). Among these, corticosterone, a well-known stress biomarker, and selenium sulfide (SeS) were notably elevated in the depression group (Fig. 1). Interestingly, corticosterone was predominantly detected in hair segments corresponding to the later phase of stress exposure, suggesting a correlation with more severe depressive symptoms. In contrast, SeS was detectable at much earlier stages, even before overt depressive behavior was observed. This suggests that SeS may serve as an early indicator of mild psychological stress, potentially marking the transition from wellness to pre-depression states.

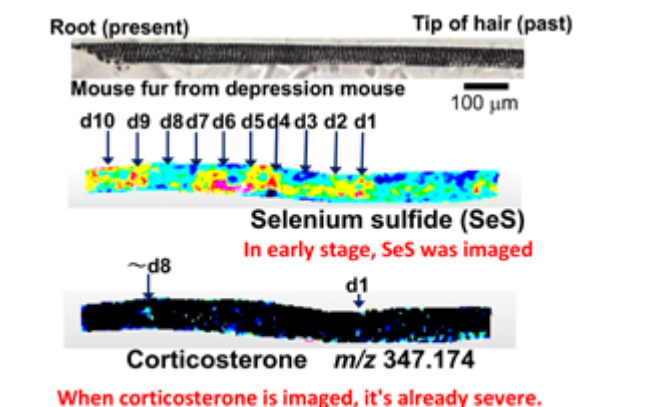


Figure 1 Nano-PALDI imaging mass spectrometry of depression mice fur.

We further applied Nano-PALDI IMS to longitudinally sectioned human hair samples. Both cortisol (the human analog of corticosterone) and SeS were successfully detected and spatially imaged. While cortisol levels showed no consistent differences between healthy individuals and depression patients, SeS levels were significantly elevated in the latter. This highlights the potential of SeS as a sensitive and discriminative biomarker for human stress and depression.

Our results demonstrate the feasibility of using longitudinal hair sections as a temporal archive of metabolic states, and highlight the potential of Nano-PALDI IMS as a powerful tool for non-invasive mental health diagnostics. These findings suggest that SeS may serve not only as an early biomarker for stress-related mood disorders, but also as a potential target for longitudinal health monitoring in clinical and occupational settings. This methodology offers a paradigm shift in mental health diagnostics by enabling molecular-level insights from a single, non-invasive hair sample.

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O-009**ELEMENTAL MAPPING TO STUDY THE INTERACTION OF MRI CONTRAST AGENTS WITH EXTRACELLULAR MATRIX COMPONENTS****Traub, Heike¹; Schannor, Mathias¹; Stach, Anke²; Seim, Christian³; Ludwig, Antje².***¹Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany; ²Deutsches Herzzentrum der Charité, Berlin, Germany; ³Technische Universität Berlin, Berlin, Germany.*

Non-specific, low-molecular-weight Gd-based contrast agents (GBCAs) have long been routinely used in clinical magnetic resonance imaging (MRI) diagnostics. Nevertheless, the interaction of such contrast agents with tissue components is not yet fully understood. Typically, they go into extracellular space. The extracellular matrix (ECM) is a three-dimensional network of macromolecules providing structural and biochemical support of the surrounding cells in all mammalian tissues. It is composed of structural proteins (e.g., collagen, elastin) and proteoglycans, which consist of glycosaminoglycans (GAGs) covalently bound to a protein core. GAGs are long, linear polysaccharides composed of repeating disaccharide units that differ in molecular mass, disaccharide structure and degree of sulfation. Many diseases, including inflammation, fibrosis, and tumor invasion, are associated with characteristic ECM changes, especially at an early stage of disease development. As disease severity increases, the amount of one or more different GAG types in the ECM and the extent of GAG sulfation increases. Characteristic of GAGs is their ability to form complexes with cations, e.g., with lanthanides. Thus, GAGs could be a potential binding partner for GBCA molecules as a whole or for dechelated Gd.

Currently, there are still many unanswered questions about the interaction of contrast agents with ECM components. In this study, therefore, the uptake and distribution of ionic Gd and various linear and macrocyclic GBCAs was investigated in spheroids as model systems mimicking the complex physiologically relevant tissue microenvironment. Chinese hamster ovary (CHO) cells and CRL-2242 cells, a CHO mutant that does not produce sulfated GAGs, were used to prepare spheroids. Afterwards the spheroids were incubated with gadolinium chloride and GBCAs for several minutes. For elemental mapping laser ablation inductively coupled plasma time-of-flight mass spectrometry (LA-ICP-TOFMS) and scanning transmission X-ray microscopy in combination with X-ray fluorescence detection (STXM-XRF) were used.

Although all spheroids were exposed to identical Gd concentrations, differences were observed in the spatial distribution and the amount of Gd taken up. After incubation with linear and macrocyclic GBCAs, Gd is detected in the interior of both types of spheroids. Furthermore, differences in the Gd amount were found depending on the GBCA used. In contrast, incubation with gadolinium chloride leads to a Gd enrichment in the outer regions of the spheroids as well as to much higher Gd contents compared to incubation with GBCAs.

Both LA-ICP-TOFMS and STXM-XRF can make an important contribution to better understand the relationship between the affinity of GBCAs and ECM components. However, to elucidate such complex interactions, further studies are needed, also with other (bio-)analytical techniques.

O-010**MULTISCALE SPECTROSCOPIC CHARACTERIZATION OF MICROPLASTICS IN SOIL: FROM RAPID SCREENING BY HANDHELD NEAR-INFRARED SPECTROSCOPY TO SUBMICRON ANALYSIS BY OPTICAL PHOTOTHERMAL INFRARED AND RAMAN MICRO-SPECTROSCOPY**

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The contamination of soil with microplastic particles represents a growing environmental concern with significant ecological and agricultural impacts. The characterization of microplastics across a range of sizes, from millimeter-scale to submicron particles, is crucial for understanding their distribution, their sources and their potential impact on soil health and food safety. The present study explores the use of advanced spectroscopic techniques for the detection and characterization of microplastics in soil, emphasizing both rapid field-based screening and high-resolution laboratory analysis.

In recent years, handheld near-infrared (NIR) spectroscopy has become a valuable tool not only for rapid, non-destructive and on-site analysis of materials and food, but also for the screening of microplastics in soil samples. In recent studies, we have demonstrated that this technique allows in-situ identification and quantification of common polymer types up to concentrations above 1000 ppm and provides a cost-effective means of soil monitoring.

To overcome the challenges of detecting very small microplastics in soil at concentrations < 1000 ppm, optical photothermal infrared (O-PTIR) micro-spectroscopy enables infrared (IR) analysis at the sub-micron level while simultaneously acquiring Raman spectra, providing both high spatial resolution and chemical specificity. O-PTIR combines IR absorption and thermal expansion detection, allowing label-free, non-contact submicron chemical imaging of microplastics within complex soil matrices. In addition, the simultaneous acquisition of Raman spectra with O-PTIR provides complementary molecular fingerprinting at the same submicron spatial resolution, enhancing microplastic identification in complex soil environments.

Integrating these spectroscopic techniques enables a comprehensive two-step characterization approach, bridging the gap between large-scale screening and fine-scale chemical imaging. This study highlights the advantages, limitations, and synergies of these methods in microplastic analysis in soil, offering insights into their application in environmental monitoring, soil health assessments, and future research directions.

O-011

VISUALIZE INVISIBLE WITH HYPERSPECTRAL IMAGING FOR REAL-TIME, IN-LINE AND NON-DESTRUCTIVE INSPECTION IN FOOD, PHARMA & RECYCLING

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Sales Application Engineer, Valencia, Spain.

Hyperspectral imaging is used as a technology in both industrial and research domains, offering capabilities in material characterization and quality assessment. By capturing detailed spectral information across a wide range of wavelengths and spatial information, hyperspectral imaging enables the identification and analysis of materials based on their unique spectral signatures.

In this presentation, Lea Butruille, Sales Application Engineer at inno-spec GmbH, a member of the Headwall Group, will provide an overview of hyperspectral imaging possibilities and diverse applications. The talk will explain how this technology can be used for both the industrial environment and also laboratory applied research.

The discussion will then transition to specific application areas where hyperspectral imaging has demonstrated significant impact:

- **Food Quality and Safety:** In food processing, it facilitates the detection of contaminants, assessment of freshness, and verification of quality parameters such as brix, acidity in fruits. Its ability to identify foreign objects for quality control purposes.
- **Textile and Plastics Recycling:** It enhances recycling processes by accurately identifying and sorting materials based on their spectral characteristics. For instance, in plastics recycling, it can differentiate between polymers such as PE, PP, and PVC, improving the purity of recycled materials. In textile recycling, it aids in classifying materials or estimating concentration of mixtures contributing to more efficient recycling workflows.
- **Pharmaceutical Industry:** It serves as a powerful tool for enabling real-time, non-destructive monitoring of pharmaceutical products. By analyzing the spectral properties of tablets and capsules, it can detect variations in chemical composition, ensuring consistent product quality and compliance with regulatory standards.

Furthermore, the presentation will show a broad range of feasible applications using hyperspectral imaging. The set-up including hardware and software covers the workflow from acquisition until processing of data. The purpose is to enhance that anyone can record and analyze spectral data without any chemometrics or machine learning knowledge within a few minutes. The main goal is to show how easy-to-use this powerful technology is.

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[Towards non-contact pollution monitoring in sewers with hyperspectral imaging](#)

O-012**EXPLORING FACTORS INFLUENCING THE REFLECTANCE SPECTRA IN MOULDY CORE APPLES AND VALIDATION OF LC-MS/MS FOR MYCOTOXIN DETECTION**

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The consumption of apples has progressively increased over the last 20 years, proving to be essential for a healthy diet. However, during the postharvest period and storage, apples can deteriorate due to phytopathogenic microorganisms. One of the fungal genera responsible for this deterioration is *Alternaria*, which can cause a pathology in apples known as mouldy core (MC). This condition is characterized by internal damage that is challenging to detect, as it lacks visible external lesions. Moreover, MC poses a toxicological risk to human health due to the production of mycotoxins, secondary metabolites with harmful effects. Therefore, it is necessary to optimize early a non-destructive hyperspectral-based method for the detection of MC and prediction of mycotoxins, and to understand the factors affecting the reflectance spectra for ensuring an accurate detection. Although hyperspectral imaging (HSI) facilitates the prediction of contaminants in the fruit, mathematical models need to be developed. With this in mind, during the model training and validation phase, mycotoxin concentrations obtained through other methods, such as LC-MS/MS, are required.

Thus, the objectives of this work were: i) to validate an LC- MS/MS method for the simultaneous quantification of six *Alternaria* mycotoxins; ii) to study the light penetration in apples in the VNIR and NIR regions; iii) to study the factors affecting the reflectance spectra of apple fruit infected with *Alternaria*. Two apple varieties, namely *Golden Delicious* and *Jonagold*, were used under different conditions for detecting MC and mycotoxins production.

For the first objective, validation of the LC-MS/MS method for the quantification of six *Alternaria* mycotoxins was done following the criteria established in SANTE /12089/2016 and Commission Regulation EU 2023/2782; limits of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), repeatability, precision, and recovery were calculated. All parameters were compliant, achieving LODs below 0.6 µg/kg and recoveries greater than 80%, producing reliable results.

For the other objectives, three experiments were conducted. A light penetration experiment where apples were cut into various thicknesses and imaged on a 98% reflective white background and a 4% reflective black background; the second was an experiment with increasing concentrations of *Alternaria* injected into the apples, with images taken at 0, 24, 45, and 120 days; the last experiment aimed to check for differences in the reflectance spectra when apples were injected with PBS, or whether injection itself caused variations in the images, thus altering the results. This final experiment was conducted at 0, 35, and 56 days. In all three experiments, apples were imaged both vertically and horizontally before the experiments and on the specified days with the Specim FX10 (400–1000 nm) and FX17 (900–1700 nm) cameras.

The difference between the spectra with a white and black background was used to determine the light penetration depth. The results show that in the VNIR region, light penetrates the apples up to a distance of 20 mm and in the NIR 25 mm, not reaching the apple core, where the MC originates. However, when analysing the concentration experiments over several days, a change in the reflectance spectra was observed due to the disease and the subsequent production of mycotoxins. Furthermore, no significant differences were detected when PBS was injected or when the apples were injected directly.

The results demonstrate the potential of HSI as an effective tool for early detection of MC and mycotoxins in apples, which could improve food safety and quality control processes.

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O-013**CONNECTING PAST AND PRESENCE: NOVEL STRATEGIES TO STUDY LOCALIZED PROTEOME AND LIPIDOME CHANGES IN PARCHMENT VIA MULTIMODAL RAMAN, FTIR AND MALDI MS IMAGING****Malissa, Antonia¹; Kleibl, Jan¹; Cappa, Federica²; Schreiner, Manfred³; Marchetti-Deschmann, Martina¹.**¹TU Wien, Vienna, Austria; ²Academy of Fine Arts Vienna, Vienna, Austria; ³Academy of Fine Arts Vienna and TU Wien, Vienna, Austria.

Proteomic and lipidomic technologies offer powerful insights into the molecular composition and degradation of parchment manuscripts [1]. Despite its delicate, paper-like appearance, parchment is a highly processed biomaterial derived from calf, sheep, or goat skin. The labor-intensive production reduces the skin to its dermal layer, yielding a dense, collagen- and lipid-rich fibrous matrix. Structural and molecular alterations in this dermal network, triggered by environmental stressors such as light, humidity, and atmospheric pollutants, are closely related to degradation processes. Particularly, oxidative stress promotes lipid peroxidation and collagen modifications, compromising the material's integrity and long-term preservation. While traditional spectroscopic techniques have monitored such damage by tracking alterations in the protein backbone, they lack detailed molecular specificity [2]. This study presents the first multimodal approach combining proteomic and lipidomic analyses with spectroscopic imaging to resolve degradation-induced changes in parchment at the fibrillar level. Beyond heritage science, these findings have implications for understanding oxidative processes in skin and other collagen-rich tissues.

Parchment samples were submitted to accelerated light- and SO₂-aging under controlled laboratory conditions. Following OCT-embedding and vertical sectioning at 8 µm, spectroscopic imaging was performed using FTIR and Raman spectroscopy at spatial resolutions below 10 µm. Adjacent sections were analyzed by MALDI FTICR MSI at a lateral resolution of 15 µm. For lipid imaging, samples were coated with 1,5-DAN via sublimation using a custom-built (patent-pending) system. Protein imaging involved enzymatic treatment with COLase III and trypsin/LysC, respectively, applied via spray deposition (Shimadzu iMLayer Aero), followed by incubation at 37 °C under high humidity before CHCA was applied as MALDI matrix by spraying. High mass accuracy measurements in positive and negative ionization mode confirmed lipid identification with an achieved mass error below 2 ppm. Peptide identities were confirmed by comparison with MS¹ and MS² data from established in-solution digestion protocols and comparisons against theoretical in-silico digestions. MSI data analysis and visualization were conducted using SCI_{LS} Lab software (Bruker Daltonics).

The delicate, collagen-rich tissue, characterized by fibrillar and gelatinous structures, posed significant challenges for sample preparation. Nevertheless, OCT-embedding and optimization of sample preparation for MALDI MSI facilitated a reproducible preparation of sections and subsequent imaging experiments. Spectroscopic analyses assess the structural degradation of collagens in parchment by extensive analysis of amide I, II, and III bands. MALDI MSI allows localizing and distinguishing of different lipid classes and collagen types and their relative variation between unaged and aged parchment samples. Among the identified lipid species are sphingolipids, glycerolipids, fatty acyls, and sterols, showing distinct distributions on the hair and flesh side of the samples. In particular, an increased presence of sphingolipids is observed on the flesh side of unaged samples, whereas glycerolipids, sterols, and fatty acyls are distributed on the hair side. The respective distributions reveal striking differences between unaged and aged samples. Comparative analysis of proteomic MSI data with in-solution digestion protocols and theoretical in-silico digests enabled the identification of collagen type I and less abundant dermal collagen types. Notably, preliminary data following artificial aging reveal distinct mass shifts of +1 Da, +16 Da, and +64 Da consistent with deamidation, oxidative modifications, and SO₂-adduct formation, respectively. These findings highlight the potential of MALDI MSI to capture meaningful molecular characteristics in the context of parchment degradation.

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O-014

AN ORIGINAL METHOD OF FUNCTIONALIZING MESOPOROUS CARBON WITH LAWSONE AS A NEW SENSOR FOR HIGHLY SENSITIVE HORMONE DETERMINATION

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Design, characterization and application of chemical sensors is currently one of the fastest developing scientific disciplines. They play a key role in all sectors of the economy, monitoring environment pollution and vital functions of patients. A significant role in the development and improvement of sensor parameters is played by nanomaterials, used mainly as components of multi-component nanostructured composites, acting as a modifier applied to the surface of stationary electrodes intended for voltammetry and potentiometry. The advantage is the ease of preparation of such sensors and an unlimited spectrum of possibilities of composing the layer, which determines the sensor parameters, its selectivity, sensitivity and durability.

The aim of this work was to develop and produce voltammetric sensors based on glassy carbon electrodes (GCE) modified in a simple and reproducible way using a stable composite layer. The basic element of the composite was mesoporous carbon. Due to its specific physicochemical properties, such as strong surface development, very good electrical conductivity and high chemical and physical stability, this material is often used as a surface modifier of substrate electrodes, providing excellent sensitivity and selectivity compared to unmodified electrodes. To make the composite, mesoporous carbon was combined with lawsone (2-hydroxy-1,4-naphthoquinone) - a compound from the quinone group, which is characterized by unique redox properties and has not been used as a electrodes modifier so far. The conducted studies confirmed the improvement of the metrological parameters of the sensor, among others, due to their electrocatalytic properties. The sensor prepared in this way was used for highly sensitive determinations of the hormone – ethinylestradiol.

The modifier was characterised using ultra-high-resolution scanning microscope (SEM) with X-ray microanalysis system (EDS), infrared spectroscopy (FTIR) and other methods. For high sensitive ethinylestradiol measurements DPV method was used, resulting in determining the analyte at the concentration as low as 0.009 mg ml^{-1} . Such high sensitivity of the performed measurements is connected with the effectiveness of ethinylestradiol accumulation on the LAW-MC/GC electrode surface. The proposed method can be successfully applied for EE determination in complex matrix, such as biological fluids, pharmaceutical formulations with very good repeatability and reproducibility.

Acknowledgements: Research project supported by program „Excellence initiative – research university” for the AGH University of Krakow.

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O-015

ADVANCING POINT-OF-CARE DIAGNOSTICS: MICROFLUIDIC ELECTROCHEMICAL BIOSENSOR FOR DETECTING IMMUNE RESPONSES CAUSED BY MULTIDRUG-RESISTANT PATHOGENS

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Antibiotic resistance has become one of the mayor health challenges of the 21st century, due to the increasing prevalence of infections caused by multidrug-resistant (MDR) pathogens. The effective management of MDR infections is critical to reduce mortality, improve patient outcome, and decrease costs related to operational burden on hospitals. However, current diagnostic methods and tools are often too slow, centralized, and complex to provide rapid and appropriate solutions and therapies to the patients. [1] Among all MDR pathogens, *Klebsiella pneumoniae* stands out due to its high mortality rate, prevalence, and resilience. This opportunistic pathogen accounts for 10% of the total of nosocomial (hospital-acquired) infections. Therefore, we select it as a model for the development of this novel Point-of-care (PoC) device.

To overcome the shortcomings of current diagnostic methods, we present here a rapid, field-deployable POC device for monitoring the immune response to MDR infection and vaccination. Built on a miniaturized and multi-parametric electrochemical immunosensor, the device detects anti-*K. pneumoniae* antibodies in serum samples quickly and with high specificity. This device was designed for on-site use by non-trained personnel, making it well-suited, but not limited to, hospital laboratories, emergency rooms, primary care or low-resource settings.

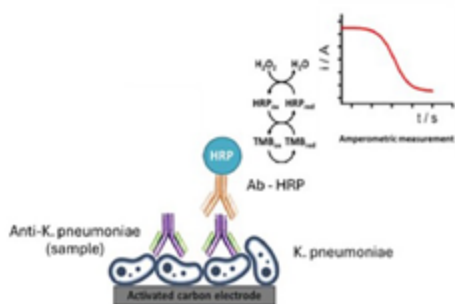


Fig. 1 Amperometric detection principle of the immunosensor

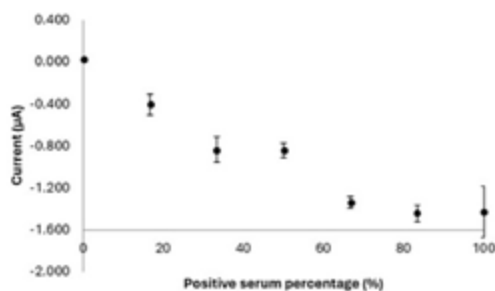


Fig. 2 Calibration with different percentages of positive serum with electrode functionalized with *K. pneumoniae*

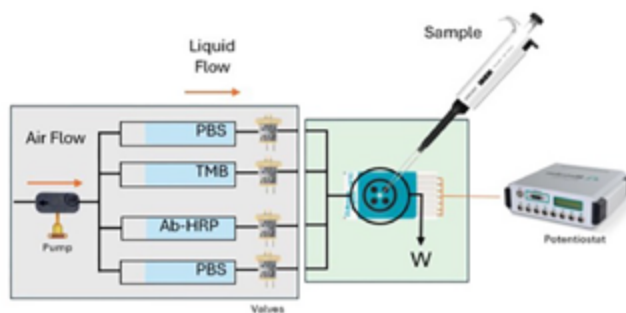


Fig. 3 Schematic representation of the microfluidic design



Fig. 4 3D-printed microfluidic system

The core sensing element consists of a screen-printed carbon electrode featuring four working electrodes functionalized with *K. pneumoniae* bacteria. Detection of anti-*K. pneumoniae* specific antibodies in the sample is done by amperometry, by means of secondary antibody conjugated to horseradish peroxidase (HRP) in the presence of tetramethylbenzidine (TMB) as electrochemical substrate. Calibration was successfully carried out using positive serum samples. These samples were obtained from vaccinated mice and diluted with different percentages of negative serum samples. Results clearly showed that an amperometric signal is only produced in the presence of positive serum samples, but not when only the negative one is used. This demonstrates the biosensor's ability to detect antibodies generated (humoral response) as a response to both infection by *K. pneumoniae* and immunization.

To further enhance automation and scalability, a portable microfluidic module was developed to automate the analysis process. This module contains the reagent reservoirs (HRP-Ab, TMB, PBS and water), a micropump for pressure-driven flow and four valves for the reagent's delivery. The micropump generates an increment of pressure

in the headspace of the reservoirs, pushing the reagents. The valves are electronically controlled to sequentially inject each reagent for the required time. The module is connected to a microfluidic chip that houses the biosensor, sample inlet, and waste tank. This configuration allows full automation of the assay, from sample input to signal readout, using an external potentiostat. The integration of custom electronics, user interface, and system housing is ongoing.

This result represents a significant step toward an automated real-time diagnostic tool for managing *K. pneumoniae* infections and vaccination monitoring. Its portability and low complexity make it ideal for decentralized testing, helping to relieve pressure on hospital infrastructure, accelerate patient triage, and strengthen outbreak response capabilities.

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O-016

HIGHLY EFFICIENT COREACTANT-FREE ELECTROCHEMILUMINESCENCE SENSING PLATFORM WITH MULTIPLEXED INTERDIGITATED SPIRAL MICROELECTRODES

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We present a novel fully integrated, miniaturized multiplexed electrochemiluminescence (ECL) sensing platform that enables on-site analyte monitoring without requiring hydrogen peroxide (H_2O_2) as a coreactant. Traditional luminol-based ECL systems rely on H_2O_2 , which is unstable and impractical for real-time on-site detection[1–4].

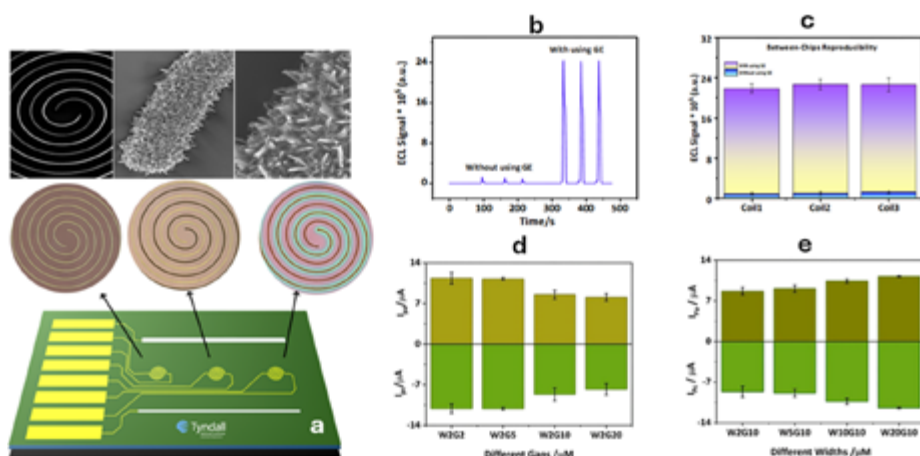


Fig1. (a) Fully integrated silicon device consisting of 3 G&C microelectrodes, counter, and reference electrodes, with optical and SEM images after gold NPs and chitosan nanocomposite deposition, (b) ECL signal with and without applying -0.6 V to the GE. (c) Reproducibility of the ECL signal across 3 devices for 9 sensors. (d & e) histogram of peak currents for different gaps and widths of bare gold microelectrodes.

By leveraging microfabrication technology[5–7], we developed an interdigitated spiral microelectrode design integrating generator and collector (G&C) functionalities to enhance reactive oxygen species (ROSs) generation and ECL emission (**Fig1.a**). In a single-electrode setup, the collector electrode (CE) produced only a weak ECL signal when its potential was swept from 0 to 0.8 V. However, applying a constant -0.6 V potential to the generator electrode (GE) enhanced the signal 11-fold under neutral pH conditions, showing high reproducibility both within and between devices (**Fig1.b & c**). Additionally, a CE potential sweep from -0.6 to 0.8 V provided a 2.5-fold enhancement but was less effective than the constant GE bias. Therefore, this G&C configuration, with its precise microfabrication capabilities, enables the simultaneous generation of a higher density of ROSs, which act as co-reactants to amplify the ECL signal. We systematically evaluated the electrochemical and ECL responses of seven device designs (W2G2, W2G5, W2G10, W2G20, W5G10, W10G10, W20G10) with varying gaps and widths dimensions. While the electrode width had minimal impact, increasing the gap size significantly reduced the luminol ECL response, highlighting its dominant role. Additionally, we examined the effects of applied potential on the GE, luminol concentration, and the role of dissolved oxygen. Coreactant-free ECL was also compared to luminol ECL in the presence of hydrogen peroxide, showing that the luminol-ROSs system generated an equivalent signal to the luminol- H_2O_2 system (with 500 μM H_2O_2). The W2G2 device exhibited the highest ECL signal and was used for Trolox antioxidant detection, demonstrating strong sensitivity and stability. Furthermore, the applicability of this novel ECL sensing platform for the immunodetection of IgG protein was assessed on the W2G10 device. The biosensor was prepared by selectively modifying the CE with a chitosan-gold nanocomposite (**Fig1.a**), enabling further attachment of anti-IgG antibodies to the CE. The biosensor exhibited excellent sensitivity toward IgG, with a detection range from 1 fg mL^{-1} to 100 pg mL^{-1} . These findings highlight the platform's potential as a highly sensitive, coreactant-free ECL immunosensing solution, enabling multiplexed, multi-analyte detection in a compact, integrated format suitable for portable point-of-care applications.

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O-017**ELECTROCHEMICAL DETECTION OF ISOTHERMALLY AMPLIFIED LONG NON-CODING RNAs: A PROMISING APPROACH FOR CLINICAL CANCER MANAGEMENT****Cobzariu, Madalin Alexandru¹; García-Flórez, Lucía²; González-Burgo, Nerea²; Miranda-Castro, Rebeca¹; Lobo-Castañón, María Jesús¹.**

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Early diagnosis is essential for reducing cancer mortality rates. Research in this area should focus on two pivotal aspects: identifying selective tumor biomarkers and developing tests for their clinical validation. Long non-coding RNAs (lncRNAs) are non-protein-coding components of the human transcriptome that exceed 200 nucleotides in length. They play significant roles in various gene regulatory mechanisms and are dysregulated in different cancer types, exhibiting either oncogenic or tumor-suppressive functions. As a result, lncRNAs have become promising biomarkers for the diagnosis and treatment of cancer [1]. However, to fully integrate lncRNAs into clinical practice, there is a need for robust methods to detect their expression in biological fluids and tissues.

Hybridization-based electrochemical biosensors offer an appealing strategy for lncRNA detection, promising high selectivity, sensitivity, ease of use, portability, and multiplexing capabilities [2]. Using this approach, we developed bioelectrochemical platforms for detecting the lncRNA urothelial carcinoma-associated 1 (UCA1), aberrantly expressed in colorectal cancer [3], along with beta-Actin mRNA, serving as an internal RNA control of stable expression. These platforms integrate a rapid isothermal amplification technique called recombinase polymerase amplification (RPA) on gold surfaces to produce immobilized amplicons electrochemically detected by chronoamperometry after tagging with peroxidase enzyme [4]. Under optimized conditions, the RPA-based platforms allow the reliable detection of synthetic oligonucleotides at the femtomolar level after 30 min of amplification at 40°C. The applicability of these biosensors was tested in both colorectal cancer and non-cancerous cell lines, demonstrating analytical performance in good agreement with a standard RT-qPCR method. These results highlight the potential of our RPA-based electrochemical platform for efficient and accurate cancer diagnosis.

Acknowledgments

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O-018

PRECLINICAL VALIDATION OF AN ELECTROCHEMICAL SENSOR FOR CHRONIC ALCOHOL ABUSE

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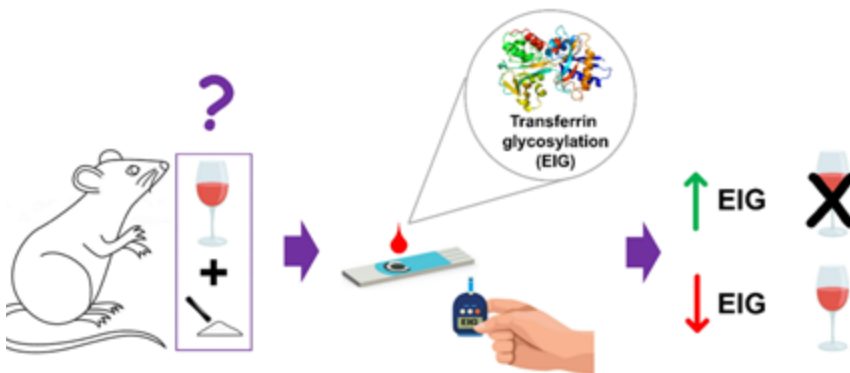
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In the context of glycoprotein biomarkers, transferrin (Tf) has received a lot of attention. Tf is a serum glycoprotein which exhibits two N-linked disialylated biantennary oligosaccharide chains (Asn413 and Asn611). The main Tf glycoform contains two N-biantennary glycans with a total number of four sialic acids (tetrasialo-Tf). However, there are other minority glycoforms with different number of sialic acids: from two to six. Under certain pathological states or due to genetic defects, the profile of glycoforms suffers modifications and minority glycoforms with lower amount of glycans (asialo-, disialo- and trisialo-Tf) are increased respect to the main glycoform (tetrasialo-Tf). This low glycosylated Tf is called carbohydrate deficient transferrin (CDT) and it is currently the clinically used biomarker for the detection of congenital disorders of glycosylation, chronic alcohol abuse and cerebrospinal fluid loss.

Typically, %CDT is measured by liquid chromatography and capillary electrophoresis separation of the Tf glycoforms, providing excellent diagnosis performance. However, these techniques require benchtop equipment and skilled personnel, so the analysis is done in clinical laboratories. In this sense, our group proposed a new *in-situ* analytical approach for evaluation of Tf glycosylation level based on an electrochemical tag (Os(VI) complex), an immunopurification process using magnetic beads functionalized with anti-Tf antibodies, and disposable screen-printed electrodes to carry out the electrochemical measurements. The result obtained by this electrochemical analysis is called electrochemical index of glycosylation (EIG).¹ EIG showed an excellent inverse linear correlation with the parameter %CDT. Later, EIG was used for evaluating chronic alcohol abuse in animal model using two different groups (rats passively fed with alcohol and rats fed with saline solution).² The electrochemical approach was analytically validated, showing a good limit of detection ($LOD = 0.7 \text{ g L}^{-1}$) well below the normal range of Tf in serum (ranging from 2 to 3.6 g L^{-1}). In addition, the diagnosis capabilities of EIG were preliminarily evaluated by analysing 31 samples of rat plasma, yielding a clinical sensitivity and specificity of 81% and 87%, respectively.

In this work, a more complex and realistic animal model was devised to perform a deeper preclinical validation of the proposed electrochemical sensor for chronic alcohol abuse identification. It consists of a polydrug self-administration animal model with a higher number of individuals (121 rats, 65 female and 56 male), trying to imitate the human pattern of drug use and considering

also sex dimensions. In this case, three rat groups were designed: saline group, cocaine group and cocaine-alcohol group. Moreover, two periods of withdrawal were studied, 2 days and 30 days. The alcohol-cocaine group after 2 days of withdrawal showed significantly lower EIG values ($p < 0.1$) than the rest of the groups and alcohol-cocaine group after 30 days of withdrawal, so EIG was able to identify the alcohol consumption in rats and to monitor the recovery of glycosylation level after 30 days of withdrawal too, even combined with cocaine. Receiver operating characteristic (ROC) curves were developed for each sex and the corresponding cut-off values were determined. The electrochemical approach showed a clinical sensitivity of 70% for male and 75% for female, and a specificity of 67% for both sexes. This preclinical validation demonstrated the possibilities of this sensor for point-of-care testing of alcoholism, even in cocaine addicts, making it a potential tool for diagnosis and monitoring of alcohol consumption in detox treatments for humans.³



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O-019

BRAIN DERIVED EXOSOMES IN PLASMA AS NOVEL BIOMARKERS FOR EARLY ALZHEIMER'S DETECTION AND RISK STRATIFICATION OF PATIENTS: A NOVEL POINT-OF-CARE DIAGNOSTIC APPROACH

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Alzheimer's Disease (AD) is the most prevalent form of dementia, accounting for approximately 60–70% of cases worldwide, according to the World Health Organization (WHO). The disease is characterized by progressive cognitive decline, ultimately leading to severe memory loss and functional impairment. A major challenge in AD management is the difficulty in diagnosing the disease at an early stage, as clinical symptoms typically appear only after significant neuronal loss and brain atrophy have occurred(1). For this reason, AD is currently diagnosed mostly at late stages. Current diagnostic tools, such as neuroimaging techniques(2–5) and cerebrospinal fluid (CSF) analysis(6), provide valuable insights but are often expensive, invasive, and not widely accessible. These limitations highlight the urgent need for alternative, minimally invasive, and cost-effective diagnostic strategies.

Exosomes, small extracellular vesicles secreted by most cell types(7,8), have emerged as promising biomarkers for neurodegenerative diseases, including AD(9). Brain-derived exosomes (BDEs), which carry disease-associated molecules, can cross the blood-brain barrier and be detected in peripheral body fluids such as plasma, making them particularly interesting in the so-called "liquid biopsies". Also, BDEs unique composition reflects pathological changes occurring in the brain, making them ideal candidates for early disease detection(10,11). In this study, we developed a novel diagnostic approach based on the isolation and analysis of BDEs from plasma to identify AD-related biomarkers.

To set-up our method, we first optimized the isolation of BDEs using the SH-SY5Y neuroblastoma cell line as a model of disease. Exosomes were purified via differential ultracentrifugation, followed by immunocapture using tosyl-activated magnetic particles conjugated with an anti-NLGN3 antibody (antiNLGN3-MPs). This enabled the selective enrichment of BDEs based on the presence of NLGN3, a neuronal surface marker(12). Subsequently, on the surface of captured BDEs, we targeted β -site APP cleaving enzyme 1 (BACE-1), a key biomarker associated with AD pathology(13). BACE-1 plays a central role in the production of β -amyloid peptides, which aggregate into plaques—a hallmark of AD pathology(14,15). In Figure 1A the overall system is presented (Patent EP25382155.7). Detection uses biotinylated anti-BACE-1 and streptavidin-poly-HRP (Strep-Poly-HRP) to enhance the signal.

To detect BACE-1 levels on exosomal surfaces, we developed multiple immunoassay platforms utilizing optical, chemiluminescent, and electrochemical detection techniques. Normalized calibration curves are shown in Figure 1B, with the relevant limit of detections (LODs). Among these, the electrochemical platform demonstrated the highest sensitivity, achieving the lowest LOD. This approach was then extended to analyze plasma samples from patients with mild cognitive impairment (MCI) and those with suspected AD. The analysis of exosomes from plasma of patients was carried out using a miniaturized portable device operated by batteries integrated with a screen-printed electrode for the Point of Care application (Patent PCT/EP2022/071078).

Our preliminary results are shown in Figure 1C indicating that this method not only could enable early AD detection but also allows for risk stratification of patients, offering a valuable tool for precision medicine and clinical decision-making.

This exosome-based diagnostic strategy presents significant advantages over existing approaches. By leveraging blood-derived exosomes, our method provides a minimally invasive alternative to CSF analysis, improving patient compliance and accessibility. Additionally, the high sensitivity and specificity of the electrochemical detection platform enhance the reliability of the test. Future work will focus on validating this method in larger patient cohorts, refining detection techniques, and exploring the potential for integrating this platform into point-of-care diagnostic systems.

Overall, our study highlights the potential of exosome-based biomarkers in revolutionizing AD diagnosis and patient management. By enabling earlier and more accurate detection, this approach could pave the way for improved therapeutic interventions, ultimately slowing disease progression and enhancing patient outcomes.

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O-020

NANOUHPLC-HRMS WITH A Q-LIT-ORBITRAP TRIBRID MS FOR CHARACTERIZING ASTAXANTHIN-LOADED NANOPARTICLES IN OCULAR APPLICATIONS

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Astaxanthin (AST) is a carotenoid from the xanthophyll family, known for its antioxidant and anti-inflammatory properties [1]. Mainly produced by microalgae, AST has gained increasing interest in biomedical research due to its ability to counteract oxidative stress, a key factor in numerous ocular diseases. Despite its therapeutic potential, its clinical application is limited by poor stability (photo-oxidation) and low water solubility [2]. To address these challenges, several studies have been focused on developing innovative nanoparticle (NP)-based delivery systems to protect AST from degradation and enhance its absorption into ocular cells [3,4]. In this context, our collaborators have optimized AST-loaded nanoparticles (NPs [AST]⁺) with surface functionalization to improve permeability and increase AST bioavailability, hypothesizing that these newly synthesized nanoparticles may serve as a promising therapeutic strategy for ocular disease management.

This study aims to integrate multiple analytical techniques, both spectroscopic and spectrometric, to characterize the newly synthesized NPs [AST]⁺ and provide a compositional-qualitative and quantitative determination of their molecular effects in retinal cells.

The physico-chemical properties of the optimized NPs were characterized by dynamic light scattering (DLS) measurements using the Nano ZetaSizer ZS, Zen 3600 Malvern instrument. Cell viability assays on ARPE-19 retinal cells exposed to hydrogen peroxide (H₂O₂), with or without treatments (AST, NPs, NPs [AST]⁺) were conducted, as well as the uptake of NPs [AST]⁺ by retinal cells was evaluated through confocal microscopy. Proteomics analysis was employed to investigate the molecular mechanisms underlying the effects of NPs [AST]⁺ with retinal cells treated under oxidative stress (H₂O₂). Pre-analytical stages included protein extraction, digestion and peptide purification by solid-phase extraction (SPE). Peptides were then analyzed using an Ultimate 3000 nano-UHPLC system coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer. Label-free quantification (LFQ) was performed with abundance calculations based on pairwise ratio-based comparisons, and statistical univariate analysis was performed applying a Student's t-test.

Moreover, a multivariate analysis was conducted on the obtained datasets using the R/Bioconductor packages DEP and limma [5]. The data were log-transformed, variance-stabilized, intensity values were normalized, and missing data were imputed using a combined approach. Then, a single model incorporating condition and time was fitted, allowing detection of both main effects and interactions. For both analyses, proteins with p-value < 0.05 and fold change ≥ ±1.3 were considered statistically significant differentially expressed proteins (DEPs). Functional enrichment analysis was performed using bioinformatic tools (Cytoscape v.3.10.1 and STRING v.12).

DLS results confirmed the AST loading efficiency in NPs ranging between 37.3% ± 2.4 and 80.9% ± 6.4. Spectroscopic data on cell viability demonstrated that empty and NPs [AST]⁺ were non-toxic, maintaining viability > 80% after 24h. Notably, NPs [AST]⁺ preserved ARPE-19 cell vitality above 90% under oxidative stress. Confocal microscopy revealed the NPs [AST]⁺ uptake at 6h and 24h, showing significant NPs migration and cellular internalization.

The nanoUHPLC-HRMS analysis, integrated with univariate and multivariate statistical approaches, revealed dysregulated proteins in ARPE-19 retinal cells treated with NPs [AST]⁺ compared to controls after 6h of H₂O₂ exposure, which are related to execution phase of apoptosis, nitric oxide biosynthetic process and lipid biosynthesis (i.e. apoptosis-stimulating of p53 protein 2 and ORM1-like protein 2). In addition, the LFQ proteomic analysis allowed the detection in NPs [AST]⁺ retinal cells of up-regulated proteins which influence camera-type eye development (i.e. collagen alpha-1(IV) chain and fibrillin-1 proteins) and steroid biosynthesis pathways (i.e. delta (24)-sterol reductase) compare to the empty NPs retinal cells.

In conclusion, this research paves the way for characterizing astaxanthin-loaded nanoparticles in ocular applications, leading to the detection of the molecular mechanism implicated in their beneficial effects and the subsequent development of an improved treatment for ocular diseases

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O-021

FLEXiGUT: UNRAVELING THE IMPACT OF ENVIRONMENTAL AND DIETARY CONTAMINANTS ON GUT MICROBIOME COMPOSITION AND INFLAMMATION

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Exposure to environmental and dietary contaminants, such as toxins, man-made chemicals and air pollution, is increasingly recognized as a disruptor of gut homeostasis with significant implications for human health. Alterations in the gut microbiome have been linked to chronic diseases such as obesity, diabetes, and colorectal cancer. The Bacteroides2 enterotype, in particular, has been associated with both gut and systemic inflammation, suggesting the existence of a gut microbiome-inflammatory axis [1]. The gut microbiota appears to play a central role in inflammation owing to its ability to release inflammatory products, which may contribute to biological processes that increase the risk of chronic diseases and/or accelerate biological ageing.

FLEXiGUT, the Flemish exposome project, is the first large-scale study investigating the role of environmental and dietary exposures on gut microbiome composition and inflammation both in children and adults [2]. This initiative integrates exposure records, high-throughput omics technologies and epidemiological studies from two Flemish prospective cohorts: the ENVIRONAGE mother-child cohort, which tracks participants (N=400) from gestation to 10 years of age, and the Flemish Gut Flora Project longitudinal cohort of predominantly healthy adults (N=400). FLEXiGUT leverages three key data sources, positioning us at the forefront of microbiome-exposome interaction research: 1) rich metadata including geolocation, drug intake, dietary intake and lifestyle; 2) biomonitoring of environmental and dietary contaminants in biofluids and tissues such as urine, blood and placenta; and 3) extensive multi-omics data including fecal metagenomics, metabolomics, DNA adductomics, telomere length, and inflammatory markers.

Our human biomonitoring results in biofluids and tissues reveal widespread exposure to contaminants such as organophosphate flame retardants, plasticizers, polychlorinated biphenyls (PCBs), mycotoxins, and particulate air pollutants among the Flemish population. Notably, a sex-dependent association was observed between exposure to air pollution (black carbon, and particulate matter -PM_{2.5} and PM₁₀-) and gut microbiome diversity in Flemish children. In boys, particulate air pollution exposure was negatively associated with gut microbiome richness and Shannon diversity, whereas in girls, non-significant (positive) associations were observed with species evenness and Shannon diversity. These findings suggest boys may be more vulnerable to gut microbiome diversity loss associated with childhood exposure to particulate air pollution.

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O-022**COMPARATIVE ASSESSMENT OF HILIC-HRMS VS. CE-HRMS IN METABOLOMICS FOR BIOMARKER DISCOVERY OF PCB AND BPA EXPOSURE**

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Hydrophilic interaction liquid chromatography (HILIC) and capillary electrophoresis (CE) coupled to high-resolution mass spectrometry (HRMS) emerge as two of the most powerful analytical techniques for probing the intricate polar fraction of the metabolome. Their combined use is especially recommended to enhance coverage of the polar metabolome, particularly in scenarios where multiple metabolic pathways may face disruption, as seen in toxicological studies examining exposure to chemicals like polychlorinated biphenyls (PCBs) or bisphenol A (BPA)].

Within the framework of this study, HILIC-HRMS and CE-HRMS methods were compared across four key criteria: (1) compounds detectability, (2) Pezzatti's score [1], (3) intra-day precision, and (4) ease of automatic data analysis. The HILIC-HRMS method proved to be a more practical tool for polar metabolome analysis; however, the CE-HRMS method contributed to the identification of additional biomarkers, helping to expand overall metabolome coverage in metabolomics studies [2]. Together, these methods encapsulate a profound complementarity.

Leveraging their proven complementarity in metabolomics, the HILIC-HRMS and CE-HRMS methods were applied in two separate studies to extend polar metabolome coverage and better illustrate the effects of contaminant exposure—an emerging approach in chemical risk analysis [3]. These studies aimed to identify biomarkers of effect associated with low-dose exposure to non-dioxin-like (NDL)-PCBs and BPA in pig metabolism, contributing to the risk assessment of these chemicals. Dietary exposure to Aroclor 1260 (a commercially available mixture consisting of 98% NDL-PCB congeners) at doses of 20 ng/kg body weight (b.w.) per day for 21 days was selected based on reported human exposure levels to NDL-PCB. Similarly, dietary exposure to BPA at doses of 4 µg/kg body weight (b.w.) per day for 21 days was based on the tolerable daily intake (TDI) established until 2023 (EFSA, 2015).

The combined use of HILIC-HRMS and CE-HRMS provided valuable insights into the metabolic effects of NDL-PCBs and BPA in pigs. Importantly, both contaminants were shown to affect the arginine biosynthesis pathway. Additionally, NDL-PCB exposure disrupted glyoxylate and dicarboxylate metabolism, as well as valine, leucine and isoleucine biosynthesis [4]. In contrast, BPA exposure was associated with alterations in alanine, aspartate and glutamate metabolism. Notably, neither analytical method alone provided a comprehensive overview of metabolic changes in pigs, underscoring the importance of employing multiple analytical techniques to fully capture the complexity of the metabolome.

Keywords: Non-dioxin like polychlorinated biphenyls, bisphenol A, metabolomics, hydrophilic interaction liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry

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O-023

NON-TARGET METABOLOMIC APPROACH OF THE TOXIC EFFECTS OF GLYPHOSATE IN ZEBRAFISH (*D. RERIO*)

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Glyphosate (GLY), also known as *N*-(phosphonomethyl)glycine, is a phosphonate compound extensively utilized as the primary component in most globally manufactured and applied herbicides. The more extended use of GLY is translated into a higher risk of exposure. Even though GLY is known for not being a persistent organic pollutant (POP), it has been detected in air during spraying, in food, and in several aquatic environments [1], [2].

This study assessed the toxic effects of GLY exposure in zebrafish (*D. rerio*) using a non-targeted metabolomic approach. Metabolic profiling was performed on the brain, gut, and fecal biological samples exposed to this broad-spectrum herbicide's environmentally relevant concentrations (0.3 and 3 µg L⁻¹) for two weeks. Data analysis using the ROIMCR approach [3] resolved up to 70 components depending on the tissue, accounting for over 99% of data variance. Compound annotation via the MSident toolbox [4] revealed diverse biomolecules, including amino acids, sugars, nucleotides, organic acids, and other metabolites. A total of 109, 294, and 170 metabolites were tentatively identified in the brain, gut, and feces, respectively, being 92, 182, and 117 further confirmed as part of the zebrafish metabolome, according to the KEGG database [5].

Statistical analyses, including partial least squares discriminant analysis (PLS-DA) and hierarchical clustering, revealed key metabolites (within the confirmed *D. rerio* metabolome) that distinguished Control and GLY-treated samples, with the most evident separation observed between the Control group and samples exposed to high GLY doses. Notably, 37 metabolites were identified in the brain, 75 in the gut, and 31 in feces, showing significant differences between sample groups. Additionally, 15 common metabolites were found in both brain and gut tissues, suggesting a potential link between the gut microbiome and the central nervous system (gut-brain axis) affected by GLY.

Functional enrichment analysis revealed that GLY exposure down-regulated 17 metabolic pathways in the gut, including protein, amino acid, lipid, and short-chain fatty acid metabolism, leading to potential energy deficits. In the brain, most metabolites were also down-regulated, while fecal samples showed up-regulated metabolites linked to conditions like rheumatoid arthritis and colorectal cancer [6], [7].

These findings demonstrate that GLY exposure disrupts metabolic pathways in zebrafish, affecting the gut-brain axis and energy metabolism, and may contribute to neurological and systemic health issues.

Keywords: Non-target metabolomics; ROIMCR; glyphosate; gut-brain axis; *Danio rerio*

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O-024**INVESTIGATING OLIVE NATURAL RESISTANCE TO VERTICILLIUM WILT USING LC-IMS-MS-BASED NON-TARGETED METABOLOMICS****Serrano-García, Irene¹; Olmo-García, Lucía¹; Martakos, Ioannis C²; León, Lorenzo³; De La Rosa, Raúl³; Dasenaki, Marilena E²; Thomaidis, Nikolaos S²; Carrasco-Pancorbo, Alegría¹.**¹University of Granada, Granada, Spain; ²National and Kapodistrian University of Athens, Athens, Greece; ³IFAPA Centro Alameda del Obispo, Córdoba, Spain.

Verticillium wilt of olive (VWO), caused by the soil-borne fungus *Verticillium dahliae*, is currently one of the most serious threats to olive-growing regions worldwide. This devastating disease leads to plant wilting and considerable economic losses. In the absence of effective preventive or curative chemical fungicides, exploiting the natural resistance of olive cultivars is considered a key component of integrated disease management strategies.

This study aimed to unravel the metabolic traits linked to VWO resistance in olives, using a broad, untargeted metabolomic strategy. To that end, roots, stems, and leaves from 43 olive genotypes with varying levels of resistance to VWO were sampled from three healthy one-year-old plants (biological triplicates), resulting in a total of 387 samples across all organ types. An advanced UHPLC-ESI-TimsTOF MS/MS platform was employed to profile the basal metabolome of the samples.

In the first phase of the study, the metabolic profiles of roots, stems, and leaves were comprehensively characterized. Over 80 metabolites, spanning various chemical classes—including organic acids, simple phenols, iridoids, coumarins, flavonoids, lignans, secoiridoids, pentacyclic triterpenes, and fatty acids—were annotated. The organ-specific presence or absence of these secondary metabolites was established. Notably, incorporating ion mobility enabled the detection of isomeric species in olive tissues for the first time. Furthermore, the dataset was used to establish a preliminary ^{TIMS}CCS_{N₂} experimental library, which is expected to improve compound identification accuracy in future studies.

In the second phase, multivariate statistical analyses were applied to explore metabolic differences among the cultivars in relation to their resistance to VWO across the three organ types. Clear metabolic distinctions were observed between highly resistant (HR) and susceptible (S) genotypes. Principal component analysis (PCA) revealed natural clustering of samples based on resistance level. Then, supervised statistics (partial least squares-discriminant analysis) were used to further discriminate genotype groups.

These classification models pointed out key metabolic markers associated with VWO susceptibility and contributed to defining their compositional patterns. In stems of HR genotypes, secoiridoids and flavonoid derivatives emerged as key discriminant compounds, while lignan-related metabolites contributed to the separation of S genotypes. In leaves, HR cultivars were differentiated by specific profiles involving iridoids and fatty acids, whereas a broader array of compounds—including flavonoids, simple phenols, and fatty acids—played a role in distinguishing S genotypes.

These results demonstrate the analytical power of non-targeted metabolomics combined with ion mobility spectrometry for resolving complex plant metabolomes. The integration of high-resolution separation techniques and multivariate analysis provided a robust framework for biomarker discovery, offering valuable insights for both functional studies and applied breeding programs focused on disease resilience.

O-025**VALORIZATION OF A VARIETY OF HISTORICAL WHEAT (SAN PASTORE) ALONG THE BREAD PRODUCTION CHAIN USING OMICS AND MULTIVARIATE METHODS**

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In the perspective of the protection and valorisation of typical products, the role played by analytical chemistry appears of primary importance in order to provide consumers and workers in the production chain with useful information on the characterization of the products. The study presented here was developed within the “Pa.N.E.” project. – Panem Nostrum Every Day” (PSR 2014-2020, Measure 16 – 16.1.1), aimed at the recovery of a historical variety of soft wheat, the San Pastore variety, widely used in the Piedmont area until the 1960s, then fallen into disuse, and now the almost exclusive prerogative of a few research and conservation centres, the most authoritative of which in Italy is the Morando Bolognini Foundation. The project involved the valorisation of San Pastore wheat along the entire supply chain, from wheat to flour to bread, rediscovering the modernity of a traditional wheat, with the aim of combining innovation with tradition. From a characterization point of view, the supply chain was followed from different points of view:

- Characterization of wheat, comparing 4 different varieties of San Pastore with 2 modern and 2 historical wheat varieties;
- Flour authentication study on mixtures of stone-milled San Pastore wholemeal flour with increasing contents of flours of two modern soft grains, to identify possible adulterations;
- Authentication study on bread, on which the present contribution will focus: the bread produced from San Pastore flour currently in production was studied to evaluate whether variations to the recipe could be detected (type of oil, yeast, flour, operator), as part of the identification of commercial frauds. The study was approached using experimental design techniques to study the effect of the recipe on the chemical characteristics of the final product.

All phases of the research were followed through a characterization obtained by a multi-omics approach. Wheat, flour and bread samples were pre-treated to extract proteins, lipids and small molecules which were identified and quantified using UHPLC-HRMS (proteomics and lipidomics) and GCxGC-MS (small molecules). The data were processed using chemometric pattern recognition techniques (PCA, cluster analysis, Kohonen autoassociative maps, MFA) and classification methods (PLS-DA, SIMCA, Artificial neural networks, etc), also coupled with variable selection methods.

Acknowledgements:

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O-026

DEVELOPMENT AND CHARACTERIZATION OF A MONOLITHIC APTAMER-BASED SORBENT FOR SELECTIVE EXTRACTION OF A β AMYLOID PEPTIDES FROM CEREBROSPINAL FLUID COUPLED ON-LINE WITH LC-MS/MS ANALYSIS

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The amyloid-beta peptides ratio (A β 42/A β 40) in cerebrospinal fluid (CSF) has been identified as a potential better biomarker for Alzheimer's disease compared to the A β 42 peptide concentration alone. However, due to the very low levels of these peptides in CSF, their tendency to aggregate, and their adsorption onto surfaces and albumin (which is present at high concentrations in CSF), their quantification by LC-MS/MS remains a difficult task. In addition, the complex nature of CSF can lead to substantial matrix effects during LC-MS/MS analysis. To address these issues, an approach based on immobilizing biomimetic tools, specifically aptamers, was employed for the selective extraction of A β 40 and A β 42 coupled on-line to LC-MS/MS analysis.

For this, the *in-situ* synthesis of a hybrid-inorganic silica monolith within a 530 μ m inner diameter, 100 mm long capillary was considered. It was optimized to achieve both correct attachment of the monolith to the inner surface of the capillary (confirmed by scanning electron microscopy) and a sufficient permeability compatible with the intended online coupling. An aptamer described to bind A β 42, *i.e.* the oligonucleotide GCC-TGT-GGT-GTT-GGG-GCG-GGT-GCG, and modified with a spacer arm containing 12 carbon atoms and a 5' amino group was covalently grafted to the monolith surface, with a grafting density of 655 pmol/ μ L of capillary. The optimization of the extraction protocol using the monolithic oligosorbent (mOs) in pure medium was performed to ensure both retention and selectivity for A β 40 and A β 42, focusing on the volume and composition of washing and elution steps. This led to extraction yields of 80% for A β 40 and 42% for A β 42. The mOs capacity (255 ng of A β 40/ μ L of mOs) was sufficient for the target application in CSF. The method was validated for artificial CSF with A β 40 and A β 42 concentrations ranging from 0.6 to 13 ng/mL, yielding limits of quantification of 0.1 ng/mL for A β 40 and 0.15 ng/mL for A β 42 when injecting a part of the mOs elution fraction off-line to the LC-MS/MS.

To easily automate the whole analytical procedure, the on-line coupling of the mOs with LC-MS/MS was considered. For this, a trapping column was introduced between the mOs and the LC column. The elution volume on the mOs, that has to be lower than the breakthrough volume of the trapping column, was optimized and the resulting method was further validated using artificial CSF for A β peptide concentrations ranging from 0.6 to 13 ng/mL. Thanks to the online coupling, the whole elution fraction from the mOs was transferred via the trapping column into LC-MS/MS, resulting in a lowered limit of quantification of 0.03 and 0.1 ng/mL for A β 40 and A β 42 respectively, in artificial CSF. This method offers a promising tool for determining the A β 42/A β 40 ratio in CSF from Alzheimer's disease patients.

O-027**MICROWAVE AND SPE: A RECIPE FOR FASTER AND GREENER LIPID ANALYSIS****Ferrara, Donatella¹; Ruin, Nicola²; Beccaria, Marco³; Cordero, Chiara¹; Purcaro, Giorgia⁴.***¹University of Turin, Turin, Italy; ²University of Ferrara, Ferrara, Italy; ³University of Ferrara, Ferrara, Italy; ⁴Gembloux Agro Bio Tech, gembloux, Belgium.*

The analysis of sterols is performed on a daily basis in many laboratories as they serve as key markers for detecting adulteration in extra virgin olive oil (EVOO). Due to its high nutritional and economic value, EVOO is frequently targeted for fraud, often through mixing with cheaper oils. Accurately assessing the sterol profile is crucial for maintaining product authenticity and ensuring compliance with regulatory standards.

Traditionally, sterol fraction analysis requires multiple steps, including saponification, liquid-liquid extraction, thin-layer chromatography (TLC), and gas chromatography with a flame ionization detector (GC-FID). Although effective, this method has notable drawbacks: it is time-consuming (about eight hours), relies on large volumes of organic solvents, and demands intensive manual handling. These limitations reduce its suitability for high-throughput analyses and raise environmental concerns, as it conflicts with the principles of green analytical chemistry.

To overcome these challenges, this study developed a more efficient approach by combining the traditionally separate steps of saponification and liquid-liquid extraction into a single, integrated microwave-assisted saponification and extraction (MASE) step. This approach not only reduces processing time but also minimizes solvent consumption and waste generation.

Additionally, the solid-phase extraction (SPE) purification phase was optimized to further enhance efficiency and sustainability. By modifying the solvent mixtures and reducing the number of fractionation steps, the method achieved improved separation of sterols while lowering solvent use. The optimized approach demonstrated comparable analytical reliability to the official method, with no significant differences observed.

Moreover, its environmental impact was evaluated using the AgreePrep software, yielding an higher score than the score of the traditional method. Overall, the proposed method is faster (about 2 hours), more sustainable, and equally reliable for detecting sterol adulteration in olive oil. Its reduced analysis time and lower environmental footprint make it a valuable alternative for routine laboratory use, supporting quality control and regulatory compliance while aligning with green chemistry principles.

O-028**GREEN PRECONCENTRATION METHODS BASED ON DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING HYDROPHOBIC NATURAL DEEP EUTECTIC SOLVENTS FOR THE DETERMINATION OF EMERGING CONTAMINANTS IN WATER SAMPLES****Gómez-Nieto, Beatriz; Fernández-Jiménez, Marta; Abad-Gil, Lucía; Rodríguez Procopio, Jesús.***Universidad Autónoma de Madrid, MADRID, Spain.*

The widespread use of personal care products has led to a significant increase in the environmental concentrations of many chemical substances such as preservatives or UV filters. These compounds are classified as emerging contaminants (ECs) due to the lack of regulatory control over their release and their potential adverse effects on human health and ecosystems. In this context, the development of accurate analytical methods is critical for monitoring their presence in environmental samples.

Considering the typically low concentration of ECs in the environment and the complexity of most environmental sample matrices, it is essential to perform a sample preparation step prior to the analytical measurement to extract and preconcentrate the target analytes. Dispersive liquid-liquid microextraction (DLLME) is one of the most widely used methods for the extraction and preconcentration of compounds from liquid samples due to its simplicity, cost-effectiveness, minimal consumption of extractants, and low generation of waste. To improve the sustainability of analytical methods and fully comply with the principles of Green Analytical Chemistry, current research is focused on replacing conventional organic extractants used in DLLME with non-toxic alternatives, such as Natural Deep Eutectic Solvents (NADES). NADES are binary or ternary mixtures of natural compounds such as fatty acids, carbohydrates and/or alcohols, combined in specific molar ratios to yield liquids with melting points lower than those of their individual components. NADES are characterized by their easy and economical synthesis and the adaptability of their physicochemical properties

In this study, an analytical method based on DLLME and high-performance liquid chromatography with Diode Array Detector (HPLC-DAD) was optimized for the simultaneous determination of alcohol-based preservatives with varying polarities in water samples. To develop this method, different hydrophobic NADES composed of water-insoluble monoterpenes and/or non-polar fatty acids were evaluated as extraction media and the chromatographic conditions for the direct determination of the analytes in the extracts were optimized. The influence of key experimental variables such as the pH of the aqueous sample, the ratio of NADES phase to sample volume or the extraction time on the extraction efficiency was investigated using a multivariate optimization. Under optimal conditions, extraction efficiencies of 50–70% were obtained for the more hydrophilic analytes, while efficiencies of 81–96% were achieved for the more hydrophobic compounds. The extraction and preconcentration step prior to HPLC-DAD analysis has enabled to reduce the limits of detection, reaching values in the range from 0.5 to 2.0 $\mu\text{g L}^{-1}$.

O-029

INNOVATIVE ALL-IN-ONE SAMPLE TREATMENT PLATFORMS BASED ON SUPRAMOLECULAR SOLVENTS WITH LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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The analysis of chemical contaminants, both targeted and non-targeted, using liquid chromatography–mass spectrometry (HPLC-MS) faces increasingly demanding challenges across diverse fields such as agri-food, environmental monitoring, toxicology, doping control, and epidemiological studies. Thus, it is common to require the rapid, sensitive, and selective identification and/or quantification of a large number of structurally diverse contaminants across numerous samples. Consequently, sample preparation must be aligned with these challenges, requiring approaches that are not only simple, fast, and efficient, but also sustainable [1].

Supramolecular solvents (SUPRAS) offer significant opportunities for the development of innovative sample treatment platforms suited to both targeted and non-targeted analysis by HPLC-MS. SUPRAS are nanostructured liquids generated from colloidal solutions of amphiphilic molecules through spontaneous self-assembly and coacervation processes. Their synthesis is spontaneous, atomically efficient, and requires minimal or no external energy input. Furthermore, SUPRAS can be generated *in situ* within liquid samples, with which they are immiscible. These solvents provide microenvironments of different polarity, multiple binding sites, large surface areas, and tuneable properties, which can be modulated by selecting specific amphiphilic molecules or adjusting self-assembly conditions.

This presentation will address the major advances made in fundamental aspects of SUPRAS, focusing on their synthesis, characterization, and property modeling. It will also explore the knowledge gained from their application in extraction processes, such as SUPRAS–solute interactions and preconcentration factors, and the strategies developed to ensure compatibility of supramolecular extracts with HPLC-MS. Particular attention will be given to recent progress in the design of novel sample preparation platforms where conventional organic solvents are either unsuitable or inefficient. These include, among others, all-in-one extraction methods capable of solubilizing structurally diverse contaminants spanning a broad polarity range from both solid and liquid multi-matrix samples. In addition, SUPRAS enable the integration of extraction and clean-up steps into a single operation.

Finally, the presentation will address the remaining challenges that should be addressed to facilitate the implementation of SUPRAS in routine analytical laboratories for targeted and non-targeted contaminant analysis.

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O-030

EXPLORING THE POTENTIAL AND CHALLENGES OF MICROWAVE-ASSISTED PRETREATMENTS COUPLED WITH PY-GC-MS FOR THE QUANTIFICATION OF MPs AND ASSOCIATED CONTAMINANTS

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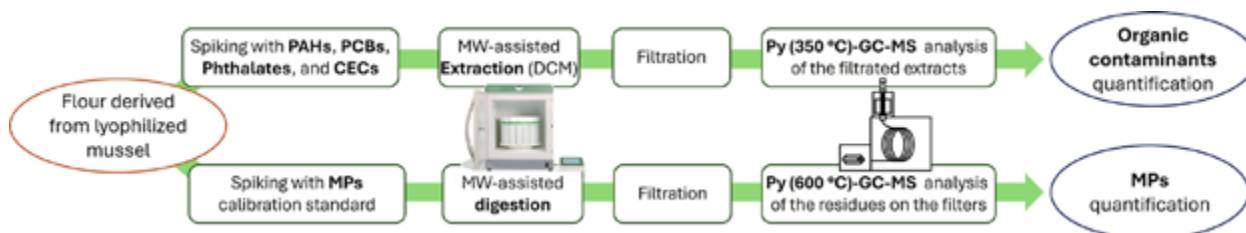
Microplastic (MP) pollution has been acknowledged as a global threat and one of the most relevant environmental issues. Different analytical techniques have been applied and optimized for microplastic analysis along with non-polymeric organic compounds associated with them, such as additives, persistent organic pollutants, and polymer degradation products. Nevertheless, there is still a major lack of understanding of the interaction mechanisms between these classes of pollutants and the ecosystems. Analytical pyrolysis-based techniques have proven to be effective in the analysis of microplastics in environmental samples, providing mass-based quantification of the different polymers along with quantification of MPs-associated organic contaminants. However, depending on the environmental matrix, sample pretreatment before analysis can require numerous and time-intensive steps.

We combined microwave-assisted digestion and extraction, together with pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), to characterize and quantify MPs and different classes of pollutants, frequently associated with MPs, in environmental samples. Microwave-assisted extraction (MAE) under mild conditions was performed to isolate phthalate plasticizers, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and contaminants of emerging concerns (CECs), from the matrix. MAE parameters (extraction time and extraction temperature) were optimized through experimental design for the extraction of PAHs, PCBs, phthalates and CECs from the matrix.

Microwave-assisted digestion under harsh conditions was instead employed to isolate microplastics, separating them from the matrix.

Recoveries of both organic contaminants and microplastics were evaluated using an organic-rich matrix (flour derived from lyophilized mussels), spiking it with the analytes and subjecting it to the developed analytical protocol (Figure). This method allowed the quantification of more than 30 different organic contaminants in a single chromatographic run, with recoveries higher than 87% in most cases and limits of detection (LOD) and quantitation (LOQ) in the nanogram range. Regarding MPs, recoveries higher than 84% were obtained in most cases.

After validation, the method was tested for the quantification of MPs in different environmental samples, such as lettuce, and marine biota samples provided in the context of national and international projects. Preliminary analyses highlighted the presence of polyethylene (PE) and polystyrene (PS) MPs in biota samples.



The combination of microwave-assisted pretreatments and Py-GC-MS analysis represents an efficient, and fast analytical strategy for the detection and quantification of both polymeric and non-polymeric pollutants, contributing to the development of high-throughput approaches that could be employed for environmental monitoring.

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O-031**SUSTAINABLE CELLULOSE BASED MODIFIED SORBENTS FOR BIOANALYSIS****González-Bermúdez, María Sol; López-Lorente, Ángela Inmaculada; Lucena, Rafael; Cárdenas, Soledad.***Affordable and Sustainable Sample Preparation (AS2P) Research Group, Analytical Chemistry Department, Chemical Institute for Energy and Environment (IQUEMA), University of Córdoba, E-14071, Córdoba, Spain, Córdoba, Spain.*

Sample preparation is a critical step of the analytical process having an inherent environmental impact, which is in many cases unavoidable because of the low analyte concentration or the complexity of the sample matrix, such as in the case of biofluids. In this context, the development of sustainable sorbent phases for the extraction of analytes of different nature is a current trend of Green Sample Preparation [1]. Among the different natural materials that have been reported in sample preparation, (ligno) cellulosic materials, including paper, cotton, wood or cork, have been extensively used in the last years due to their affordability, versatility, low cost and valuable physicochemical properties [2,3]. In this communication, the modification of cellulose materials, namely paper and cotton, with either natural sorptive phases, i.e., beeswax and oleic acid, or polymeric phases from recycled waste source is presented, along with their application in bioanalysis.

Cellulose paper modified with natural beeswax was prepared by drop-casting of beeswax dissolved in hexane and evaporation of the solvent, rendering a hydrophobic paper, capable of extracting tricyclic antidepressants (i.e., imipramine, desipramine, amitriptyline and trimipramine) from saliva samples via hydrophobic interactions [4]. The analytical workflow combines a straightforward sampling, simultaneous extraction of 30 samples in 1 h, and the rapid (<2 min) determination of the analytes via direct infusion mass spectrometry.

On the other hand, cotton has been modified with oleic acid via dip-coating method, immersing the cotton in pure oleic acid followed by incubation in an oven. The modified cotton was employed for the in-tip fabric phase extraction of tricyclic antidepressants from urine samples followed by their analysis by direct infusion mass spectrometry.

Moreover, paper can be modified with molecularly imprinted polymers (MIP) obtained via dissolution of nylon polymer and rearrangement upon solvent evaporation [5]. We have explored the possibility of preparing the MIPs from polymer waste source, thus giving a second life to this material, increasing the circularity of the plastic polymer. The obtained MIP papers were applied to the determination of naproxen drug via fluorescence measurements directly on the paper by using a custom-built platform, as well as after elution in methanol.

Acknowledgements

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O-032

EMPOWERING LEARNERS: STUDENT-CENTERED STRATEGIES IN ANALYTICAL CHEMISTRY FOR UNDERGRADUATE AND GRADUATE EDUCATION

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In recent years, one of the main objectives of the teaching innovation group "Analytical Chemistry in Sciences and Health Sciences (QAACS)" at the University of Barcelona has been the implementation of active and student-centered methodologies. These approaches aim to enhance students' ability to connect, integrate, and apply theoretical knowledge in practical contexts. By engaging with real-world scenarios, students are encouraged to analyze complex situations, make informed decisions, and collaborate to solve problems. This not only fosters cooperative learning but also strengthens their critical thinking and reflective skills. Such methodologies support meaningful learning, where knowledge is not merely acquired but deeply understood and retained through experience and personal relevance. Also, this pedagogical approach better prepares students for the demands of the professional world, equipping them with the analytical and interpersonal skills necessary to face complex challenges in research and industry contexts.

This work presents a selection of learning methodologies and strategies that have been implemented across various undergraduate and master's degree courses in the field of analytical chemistry. A recurring challenge in experimental teaching is the disconnection students often experience between theoretical content and its practical application, especially when theory and laboratory work are taught in different academic semesters. To address this issue, in the subjects "*Analytical chemistry*" and "*Basic laboratory of analytical chemistry*" within the chemistry degree, specific activities have been designed to bridge the gap between theory and practice, thereby enhancing student comprehension and engagement [1]. These activities aim to foster meaningful learning by encouraging students to actively construct knowledge through contextualized experiences. In the advanced course "*Analytical chemistry laboratory*", a problem-solving methodology has been adopted, incorporating webquests as a didactic tool. These web-based inquiry activities guide students through the resolution of real analytical problems related to environmental and food safety, promoting collaborative learning and the development of critical thinking skills.

At the master's level, active learning strategies have been implemented in two quality management subjects to enhance student engagement and deepen understanding of complex regulatory frameworks. In the subject "Analytical process quality", traditional lectures on the ISO/IEC 17025:2017 standard have been replaced by a flipped classroom model, in which students complete guided reading assignments prior to class. This shift allows classroom time to be dedicated to collaborative interpretation and practical application of the standard's requirements [2]. In parallel, team-based learning has been introduced to foster peer interaction and collective problem-solving, reinforcing key concepts through discussion and shared responsibility. Furthermore, in the "Audits" module, simulation-based learning has been integrated to recreate realistic professional scenarios, with a particular focus on analytical laboratory audits. These simulations offer a safe and controlled environment in which students can practice audit techniques, make decisions, and reflect on their performance [3]. Together, these methodologies aim to bridge theory and practice, preparing students to face real-world challenges in quality assurance and laboratory management.

Implemented across several academic years, these teaching experiences have consistently demonstrated their effectiveness in enhancing student learning outcomes and motivation. The application of active and student-centered methodologies has not only increased students' engagement with the content but has also significantly contributed to the development of essential professional competencies.

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O-033**EMBRACING ERROR: A TRANSFORMATIVE APPROACH TO SKILL ACQUISITION IN ANALYTICAL CHEMISTRY EDUCATION****Subirats, Xavier; Bastos-Arrieta, Julio; Díaz-Cruz, José Manuel; Pérez-Ràfols, Clara; Serrano, Núria.***University of Barcelona, Barcelona, Spain.*

In the current learning system, error is conceptualized as an obstacle to be avoided as it deprives the student of success, efficiency, and the desired judgement skills. This can generate stress and anxiety, reducing classroom participation. This negative connotation of error is especially rooted in traditional learning processes where the teacher transmits knowledge and expects the student to achieve it, grading the final level of accomplishment. In active learning methodologies, where the student constructs knowledge from experience, it is necessary to move beyond the view of error as a punishable deviation from the norm and reconceptualize it as a mismatch between the obtained and desired results. Acceptance and analysis of error can thus become a constructive, student-centered element that provides learning opportunities. In this context, the teacher's role is to diagnose through error, create learning situations, guide with a flexible attitude toward the initial plan, and evaluate processes and strategies. Thus, the student takes a leading role that integrates individualization (own error) and socialization (peer error), achieving deeper and more permanent learning.

Learning through error is particularly significant in contexts where the process is as important as the result or even more. In skill acquisition, practice is indispensable, and the student must be able to develop in a safe and tension-free environment. However, constructing this learning requires the student to be able to identify possible sources of error and evaluate their impact on the result to perform the task in the best possible way. Currently, most teaching practices are based on observing model roles that correctly perform the proposed task. However, this practice has deficiencies, including a passive attitude on the part of the student that hinders effective learning and a lack of critical evaluation of the different stages of the procedure, which favors superficial mimicry lacking awareness of error.

To overcome the limitations of skill acquisition based on model observation, this work presents a teaching strategy based on learning from error. Thus, it aims to develop a series of audiovisual supports that reflect the key points that are the most common sources of error in Analytical Chemistry laboratories. The first part of this audiovisual support, intended to be watched by the students before the laboratory session, shows an experimental procedure correctly performed. The second part, played in class, displays the same procedure but with the most frequent mistakes in the acquisition of a particular skill. The students are asked to evaluate the procedure to detect the errors made, share them, discuss their impact, and propose a correction.

This strategy seeks the student's involvement and proactivity to become aware of the error and how to avoid it, thus internalizing the correct practice and contributing to the development of the competence of learning ability and responsibility (capacity for analysis, application of knowledge to practice; ability to make decisions and adapt to new situations).

The project is developed within the framework of the practical classes of the Basic Laboratory in Analytical Chemistry, a mandatory subject in the fourth semester of the curricular itinerary of the Chemistry Degree at the University of Barcelona.

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O-034

INTEGRATING COMPUTATIONAL THINKING AND MACHINE LEARNING INTO ANALYTICAL CHEMISTRY EDUCATION: A HANDS-ON STRATEGY USING VISUAL AND SCRIPT-BASED TOOLS

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The increasing availability of high-dimensional chemical data, alongside the rapid emergence of artificial intelligence (AI), has made machine learning (ML) an essential tool in analytical chemistry. However, the integration of ML into chemistry education remains limited, hindered by curricular rigidity and insufficient computational training among both students and faculty. To address this challenge, we have developed and implemented a multilevel, research-informed educational strategy rooted in computational thinking and open-source tools such as Orange and MATLAB Live Scripts [1–3].

Over the past three academic years, our group has deployed this strategy across undergraduate, postgraduate, and faculty development contexts. At its core, the approach emphasizes hands-on learning, intuitive visual workflows, and scaffolded exposure to multivariate data analysis in real-world chemical problems. Orange, a Python-based, drag-and-drop visual programming platform [4], allows students to build Principal Component Analysis (PCA), Partial Least Squares (PLS), Support Vector Machines (SVM), and Random Forest models without coding. Meanwhile, MATLAB Live Scripts support deeper algorithmic understanding through executable code combined with narrative and equations.

At the undergraduate level, we assessed computational skills and perceptions through structured surveys (N=56) administered to chemistry students at the University of Valencia. Results revealed serious deficits: although 39% of first-year students reported some programming knowledge, this dropped to 0% by the third and fourth years. Despite this, interest in computational skills was high, and students identified clear relevance to solving analytical problems. These findings underscore the need to integrate programming across the degree, not just in isolated modules in first years.

In postgraduate programs, we incorporated ML teaching modules into courses such as "Analytical Strategies for Solving Socioeconomic Problems" and "Transversal Skills" using, for example, Infrared (IR) spectroscopy datasets to predict nutritional content in food matrices. Students applied PCA, PLS, and supervised classification models using both Orange and MATLAB, gaining confidence in applying these tools to their MSc research. Comparative surveys from in-person (2022/2023) and online (2023/2024) cohorts showed consistently positive reception, particularly for the low-entry barrier and versatility of Orange. Also, we introduced Matlab Live Scripts in "Electroanalysis" module for basic voltogram plotting and analysis.

At the faculty level, we have offered three editions of a cross-disciplinary training course for university instructors focused on teaching machine learning and chemometrics with accessible, open-source tools. The course materials—including datasets, video tutorials, and guided exercises—are available under a Creative Commons license via Zenodo [5]. Participants highlighted the utility of visual interfaces like Orange for integrating ML into non-computational chemistry contexts and appreciated the ready-to-use resources for immediate implementation in their teaching.

Our results confirm that combining interactive environments with real chemical data enhances student engagement and overcomes initial resistance to statistical and programming concepts. This strategy supports the broader goal of embedding computational thinking into chemistry education as a core skill, rather than treating it as a niche elective. Students learn to conceptualize workflows, apply and validate models, and interpret multivariate results with greater confidence—preparing them for the demands of data-intensive research and industry applications [6,7].

This contribution shares our evaluation data, teaching resources, and pedagogical insights from three years of innovation. We argue for a systemic curricular reform wherein machine learning is integrated into the analytical mindset, not as an add-on but as an essential tool for the chemist of the future.

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O-035**SPECTROELECTROCHEMISTRY AS A TOOL FOR IRON SPECIATION**

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Spectroelectrochemistry (SEC) is based on simultaneous spectroscopic and electrochemical measurements of substances both optically and electrochemically active [1]. Beyond the classical applications for characterizing materials and electrochemical reactions, recent works show interesting possibilities for determining analytes, especially in the UV-vis region and using commercial screen-printed carbon electrodes (SPCE) [2,3].

Regarding quantification, the evolution of SEC signals as a function of time at constant potential appears to be quite convenient. In a previous study [3], the system Fe(III) + ortho-phenanthroline (OP) was submitted to chronoamperometric measurements by using a commercial SPCE and a reflection probe attached to a commercial cell. Then, a fixed reducing potential was applied to promote the reduction of Fe(III)-OP complexes, slightly coloured, to the predominant 1:3 Fe(II)-OP complex, strongly coloured in red (it adsorbs at 510 nm). As a consequence, the absorbance A (defining $A=0$ just before the application of the potential) is increasing with the time t according to the equation:

$$A = k_1 [\text{Fe(III)}] t^{1/2} \quad (1)$$

where k_1 is a constant. Therefore, the plots of A vs. $t^{1/2}$ at different concentrations of Fe(III) provide slopes proportional to such concentrations, which can be used to build a calibration line.

In this work, the reverse strategy has been successfully tested: Fe(II)-ions in the presence of OP produce a red solution that is progressively decoloured as Fe(II) is oxidized to Fe(III) upon the application of an oxidant potential. This causes a decrease of absorbance according to:

$$A = -k_2 [\text{Fe(II)}] t^{1/2} \quad (2)$$

where k_2 is another constant (quite similar to k_1) and where negative absorbances are registered (also taking $A=0$ just before applying the potential).

Then, a procedure is proposed to determine the Fe(II)/Fe(III) ratio in a water sample. It consists on two SEC measurements in aliquots of 100 mL of the sample spiked with OP: one at a reducing potential of -0.2 V and the other at an oxidizing potential of +1.0 V. Accurate results can be achieved by comparing SEC measurements of the sample with calibration lines obtained with standard solutions of Fe(II)- and Fe(III)-ions. Anyway, reasonable estimates can be done by just comparing with each other both SEC measurements carried out on the sample.

These promising preliminary results could be the basis of a simple, fast and cost-effective methodology for Fe(II)/Fe(III) speciation in small volumes of sample.

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O-036

QUO VADIS ELECTROANALYTICAL MEASUREMENTS IN FLOWING SYSTEM

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The aim of this presentation is to show the potential, possibilities and limitations of modern electroanalytical methods in flowing systems for monitoring of biologically active organic compounds. All these aspects will be demonstrated on results recently obtained in our UNESCO Laboratory of Environmental Electrochemistry in Prague in the field of capillary electrophoresis with amperometric detection applied for monitoring of coccidiostat nicarbazin [1], amperometric monitoring of reducible organic compounds using silver amalgam electrodes [2], or high-performance amperometric flow injection analysis using a replaceable lactate oxidase-based mini-reactor and a silver amalgam screen-printed electrode [3]. Possibilities and limitations of HPLC-ED, BIA-ED and FIA-ED will be discussed as well. This presentation is an important part of dissemination process regarding IUPAC project 2023-010-2-500: Flow-through analytical systems with electrochemical detection for monitoring of biologically active species.

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O-037

DETERMINATION OF FREE METAL ION CONCENTRATIONS WITH AGNES (ABSENCE OF GRADIENTS AND NERNSTIAN EQUILIBRIUM STRIPPING)

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Metal speciation, which refers to how an element is distributed among its various chemical forms, significantly influences numerous phenomena such as precipitation, adsorption, complexation and metal transportation. Additionally, it is closely associated with metal bioavailability and toxicity. Therefore, it is crucial to develop analytical techniques that allow us to elucidate the distribution of metal among different species, and especially its **free** concentration.

The two-stage electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) [1] has been designed to determine free metal concentration and it has been commonly applied to Zn²⁺, Cd²⁺, Pb²⁺ ions, but other analytes include Sb(III) and Sn(II). After a first deposition at the electrode until reaching Nernstian equilibrium, the analyte is quantified in a second or stripping stage. AGNES technique has been applied in a variety of media including very low ionic strength systems -such as river water-, higher ionic strength systems -estuary and sea water samples-, soil extracts, systems containing nanoparticles, biological samples and wines [2-4]. The complexation of metals to Dissolved Organic Matter (DOM) has also been evaluated with AGNES.

Gallium and Indium are increasingly used in technological fields and they have been defined as Technology Critical Elements (TCE) included in the list of the fourteen non-energetic materials that are considered critical by the European Commission. The extensive use of Ga and In in various industries, combined with inadequate recycling practices, leads to increasing concentrations in different natural compartments. So, it is fundamental to understand the chemical behaviour of these elements in aqueous solution and assess their environmental bioavailability.

AGNES application for the determination of these emerging contaminants, In[3], and Ga [5] will be presented, addressing irreversibility and hydrolysis phenomena. It will also cover cases related to the speciation with ligands and solubility of nanoparticles.

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O-038**INSIGHTS INTO THE EVALUATION OF AFFINITY CAPILLARY ELECTROPHORESIS AND MICROSCALE THERMOPHORESIS FOR CHARACTERIZING MOLECULAR AND BIOMOLECULAR INTERACTIONS****Asmari, Mufarreh.***King Khalid University, College of Pharmacy, ABHA, Saudi Arabia.*

The accurate characterization of molecular and biomolecular interactions is central to understanding biological processes, designing therapeutic agents, and developing diagnostic tools. Herein, two analytical techniques—Affinity Capillary Electrophoresis (ACE) and Microscale Thermophoresis (MST)—have gained increasing attention for their sensitivity, versatility, and relatively low sample consumption among other competitive techniques such as ITC, SPR, DSF, and other conventional spectroscopic techniques. The proposed presentation highlights the principles, applications, and comparative advantages of ACE and MST in evaluating the binding interactions and give the audience in summary a brief statistics for using of both techniques in experimental part for estimating of binding parameters based on our published works and literature search engines. ACE is using in the past decades as a separative technique with variety of CE modes, including free-solution ACE (mobility shift assay), zonal methods, frontal analysis, and immobilized ACE (IACE), enabling the technique to analyze a broad range of interactions beyond simple charge alterations. ACE is often label-free and capable of quantifying dissociation constants (K_d) with relative high precision. Its applications can be implemented from drug-target screening to aptamer selection and enzyme-inhibitor studies. In contrast, MST, relies on the movement of molecules within a microscopic temperature gradient, a phenomenon that changes upon binding due to alterations in size, charge, or hydration shell. MST is highly sensitive and operates under near-native conditions, tolerating various buffer additives, serum, and even cell lysates. The method is particularly suitable for low-volume, rapid affinity screening and delivers more precise K_d values under the challenging experimental environments. Though, labeling is typically required, label-free approaches using intrinsic fluorescence are also possible. As expert in this field, we will discuss our experience in the past 10 years between ACE and MST techniques with selected case studies demonstrating how ACE and MST have been applied in the characterization of binding interactions. We will also compare the strengths and limitations of each technique, including sample requirements, throughput, buffer compatibility, and data output. By integrating both ACE and MST into a unified workflow, researchers can enhance the reliability and depth of interaction analysis, paving the way for more efficient drug discovery, biosensor development, and biomolecular engineering.

O-039

EXPLORING THE FUNDAMENTAL LIMITS AND OPPORTUNITIES OF REFERENCE ELECTRODES BASED ON ORGANIC ELECTROLYTES

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Since the beginning of the 21st century, tremendous effort in both academia and industry has been devoted to developing and commercializing sensing platforms and analytical devices for non-invasive detection and monitoring of various disease biomarkers [1]. This ambitious pursuit brought forth advancements in electrochemical recognition mechanisms while simultaneously highlighting the need for innovative solutions in reference element design. Conventional double-junction REs, although tried and tested, are challenging to miniaturize due to their reliance on large internal volumes of reference solution and bridge electrolyte. Instead, Ag/AgCl-based quasi-reference elements are commonly used, which rely heavily on the constant Cl⁻ concentration in the sample, inevitably introducing substantial measurement errors. Both constraints could be addressed through the broader adoption of recently introduced REs based on organic electrolytes. In these REs, the traditional liquid junction is eliminated by employing "water-immiscible salt bridges", such as free-flowing ionic liquids (ILs) or membranes doped with lipophilic salts. Upon contact with a sample, these electrolytes partition into the aqueous phase and establish an equilibrium distribution and a phase boundary potential that remains largely unaffected by the sample composition (Figure 1). The new approach moves away from bulky inner solution compartments while maintaining high potential stability across a wide range of samples and thus opens broader opportunities for miniaturization and task specific RE design.

Despite their promising features, REs based on organic electrolytes have yet to achieve a commercial breakthrough, primarily due to significant gaps in our understanding of their functional limits [2]. In this contribution, we critically examine these fundamental limitations and establish a direct link between observed behaviors and the lipophilicity of the used electrolyte through a combination of theoretical analysis and experimental validation. REs based on low-lipophilicity electrolytes are shown to suffer from ion-exchange interference from sample ions and rapid organic salt loss via leaching, which significantly shortens RE lifetimes [3]. In turn, the use of highly lipophilic electrolytes in RE design necessitates precise cation-anion matching, as even minor impurities are demonstrated to cause potential deviations. To overcome these issues, we leverage the flexible nature of polymeric membrane-based REs and introduce a novel design combining highly lipophilic electrolytes and ionophores in the same reference membrane. This seemingly counter-intuitive combination provides stable RE potentials across a wide range of solutions and reduces salt leaching, therefore highlighting the importance of understanding the underlying fundamental mechanisms to drive further development of this promising reference electrode type.

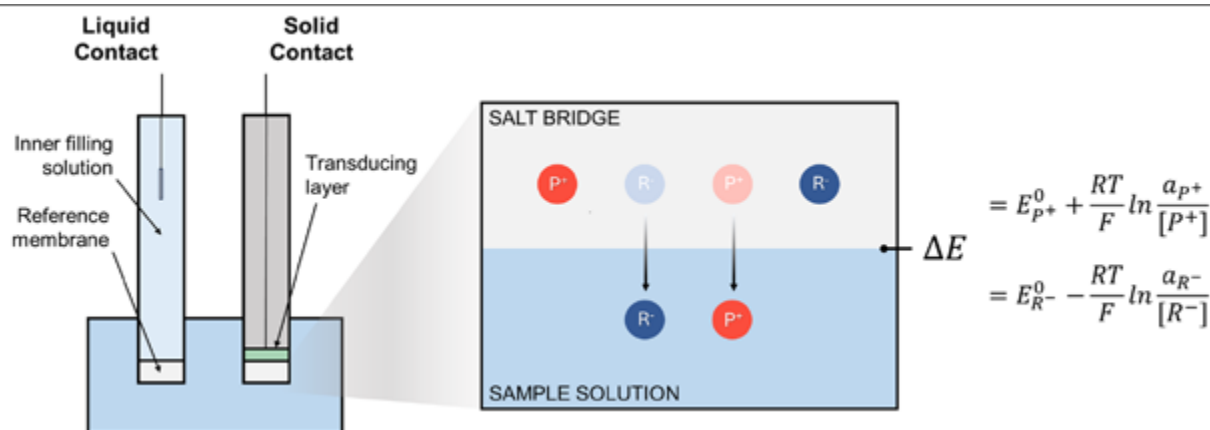


Fig. 1. Potential stabilization mechanism in REs based on an organic electrolyte P⁺R⁻.

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O-040

EVALUATING THE UNIFIED pH SCALE FOR ACCURATE AND SI-TRACEABLE MONITORING OF OCEAN ACIDIFICATION MONITORING

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Ocean acidification, driven by increasing anthropogenic CO₂ emissions, can alter seawater pH and consequently impact marine ecosystems and biodiversity [1]. pH is a master variable for monitoring ocean acidity, serving as an indicator of chemical changes occurring in seawater. Additionally, the ocean absorption of CO₂ accelerates acidification, posing significant threats to marine life and ecosystems [1]. Recognising the importance of monitoring these changes, the United Nations has included ocean acidification as a target within Sustainable Development Goal (SDG) 14.3, emphasising the need for scientific cooperation to minimise its impacts. Accurate and consistent pH measurement is essential for understanding these changes [2]. However, the complexity of seawater chemistry (ionic medium with high ionic strength) has led to the development of multiple pH scales—most notably, the total scale (pH_T), the seawater scale (pH_{sws}), and the free scale (pH_f), in contrast with the IUPAC pH scale (activity scale) [2,3]. Each scale defines pH based on specific ionic conditions and reference standards, the pH_f scale being the most widely used by oceanographers due to its accuracy and high reproducibility [3].

The adoption of the pH_f scale requires the use of high-quality buffers such as TRIS (tris(hydroxymethyl)aminomethane), with the pH characterized by Harned cell measurements [3]. These buffers are indispensable for calibrating pH sensors and thus ensuring precise pH measurements in marine environments. TRIS buffers, while traceable to the Harned Cell, lack traceability to the International System of Units (SI), are not easy to prepare and characterize, and are not commercially available.

To address this challenge, we outline the adoption of the new unified pH scale (pH_{abs}) as a promising approach for harmonizing pH measurements across different marine environments. This scale is based on the absolute standard chemical potential of the proton, defining the chemical potential of a proton in the gas phase (at 1 bar and 298.15 K) as 0 kJ·mol⁻¹, thus establishing a universal reference point (the zero point) that is the same for all media. This allows the acidity of any substance—whether gas, liquid, or solid—to be expressed on a single, consistent scale, making it especially useful for comparing acidities across different chemical environments [4]. Another advantage of this scale is its traceability to SI through IUPAC pH buffers, providing a consistent framework that improves comparability and accuracy in ocean acidification research [4]. The major drawback of this scale is the expanded uncertainty in pH measurement ($U_{\text{pHabs}} = 0.2$), which is attributed to the contribution of liquid junction potentials in seawater media [4].

Acknowledgements: This work was carried out at Instituto Português da Qualidade (IPQ), which provided the necessary infrastructure, resources, and metrological expertise for the pH measurements; their support is gratefully acknowledged.

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O-041

CHEMOMETRICS APPLIED TO THE STUDY OF NON-CANONICAL DNA STRUCTURES

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Apart from the well-known double-stranded structure, DNA may form other characteristic, non-canonical, structures, such as G-quadruplex, i-motif, triplex DNA, ... Recent studies on human genome have discovered DNA regions where these structures may be formed *in vivo*. Also, the i-motif structure has been shown to be a potential tool for the development of pH sensors in chemical analysis [Alba, 2016].

The *in vitro* study of the chemical and conformational equilibria, as well as the related kinetics aspects, of these structures is mainly carried out by using spectroscopic techniques, such as molecular absorption, circular dichroism, molecular fluorescence, or nuclear magnetic resonance. In this scenario, Chemometrics has been shown to be a useful in analyzing multivariate data recorded along the monitoring of these conformational changes or chemical equilibria.

In this presentation, a brief introduction to some of these structures will be given, paving the way to the explanation of a few examples of application of Chemometrics (hard and soft modelling, basically) to data sets of different nature.

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O-042

COMPARISON OF SPECTRAL DECONVOLUTION METHODS FOR RAPID DETERMINATION OF BETA EMITTERS BY LIQUID SCINTILLATION COUNTING

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Nuclear and radioactive facilities and NORM (Naturally Occurring Radioactive Material) industries, during their normal operation and, to a greater extent, during radiological emergencies, can release a large quantity and variety of radioisotopes into the environment (air, water, soil and biota) that need to be rapidly identified and quantified. In the decommissioning of nuclear facilities, where the number of samples to be processed is very high, or in the waste management of NORM industries, it is also necessary to characterize samples with a wide variety of isotopes and to optimize analysis times.

In the case of alpha and beta emitters present in environmental samples, building materials and waste, these isotopes must be isolated for their quantification. This radiochemical separation is sometimes not satisfactory or possible (e.g., isotopes of the same element: ⁸⁹Sr and ⁹⁰Sr), resulting in the spectra overlapping. This overlapping can be solved by applying mathematical deconvolution methods capable of adequately separating the spectra [1-5], which allows a faster identification and quantification of radioisotopes present in different matrices.

The aim of this work is to compare spectral deconvolution methods for the correct activity quantification of the radioisotopes of interest. All the techniques have been implemented in a Python open-source code, which is being developed at the Universitat Politècnica de València.

To verify the correct operation of the proven deconvolution techniques, a series of mixtures of beta emitters (natural and artificial isotopes), with known activity (0.5 to 50 Bq) and different proportions of each isotope, were prepared and measured using the 1220 QUANTULUS™ ultra-low background liquid scintillation spectrometer (PerkinElmer Inc.).

Since different measurement times can be used for each sample, the measurement data were first normalized to the measurement time. The spectrum corresponding to the blank was also subtracted from these measurements. Spectral filtering or smoothing was performed using various techniques based on Fourier series, Legendre polynomials, and Laguerre polynomials in order to eliminate the noise of the raw data extracted directly from the experimental measurements. For each isotope under study, a calibration curve was made, and the corresponding average spectrum was obtained. These parameters were used for analyzing the spectrum of the mixtures. Next, multivariate statistical calibration techniques [6] (e.g., Least Squares and Partial Least Squares regressions), have been used for predicting activities of 'unknown' samples from the spectra of the isotopes of interest with known activity. The reconstructed spectrum of the sample and the deconvolution of the spectra of each isotope were obtained too. The calculated activities were checked to ensure they are comparable with the reference values.

In view of the results obtained, different relative biases are observed in the calculation of the activities of each isotope; however, this difference is not so evident in terms of the reconstruction of the spectra. The reconstructions of the spectra provided by the three filtering techniques show almost identical spectra, with reconstruction errors less than $\pm 3.5\%$ for all cases, giving the Legendre function-PLS regression the best results. The calculated activities obtained with PLS regression fit better than those obtained with Least Squares regression, regardless of the filter used, with relative biases less than $\pm 15\%$ for all cases.

Key words: Deconvolution, discrete functions, regression techniques, radiological characterization, emergency protocols.

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O-043

A NOVEL THERMAL ANALYSIS METHOD COUPLED WITH SAMPLING THEORY FOR ANALYZING SAMPLE SIZE AND CROSS-CONTAMINATION IN PLASTIC RECYCLING

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A significant challenge in plastic recycling is the loss of quality in recycled materials. While recycling rates are often high, the proportion of high-quality plastic products manufactured from recycled materials is much lower. This discrepancy is largely due to cross-contamination¹, which occurs when different types of polymers are inadvertently mixed during the recycling process. Such mixing can result from inadequate separation during collection, processing, or sorting, as well as from process-related issues like insufficient cleaning or mechanical blending. Cross-contamination reduces the economic value of recycled plastics and limits their potential applications.

In the final stages of recycling, it is crucial to analyze washed flakes or granules for cross-contamination levels to prevent further quality loss. This analysis helps identify suitable applications for these recyclates as raw materials for new plastic products. At this stage, the emphasis shifts from speed to more detailed and accurate testing. Thermal analysis techniques, particularly Differential Scanning Calorimetry (DSC)², are well-suited to identifying the composition of polymer blends. However, conventional DSC analysis typically uses small sample sizes of just a few milligrams, which may fail to represent the variability within large-scale material streams where tons of plastic are processed.

This work presents a data analysis framework for assessing the composition and cross-contamination levels of plastic recyclate batches, while also determining the minimum sample size required to meet industry demands. By applying MADSCAN®, a scale-free thermal analysis technique, to particulate plastic samples for the first time, we overcome the limitations of conventional DSC methods that rely on milligram-scale samples. Since polyolefins are among the most abundant plastics³, this study specifically focuses on quantifying cross-contamination of High-Density Polyethylene (HDPE) with polypropylene (PP) impurities, as well as Linear Low-Density Polyethylene (LLDPE) with Low-Density Polyethylene (LDPE).

The data analysis was conducted using the Partial Least Squares (PLS) method³. The proposed PLS model was validated using external samples from different industrial plastic waste streams, ensuring its applicability to real-world conditions. Additionally, the framework integrates both analytical and sampling error estimates, using error propagation techniques and Theory of Sampling (TOS)⁴ principles, to determine the minimum representative sample size necessary to achieve the industry-allowable maximum total error of 5%.

The minimum sample size for each lot was calculated based on the 5% total error limit, incorporating both analytical and sampling errors. The analytical errors estimated from PLS analysis were used to calculate the maximum acceptable sampling errors, enabling the determination of the minimum sample size based on TOS. As illustrated in Figure 1, the minimum sample size increases exponentially as the maximum allowable total uncertainty decreases. For example,

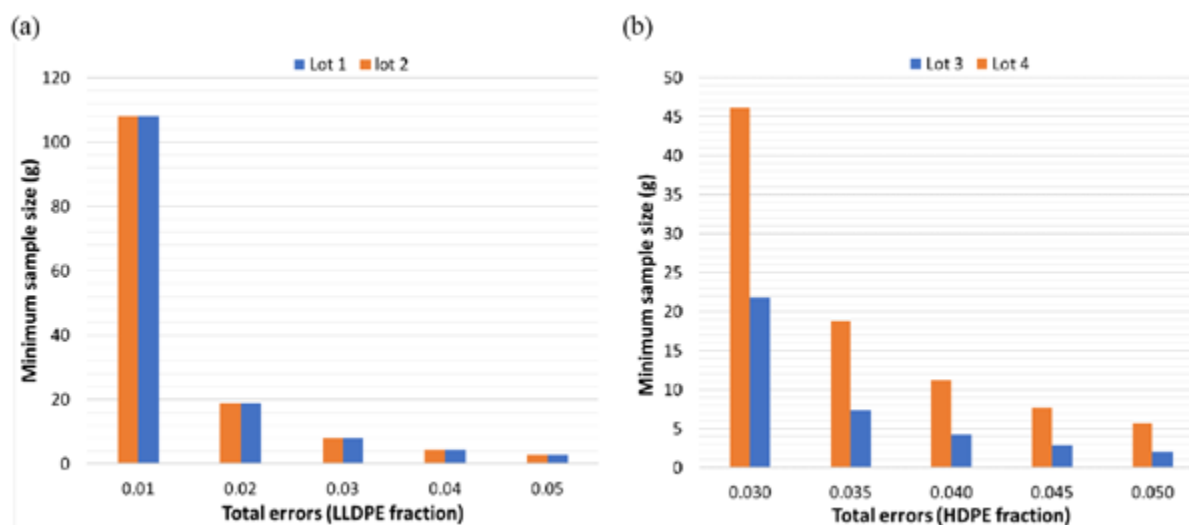


Figure 1. Minimum sample sizes needed to achieve specified levels of total uncertainty for (a) lots 1 and 2, and for (b) lots 3 and 4.

achieving a total error of 0.01 for LLDPE measurements requires a sample size of 108 g, while achieving a total error of 0.03 for HDPE measurements from lot 4 requires a sample size of 46 g. These results highlight that analyzing only a few milligrams of material is insufficient to meet industry demands or regulatory requirements when working with macro mixes or polymer flakes.

The proposed framework not only translates validated sample size measurements into evidence-based decisions but also advances the state-of-the-art in quality control for plastic recycling. This approach bridges theoretical sampling principles with practical analytical challenges, thereby enhancing both the quality and economic value of recycled plastics and contributing to the circular economy.

Plastic recycling often suffers from quality loss due to cross-contamination between polymers. This study introduces a new method combining thermal analysis (MADSCAN®) with sampling theory to accurately assess contamination and determine the minimum sample size needed for reliable analysis. Focusing on common plastics like HDPE and LLDPE, the approach shows that traditional small-sample methods are inadequate. The framework supports better quality control and strengthens the circular economy by improving recycled plastic usability.

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O-044**COMPREHENSIVE DATA ANALYSIS USING REGIONS OF INTEREST MULTIVARIATE CURVE RESOLUTION FOR ION MOBILITY MASS SPECTROMETRY: ANALYSIS OF PLASTIC ADDITIVES****Torres-Agullo, Ana¹; Tauler, Romà¹; Lacorte, Silvia¹; Simón-Manso, Yamil².**

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Ion mobility (IM) enhances liquid chromatography-mass spectrometry identification capabilities by providing an additional dimension that improves mass coverage, sensitivity, and resolving power, and it is particularly valuable for untargeted analysis. However, the high dimensionality and large datasets of IM analysis is a big challenge for data processing, enabling its broader application and its potential. The regions of interest multivariate curve resolution (ROIMCR) approach is a chemometric method based on the bilinear model intrinsic data structure of the data and represents an effective strategy for effective feature extraction, deconvolution, and resolution of overlapping signals in complex datasets without losing relevant information and maintaining instrumental mass accuracy [1]. This procedure has already been successfully applied in different research studies including LC-MS [2], GC-MS [3], or LCxLC [4]. In this work, the application of ROIMCR method is shown for the first time for liquid chromatography ion mobility mass spectrometry in data-dependent acquisition (DDA), including MS1 and MS2 datasets for the analysis of plastic additives. We show an improvement in the discrimination of isomeric species and minimization of interferences, leading to more reliable profiles. IM-MS data from 5 standards and 3 samples were simultaneously analysed, resolved and collision cross section (CCS) values were calculated for each component. The methodology allowed the direct link between the IM signals and the identification of 15 different plastic additives including phthalates, siloxanes, or phosphates present in the original polymers. Our findings demonstrate that ROIMCR provides an effective and scalable solution for extracting meaningful information from complex IM datasets.

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O-045

FAST AND NON-INVASIVE COFFEE CLASSIFICATION, CHARACTERIZATION AND AUTHENTICATION USING NEAR-INFRARED HYPERSPECTRAL IMAGING (NIR-HSI) AND CHEMOMETRICS. APPLICATION TO THE DETECTION AND QUANTITATION OF ADULTERATED COFFEE SAMPLES

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The global rise in food adulteration, particularly in coffee, has become a critical issue compromising product integrity, consumer trust, and compliance with food safety regulations. This study introduces a novel, rapid, and non-invasive method for the authentication of coffee samples, utilizing non-targeted Near-Infrared Hyperspectral Imaging (NIR-HSI) combined with chemometric analysis. This approach efficiently classifies, characterizes, and authenticates coffee, as well as detects and quantifies adulterants without needing sample preparation or chemical reagents. Notably, the run time of NIR-HSI is under 2 minutes per sample, making this method ideal for high-throughput applications.

A total of 115 samples were analyzed, including regular coffee, soluble coffee (both decaffeinated and non-decaffeinated), and chicory (a potential coffee adulterant). The hyperspectral data were processed using Partial Least Squares-Discriminant Analysis (PLS-DA) to distinguish between sample types: Coffee vs. Chicory, Regular Coffee vs. Soluble Coffee, Soluble Decaffeinated Coffee vs. Soluble Non-Decaffeinated Coffee, as well as to differentiate coffee varieties: Arabica vs. Robusta, and to distinguish between geographical origins: Vietnam, Cambodia, and Costa Rica.

The feasibility of the proposed methodology for classifying and predicting coffee samples based on type, geographical origin, and variety was assessed by paired PLS-DA. Each paired model compared a single sample class against all others, with a calibration set consisting of 70% randomly selected samples from each group and the remaining 30% used for prediction. Results were excellent, with 100% sensitivity and specificity values for all examined cases.

Additionally, Partial Least Squares (PLS) regression was applied to adulterated coffee samples to evaluate the ability of the proposed methodology to quantify adulteration levels. Two distinct adulteration scenarios were examined: Vietnamese Arabica coffee adulterated with chicory, and Vietnamese Robusta coffee adulterated with chicory. These results highlight the effectiveness of the proposed approach in both authenticating coffee and detecting and quantifying coffee adulteration, demonstrating its potential to be used in quality control and fraud prevention within the coffee industry.

Keywords: NIR-HSI; Coffee Authentication; Fast Analysis; Food Fraud Detection; Chemometrics; Non-Invasive Analysis; Quality Control.

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O-046**OPTIMIZATION OF AN LC-MS ANALYTICAL METHOD FOR THE DETERMINATION OF MULTI-CLASS POLAR EMERGING CONTAMINANTS IN RECLAIMED WATER****Pellacani, Samuele; Battisti, Marco; Pietropaolo, Mattia; Benedetti, Barbara; Durante, Caterina; Cocchi, Marina; Strani, Lorenzo.***University of Modena and Reggio Emilia, Modena, Italy.*

Food and agriculture account for most of the human demand for water, due to the huge amounts required for crops and livestock. Within the whole agricultural, municipal, industrial and energy sectors, 70% of water is used for cultures and farming. The current available water resources need an aware management to meet the growing global demand for food [1]. Thus, the possible use of reclaimed water for irrigation represents a great opportunity for water conservation and fits in the circular economy approach.

In this study, the optimization of an analytical method based on liquid chromatography-mass spectrometry (LC-MS) was carried out for the determination of several Persistent and Mobile Organic Contaminants (PMOC) in wastewater. The contaminants encompassed various classes including pharmaceuticals, personal care products, and other emerging pollutants.

Due to the high polarity of the analytes under study, hydrophilic interaction liquid chromatography (HILIC) was employed, by testing a zwitterionic stationary phase. To enhance the efficiency of the analysis, response surface methodology (RSM) utilizing a Fractional Factorial Design [2] was employed to optimize the experimental parameters. Indeed, multiple factors influence the chromatographic separation in HILIC, such as initial mobile phase percentage, gradient time, column temperature, type and concentration of salt and flow rate, which were investigated to achieve the best separation and sensitivity for the target analytes. Finally, the optimized method was combined to a suitable sample pre-treatment and applied to different reclaimed water samples. Thus, the method performance could be evaluated in real matrices and the presence of the considered contaminants in treated water was investigated.

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Project Title: EXPloitation of targeted and untargeted analytical strategies for WASTEwater monitoring: toward a sustainable water management according to the principles of circular economy- EXWASTER. Prot. n° 2022L3AH34. Principal Investigator: C. Durante.

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O-047

TOWARDS AN EPIDERMAL BIOSENSING PLATFORM FOR NON-INVASIVE DIABETES MANAGEMENT

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Blood self-testing has paved the way for the use of electrochemical sensors in our everyday life. Nonetheless, the spreading of continuous glucose monitoring devices and the intensive scientific research on minimally invasive technologies shed light on the pivotal role of comfort and handiness, besides accuracy, when it comes to the daily management of a chronic disease like diabetes. Among the different biosensing architectures, epidermal wearable biosensors adapt to various body parts, can be manufactured on several flexible substrates and are attracting interest for the real-time monitoring of physical and chemical parameters, including sweat and interstitial fluid composition (1, 2).

In this contribution, the development of amperometric and transistor-based biosensors is presented with the future scope of realizing epidermal glucose sensing (3). The devices were fabricated on flexible polyethylene naphthalate and polyethylene terephthalate foils or parylene films by ink-jet printing and are based on the organic semiconductor poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS). Functionalization of the sensing interface and transduction mechanism were characterised in detail and the performance of the sensing structures was analysed along with the miniaturization process. Moreover, particular attention was paid to the printing strategies developed to improve the sensors' robustness and figures of merit. Research on the detection of other biomarkers relevant to diabetes monitoring and on the technological shift to tattoo-like substrates is in progress, with the ambition to design a multiparametric epidermal platform.

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O-048

INVESTIGATING MICROPLASTICS THROUGH ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY: AN ANALYTICAL METHOD FOR THEIR LABEL-FREE ANALYSIS

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Molecular adsorption at immiscible interfaces is known to trigger up-concentration, leading to different chemical reactivity and interaction with solvents [1]. This process is not only fundamental for understanding life-mimicking artificial systems but also has important consequences in real-world contexts, such as the behavior of microplastics (MPs) in

natural environments. MPs can be defined as fragments of any type of plastic smaller than 5 mm that can exert toxic effects on humans for several reasons, especially due to the adsorption of toxic substances from the environment. In addition to the urgent need for rapid in situ identification and quantification that reduces the time needed compared to traditional laboratory methods, nowadays there is a growing demand for new methods to quickly assess whether MPs can absorb pollutants, especially metals cations [2]. For instance this phenomenon, mainly driven by electrostatic interactions, occurs at MPs' interfaces. To this aim, we have recently shown how electrochemical impedance spectroscopy (EIS) can quantify MPs in the absence of electrochemical mediators [3], resulting in LOD down to 0.07 % w/v. Unlike previous approaches, this method allows differentiation between virgin and lead-ions polluted MPs, by shedding light on the mechanisms involved in the electrostatic-driven pollutant adsorption at the MP surface. Our approach is based on an equivalent electrical circuit in which a constant phase element (CPE) is placed in series with the double-layer resistance (R_{dl}) and the Warburg impedance (W) modelling the electrolyte diffusion process in parallel with the double-layer capacitance (C_{dl}). The concentration of dispersed MPs is inversely proportional to the R_{dl} value. Following a simple calculation from the equivalent circuit model (Figure 1a), the R_{dl} value is chosen for MPs quantification purposes and is found to depend on the pollutant adsorbed at the MPs surface, in particular for polystyrene beads, as shown in Figure 1b. These findings are verified by independent ζ -potential analyses. Herein, preliminary results show that this method can be extended to other types of MPs of natural or artificial origin, such as cellulose-derived or polyfluoroalkylated substances, using a methodology purely based on EIS data without the need for any external electrical potential to trigger faradic responses in the cell [4]. This method permits the rapid quantification of MPs and the differentiation between polluted and virgin MPs, by implementing a simple post-analysis data treatment based on EIS circuit model fitting to obtain the R_{dl} value.

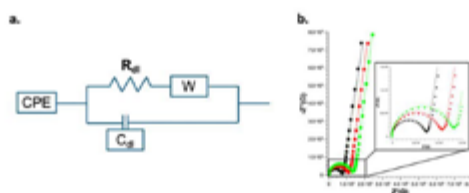


Figure 1. a. Equivalent electrical circuit used to fit the EIS data. b. Nyquist plots of polystyrene dispersed in water pre- (black dots) and post-Pb²⁺ ions adsorption at two different q_s values, namely 480 $\mu\text{g}\cdot\text{g}^{-1}$ (red dots) and 780 $\mu\text{g}\cdot\text{g}^{-1}$ (green dots).

Acknowledgments

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O-049

SMART FUNCTIONALIZATION FOR PAPER MICROFLUIDICS BY SI-PET-RAFT POLYMERIZATION

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Introduction. Microfluidic paper-based devices (μ PADs) are promising candidates for sensing platforms that can be applied outside a laboratory setting for a wide range of analytes in different matrices, since they allow passive, capillary-action-driven flow, and flow control by manipulating the paper properties. Their use, however, is limited due to difficulty to integrate advanced functionality in such devices. Here, we propose the modification of cellulose fibers in paper with stimuli-responsive polymer brushes to introduce functionalities that can be controlled by varying the chemical environmental. We grafted ionic strength-sensitive zwitterionic poly(carboxybetaine methacrylamide) brushes (Fig.1) from paper surfaces by using surface-initiated photoinduced electron transfer reversible addition-fragmentation chain transfer (SI-PET-RAFT) polymerization. This method allows growing brushes in a well-controlled fashion in an oxygen-tolerant environment [1].

Experimental. The modified papers were tested for their ability to adsorb (capture) negatively charged analytes, using bromophenol blue (BpB) dye as a model in comparison with unmodified (bare) paper. To enable the capture of BpB, papers were simply immersed in the dye solution and subsequently dried in a vacuum oven. For desorption (release) of dye, papers were submerged in water or salt solutions (Fig.2). Dye concentrations in solutions were measured with a UV-vis absorption spectrophotometer at 590 nm.

Results. Desorption studies showed that dye release occurred only in the presence of NaCl salt, as it changes the polymer structure through charge-screening (Fig.2). Optimization of the capture/ release behavior were investigated with variations in the adsorption time, initial dye concentration and [NaCl] in the desorption solution. Extended adsorption times resulted in more dye captured and released. Recovery from the desorption solution was found higher for longer adsorption time, which was related to the similar amount of dye retained in paper after desorption regardless the amount captured. Decreasing dye concentrations led to less adsorption, though dye recoveries remained similar at $83 \pm 2\%$ down to 3 ppm, below which it dropped to 60%. Dye release was assessed in varying [NaCl], where it remained similar between 1 - 0.05 M, declined below 0.01 M. Modified papers were also used for sample enrichment in flow mode. Besides BpB (Fig.3-I), 1 ppm aqueous nitrite (NO_2^-) solution was allowed to flow through modified paper to be captured and enriched, which was followed by the Griess reaction by dropping the corresponding reagents on paper in order to obtain color change (colorless to pink) for detection, resulting in more sensitive detection in comparison with direct loading of the sample on bare paper (Fig.3-II).

Conclusion. In this study, we used SI-PET-RAFT polymerization for the first time on cellulose paper, which highlights the novelty of this approach, marking a significant advancement in the field as it allows sample-content driven analyte capture and controlled release.

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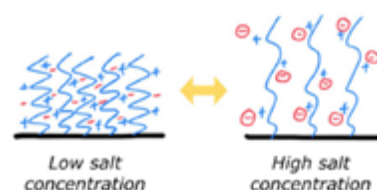


Figure 1. Ionic strength response

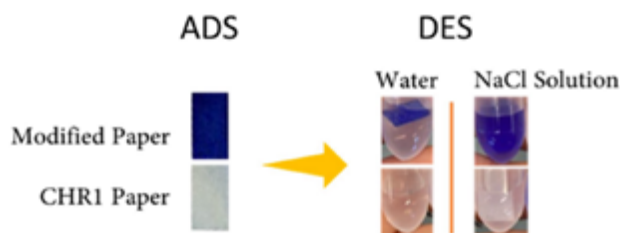


Figure 2. Selective dye capture and controlled release

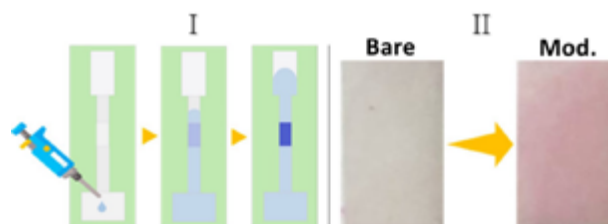


Figure 3. Enrichment of (I) BpB and (II) color detection after NO_2^- enrichment

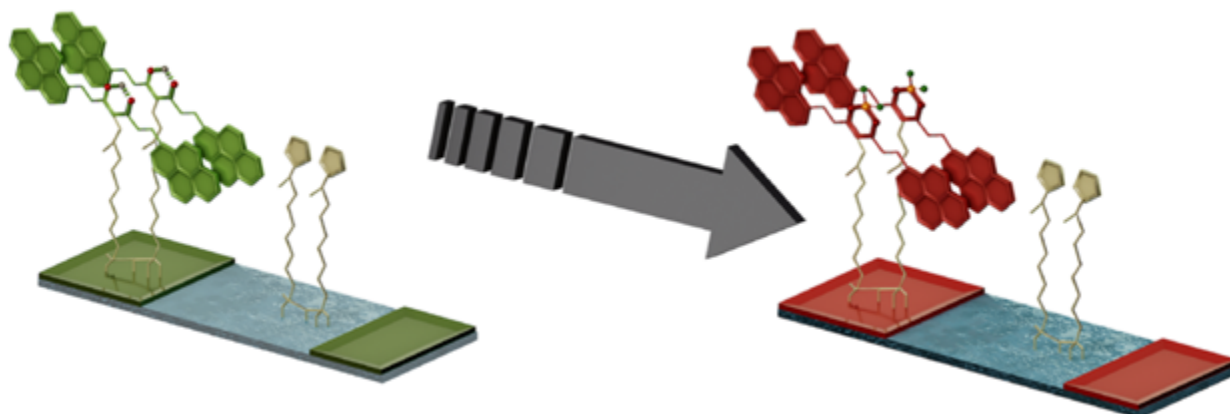
O-050

CURCUMINOID-BASED RESPONSIVE SUBSTRATES FOR THE DEVELOPMENT OF SENSORS

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The rising need for portable in-situ sensing devices emphasizes the significance of chemical sensors in advanced applications like environmental monitoring, food safety, and biosecurity. In this way, fluorescence (and colorimetric) sensors has provided a wide variety of easy-to-handle designs, developing new options for a reversible detection of analytes both in solution and in the vapor phase. Advances in molecular design have involved the preparation of multifunctional molecular materials, including the synthesis of bifunctional probes or heterogeneous sensors ^[1]. Although most of the new fluorescent probes are used in solution for their applicability in industry, it is necessary their loading in matrixes or immobilization on substrates. Here, knowing the great chemical versatility of Curcuminoids (CCMoids) and the possibility of modulating their fluorescent properties depending on the type of analyte used, ^[2] new fluorescent T-shape CCMoids and their immobilization on rigid and flexible substrates has been explored. Therefore, here we present the preparation and response of CCMoid-based active surfaces, depending on the metal or metalloid coordinated upon coordination, together with the reusability of the substrate by releasing the analyte. In this way, an increase, decrease or even a switch of the surface fluorescence signal depending on the metal/metalloid under study has been obtained in solution and gas phase, ^[3] Figure 1.



Acknowledgments

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O-051

MOLTEN SALT ACTIVATED SEWAGE SLUDGE BIOCHAR: CHARACTERIZATION AND MODIFIED ELECTRODES FOR THE ANALYSIS OF SUNSCREEN UV-FILTERS

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The transformation of sewage sludge into biochar presents a dual advantage by addressing waste management challenges while contributing to the development of sustainable energy systems [1]. In parallel, the persistent presence of organic UV filters (OUVFs) in aquatic environments has become a global concern. These anthropogenic compounds, widely used in personal care products, are resistant to degradation in wastewater treatment plants and may undergo harmful transformations, posing potential ecological risks [2].

In this study, biochar samples were synthesised via pyrolysis at a peak temperature of 800 °C in a quartz reactor under a continuous nitrogen flow, both in the absence and presence of a fivefold excess of Na₂CO₃/K₂CO₃ (1:1 w/w) molten salt, a green additive used to enhance the carbon structure. The resulting biochars were purified by treatment with aqueous HCl, followed by drying at 70 °C. The prepared biochar was then used to fabricate carbon paste electrodes. Morphological properties of the biochars were investigated using transmission electron microscopy (TEM) and Brunauer–Emmett–Teller (BET) surface area analysis.

Electrochemical assessments using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) demonstrated that the electrode made of molten salt-modified biochar exhibited superior catalytic activity, enhanced stability, and a higher oxygen evolution potential. As a case study, the electrode was applied for the determination of OUVFs, such as benzophenone-3 (BP-3), among the most commonly detected OUVFs in aquatic environments. Monitoring and quantifying this contaminant can provide valuable insight into the effectiveness of water treatment strategies and environmental exposure risks.

This approach not only offers a sustainable route for the valorisation of sewage sludge but also provides a cost-effective, high-performance alternative to conventional electrocatalysts. The findings align with circular economic principles by transforming waste into valuable functional materials with applications in advanced water treatment.

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Keywords: Biochar; Electrocatalytic properties; Sewage sludge. Electrochemical sensor

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O-052**POTENTIOMETRIC MULTISENSOR AS AN INEXPENSIVE AND RELIABLE TOOL FOR ELECTROLYTE LEVEL ANALYSIS IN BODY FLUIDS****Lenar, Nikola; Niemiec, Barbara; Piech, Robert; Paczosa-Bator, Beata.***AGH University of Krakow, Krakow, Poland.*

Nowadays, due to the dynamic socioeconomic development, greater attention is being paid to the protection of the environment and human health. At the same time, the requirements for analytical methods used to assess patient health, environmental conditions, and quality of products are increasing [1]. There is a constant search for methods characterized by a short waiting time for the analysis result, accuracy, and reliability, while at the same time generating low costs of performing a single analysis. An analytical technique that meets these requirements is potentiometry, within which a group of potentiometric sensors has been developed [2,3]. Undoubtedly, this is due to the fact that potentiometric sensors are characterized by very good analytical parameters, and at the same time, they are cheap and easy to use, which distinguishes them from other analytical tools. Potentiometric sensor is defined as electrochemical sensor that transforms the effect of the electrochemical interaction between ion and membrane into a signal [2]. The key element of the sensor is ion selective membrane, that is responsible for selective recognition of certain ion in analysed solution. The idea of this work was to combine several membranes into one sensor to ensure the possibility of simultaneous analysis of several different ions in saliva, sweat, blood serum and urine.

The body's fluids are primarily composed of water, which in turn contains electrolytes such as sodium, potassium, magnesium and chloride ions. Electrolytes play an important role in the body; they regulate the osmotic pressure in cells and help maintain the function of muscle and nerve cells. If electrolyte levels are too low or too high, cell and organ functions will decline, which could lead to life-threatening conditions [4]. Hence, the selective, accurate and precise recognition of mentioned ion content is crucial.

In this work we propose an inexpensive, easy and highly available solution for electrolyte level determination: potentiometric multisensor with four different membranes, selective to potassium, sodium, calcium and chloride ions. A new sensor body, a multiplatform, was designed, that allowed for simultaneous placement of a few different membranes onto one sensor. The electrode corpus was made of polyether ether ketone (PEEK) with four through holes drilled in it. Matching stainless steel rods were placed in the holes, leaving room for the conductive paste. In this work carbon black nanoparticles were used as a paste to ensure the charge transfer, because of its high electrical capacity and conductivity.

The research has shown that multisensor allows for K^+ , Na^+ , Ca^{2+} and Cl^- ions recognition in the concentration range of 10^{-5} to 10^{-1} M, with the satisfying potential stability (0.05 mV/h). Analysed ion content in saliva, sweat and blood serum was accurate and precise, what proves that designed multisensor is a reliable tool for body fluid analysis. What should be emphasized is that potentiometric multisensor exhibit wide linear range of ions concentrations, sufficient potential stability, and, at the same time, is small, easy to use and cheap to prepare and maintain. Proposed multisensor is universal and can be modified both in terms of analysed ions (other ions can be determined by applying suitable membranes) and the paste used as a filling.

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O-053

DEVELOPMENT OF AN ANALYTICAL METHOD TO STUDY VOLATILE ORGANIC COMPOUNDS IN SPANISH TREE LEAVES USING HS-SPME-GC-QTOF-MS COMBINED WITH MULTIVARIATE ANALYSIS

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A novel miniaturized analytical method has been optimized to study the biogenic volatile organic compounds (BVOCs) profile from tree leaves using headspace solid-phase microextraction gas chromatography-quadrupole time-of-flight spectrometry (HS-SPME-GC-QTOF-MS). Several parameters were tested, and a 50/30 μm DVB/CAR/PDMS SPME fiber, 0.20 g of sample, extraction and desorption times of 45 and 3 minutes, respectively, were selected as the optimal conditions. Subsequently, 42 samples from Ávila (Spain) were evaluated including conifers (*Pinus sylvestris* and *Juniperus oxycedrus*) and broadleaf species (*Quercus ilex* and *Quercus pyrenaica*). More than 100 different BVOCs were tentatively identified with a matching degree >85% in the NIST library. Total compounds were categorized into 12 main groups, where sesquiterpenoids, hydrocarbons, and alcohols were the dominant family groups. Moreover, a variety of chemometric tools were applied to enable data differentiation and potential classification of samples. Principal component analysis revealed that the BVOCs produced by different tree species exhibit distinct profiles, forming three well-defined clusters, while hierarchical cluster analysis indicated that the composition is influenced by the season, and sampling height. Finally, the method was classified as environmentally friendly according to the green analytical metrics and evaluated as practical by the blue applicability grade index.

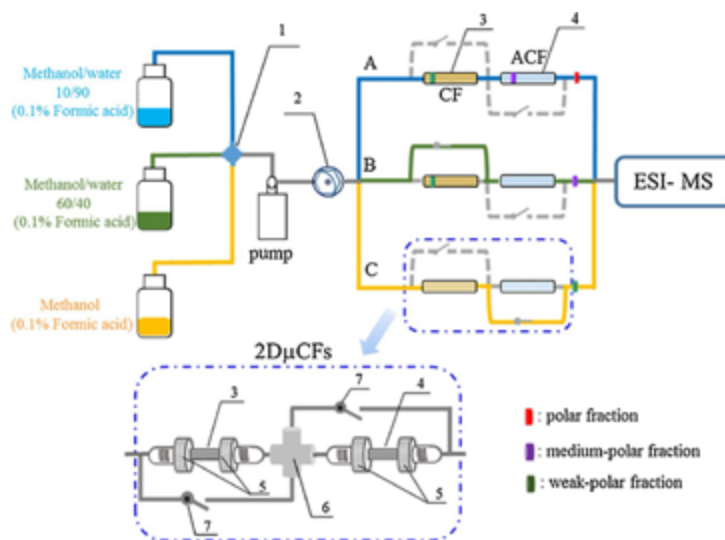
O-054

BRIDGING THE GAP BETWEEN HPLC AND DIRECT MS: A NOVEL 2D μ CFs SYSTEM FOR HIGH-THROUGHPUT AND SOLVENT-SAVING COMPLEX SAMPLES FRACTIONATION

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A novel high-throughput, solvent-saving and versatile integrated two-dimensional microscale carbon fiber/active carbon fiber system (2D μ CFs) that allows a simple and rapid separation of compounds in low-polar, medium-polar and high-polar fractions has been realized thanks to a collaboration between the Yanbian University (China) and the University of Foggia (Italy). The device has been characterized and fruitfully exploited for the screening and quantitative analyses of real samples [1]. The 2D μ CFs device is constituted by a two-dimensional system with two small columns with carbon and active carbon μ -fibers as stationary phases (Fig. 1). The adsorbing properties of these fibers have been conveniently used to create a device



able to perform a fast (< 7 min) and low-pressure separation of complex extract samples into three fractions, on the base of compound polarities. This device can be intended as an interesting compromise between the classical chromatographic separation and the direct MS approach, combining their advantages, in terms of analysis time, choice of chromatographic columns, pretreatment procedure customization, interference reduction, and ionization suppression effects. The 2D μ CFs device fills the gap between HPLC and Direct MS, giving a concrete answer to the urgent need for reliable and high-throughput screening and/or quantification methods for compound determination in real samples, ranging from low to high contents and from low to high polarities. It has been demonstrated that analysis time of 2D μ CFs–ESI–MS is considered comparable with direct MS methods but the number of detected compounds, due to the significant reduction of both background noise and ion suppression effect that expands the range of explored polarities and increases the sensitivity, is similar to those obtained with classical chromatographic methods. Numerous applications with complex real samples have been studied and published in the last year [2–5], and new research is currently underway in our laboratories.

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O-055**SORBENT PREPARATION USING ECO-FRIENDLY AND WASTE-BASED SUPPORTS: A STEP FORWARD IN SAMPLE PREPARATION**

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In the last decades, analytical scientists have been striving to develop sustainable and environmentally friendly methods for the extraction and determination of relevant/trace compounds, aligning with the principles of Green Analytical Chemistry. A clear example of this trend is the increasing focus on efficient sorbents derived from natural materials, particularly biopolymers such as cellulose, chitosan, and alginate, as well as biowaste like coffee residues and fruit peels, which have been successfully used as sorbents [1]. However, another critical waste source—packaging waste—has become a growing concern in today's world. Two of the most common types of packaging waste in the EU in 2022 were plastics, accounting for 19% of total waste, and metals (mainly tinplate and aluminum), which made up 5% [2]. While these waste materials can be recycled, plastic recycling remains particularly challenging—according to the OECD Global Plastic Outlook report (2022) [3], only 9% of global plastic waste is effectively recycled. Alternatively, these materials can be properly treated or chemically converted into high-value functional materials, such as adsorbents [4, 5]. Despite these efforts, research in this area is still emerging, and substantial progress is required to develop highly effective sorbents for sample treatment.

In this communication, we will discuss examples of eco-friendly supports, such as cellulose- or lignocellulose-based substrates, for the preparation of efficient and selective sorbents, as well as their use as fillers in the development of 3D-printed biocomposite filaments. Additionally, several strategies and case studies will be presented on the upcycling of packaging plastics (such as high-density polyethylene (HDPE) and polyethylene terephthalate (PET)) as well as aluminum cans, to develop devices incorporating valuable functional materials (e.g., metal-organic frameworks, aptamers, among others) for sample preparation applications.

Acknowledgements

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O-056**CUTTING EDGE ADVANCES TOWARD GREENER ANALYTICAL X-ray FLUORESCENCE SPECTROMETRY METHODS: A FOCUS ON SAMPLE TREATMENT****Marguí, Eva¹; Pena-Pereira, Francisco²; Queralt, Ignasi³.***¹University of Girona, Girona, Spain; ²University of Vigo, Vigo, Spain; ³IDAEA-CSIC, Barcelona, Spain.*

The truly multi-elemental character, acceptable speed and economy, ease of automation and the possibility to directly analyse solid samples (without a previous acid digestion) are the most important features among the many that have made X-ray fluorescence spectrometry (XRF) a very mature analytical tool in diverse scenarios [1].

Technological developments, including the design of X-ray miniaturized sources, the production of low-power micro-focus tubes, the novel X-ray optical devices and the improvement in signal detection systems have promoted the commercialization of benchtop and portable XRF instrumentation. These systems offer extreme simplicity of operation in a low-cost design and are attractive for both environmental and industrial fields.

One of the major limitations of XRF limiting a most frequent use in analytical chemical research has been the limited sensitivity for trace element determination. Direct XRF analysis of solutions entails also difficulties attributed to the reproducibility problems and the high spectrum background. Typical limits of detection for this type of samples are in the mg L⁻¹ range, which are unsatisfactory for most analytical applications [2].

In the last years, a great effort has been done in the design of novel “smart materials” (i.e., nanomaterials, ionic liquids, polymer inclusion membranes, surfactant-based materials, among others) and in the field of sample treatment research. Despite of the wide application of such materials for preconcentration purposes before chromatographic or atomic spectroscopic analysis, their potential in combination with XRF spectrometry has been less explored. This is undoubtedly a field of worth investigation which can open new interesting applications of XRF in the field of water analysis. For instance, the possibility to analyse directly the resulting preconcentrated sample avoids the use of an additional elution step which lead to a simplification of the whole sample treatment procedure and a considerable reduction of reagents and wastes [3]. Moreover, if the preconcentration procedure is simple and rapid enough (i.e. filtration procedure using an adequate extraction material) it can be even used for in-situ measurements in the field, in view of the availability of portable XRF instrumentation.

In this contribution, several application examples including the determination of inorganic species (i.e., Cr (III)/Cr(VI), Se (IV)/Se(VI), As (III)/As(IV)) as well as the detection and quantification of relevant metals and metalloids in water samples will be presented. Most of the proposed methods are based on miniaturized sample treatment procedures (i.e., liquid-based and sorbent based microextraction) or can be implemented in-situ and thus are in line with the green alliance priorities and the sustainable development goals. To measure the degree of compliance of these methods with the Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC) concepts, different metric tools have been applied including the Analytical greenness metric for sample preparation (AGREEprep) and the Red-Green-Blue (RGB) model.

Our findings pointed that the use of simple, effective and cutting-edge sample treatment methods can enlarge the applicability of this relatively low-cost technique and its acceptance by the analytical community as a real quantitative spectroscopic technique.

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O-057**LAB-IN-SYRINGE AUTOMATED DISPERSIVE MICRO SOLID-PHASE EXTRACTION METHOD BASED ON *IN SITU* FORMED LDHs FOR DETERMINATION OF ANGIOTENSIN RECEPTOR BLOCKERS IN ENVIRONMENTAL WATERS****Yıldırım, Sercan¹; Çevik, Hilal Rabia²; Özyiğit, Tuğçe¹; Kocaoğlu, Arzu¹; Horstkotte, Burkhard³.**

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Pharmaceutical residues in environmental waters continue to raise significant public and ecological concerns. Angiotensin receptor blockers (ARBs) are increasingly present in environmental waters due to massive human consumption, inadequate metabolism, and ineffective removal via wastewater treatment plants [1]. Due to their persistence, toxicity, and tendency toward bioaccumulation, trace-level detection of ARBs is crucial for environmental safety and public health. Nonetheless, efficient sample clean-up/preconcentration techniques are needed to determine low environmental concentrations of ARBs in complex matrices.

Dispersive micro solid-phase extraction (DMSPE) is a widely used technique in environmental analysis due to its simplicity, rapidity, and high preconcentration efficiency for trace-level contaminants [2]. In DMSPE, layered double hydroxides (LDHs) can be employed as effective sorbents thanks to their high surface area, tunable composition, and ion-exchange capacity [3]. LDHs are two-dimensional nanostructured materials composed of positively charged metal hydroxide layers with intercalated anions and water molecules, enabling strong interactions with target analytes. Lab-In-Syringe (LIS) is an analytical automation platform that integrates batch and flow techniques within a computer-controlled syringe pump, allowing precise reagent handling, in-syringe mixing via magnetic stirring, and coupling to instrumental systems such as HPLC for fully automated sample preparation and analysis [4].

In this work, a novel LIS-DMSPE method was developed to determine three ARBs in water samples. The approach was based on the *in situ* synthesis of Ni/Fe-LDHs within the void of an automatic syringe pump through controlled pH adjustment following the aspiration of precursor solutions. This enabled the rapid formation of the adsorbent and eliminated the need for pre-synthesized or magnetized sorbents. Enhanced sedimentation speed, achieved by increasing ionic strength with NaNO₃, allowed isolating the sedimented sorbent from the sample matrix without centrifugation and filtration.

After washing procedures, the LDHs were dissolved using a formic acid:methanol mixture, followed by transfer of the extract to the online coupled HPLC-DAD system. The low organic solvent content of the extract allowed large-volume injection (200 µL), which enhanced sensitivity. Systematic optimization of critical method parameters such as pH, amounts of precursors and buffers, ionic strength, stirring speed, and elution conditions was carried out. Among seven LDHs tested, Ni/Fe-based LDH displayed the optimal balance between extraction efficiency, rapid sedimentation, and easy dissolution. The method provided acceptable linearity (5–100 µg L⁻¹), recoveries (62.0–88.7%), as well as enrichment factors (13.3–25.8). The limit of detection values were between 1–1.5 µg L⁻¹ with precision (RSD < 3.5%) and accuracy (94.1–104.8%) obtained by standard addition in real water samples. To our knowledge, this is the first report on a LIS-DMSPE method that does not require pre-synthesis or magnetization of the sorbent for extraction and uses accelerated sedimentation for adsorbent isolation.

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O-058**SUPERCRITICAL FLUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM OLIVE PRUNING WASTE AND THEIR CHARACTERIZATION BY MEANS OF HYPHENATED LIQUID CHROMATOGRAPHIC TECHNIQUES****Cafeo, Giovanna¹; Russo, Marina¹; Arena, Alessia²; Zoccali, Mariosimone³; Mondello, Luigi⁴; Dugo, Paola⁴.**

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Agricultural and food wastes are included in the biomass, providing a natural source for the production of energy and bioactive compounds. *Olea europaea* is certainly among the most represented Mediterranean crops. The scientific literature on the leaves and branches of this plant shows a good amount of nutraceuticals with high antioxidant properties. Considering that phytochemicals are not involved in the process of converting biomass into energy, these compounds could be extracted from crop residues and used in the formulation of functional foods, cosmetics and pharmaceutical products.

Therefore, the objective of the present research was to extract and characterize the main bioactive compounds from *Olea europaea* pruning wastes, with a view to valorizing biomasses prior to their conversion into bio-energy. In agreement with the principles of the Green Analytical Chemistry, a supercritical fluid procedure was developed for the simultaneous extraction of several nutraceutical classes, including polyphenols, carotenoids, chlorophylls and vitamin E. A range of green co-solvents (bio-ethanol, bio-methanol, dimethyl carbonate) were tested. Furthermore, the impact of several analytical parameters (e.g. modifier percentage, flow rate, back pressure, extraction time and temperature) was investigated. Once optimized the extraction procedure, the extract was subjected to analysis by means of hyphenated liquid chromatographic techniques (HPLC-PDA-MS, HPLC-FLD).

A number of nutraceutical compounds were determined in the subject pruning waste samples. Specifically, oleuropein was identified as the predominant polyphenol in *Olea europaea* pruning wastes, accompanied by minor amounts of glycosylated phenols. Lutein and β -carotene were the most abundant carotenoids. In addition, the presence of chlorophylls and tocopherol was detected.

The present study demonstrates the potential for supercritical fluid extraction to be utilized in the recovery of various bioactive compounds from biomasses. The results obtained for *Olea europaea* pruning wastes highlight the significance of biomass reuse within the context of the circular economy.

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O-059**COMBINING PASSIVE SAMPLING, LC-HRMS/MS AND REGIONS OF INTEREST MULTIVARIATE CURVE RESOLUTION: POTENTIALITIES AND CHALLENGES****Benedetti, Barbara¹; Pérez-López, Carlos²; Mackeown, Henry¹; Magi, Emanuele¹; Tauler, Romà².**¹University of Genoa, Genoa, Italy; ²Institute of Environmental Assessment and Water Studies (IDAEA-CSIC), Department of Environmental Chemistry, Barcelona, Spain.

The main goal of non-targeted analysis (NTA) by liquid-chromatography coupled to high resolution mass spectrometry (LC-HRMS), is the comprehensive determination of the organic chemical constituents of a sample. Even though this strategy is affected by major issues (especially related to chemical coverage, quantitative analysis and data handling), NTA in environmental sciences is a promising approach to prioritize pollutants, for its usefulness in discriminating samples' groups and for the achievable semi-quantitative information ¹.

Few works in the literature have focused on NTA and passive sampling, which may represent a successful combination. While accurate quantitation using passive samplers is challenging, information from a qualitative or semi-quantitative point of view can be obtained ². Indeed, the passive sampler ability of preconcentrating chemicals in-situ for several days facilitates achieving lower limits of detection, thus widening the chemical coverage to trace-concentration contaminants. Among the possible options, polar organic chemical integrative samplers (POCIS) are well established and allow to obtain rich extracts; these, analyzed by LC-HRMS, constitute a goldmine of information, but choosing the best data acquisition and processing strategies may be very challenging.

The case study herein presented deals with the NTA LC-HRMS/MS analysis of extracts deriving from POCIS deployed in Antarctica. In particular, the devices were used to sample contaminants at the outlet of the wastewater treatment facility of the Italian Research Station Mario Zucchelli. The Data Independent Acquisition (DIA) was chosen to perform an unbiased analysis. Indeed, differently from the Data Dependent approach, the DIA mode involves the fragmentation of all precursor ions formed in the source, without filtering them based on specific thresholds.

Recently, the combination of DIA and the chemometric method Regions Of Interest Multivariate Curve Resolution (ROIMCR) have demonstrated to be successful ³. Data processing through ROIMCR provides the chemical components of a sample, resolved based on their chromatographic and mass spectra profiles. Moreover, a new tool for rapid unknowns' identification has been developed ⁴.

The complex data set obtained by the LC-HRMS/MS DIA analysis of POCIS extracts were processed through a fit-for-purpose ROIMCR workflow. The whole data set included data acquired in negative and positive ionization mode for two sampling campaigns, each one including a procedural blank, whose ROIs were carefully considered and subtracted to avoid false positives. By dividing the chromatograms in different time windows, six MCR models were computed, ending up in a total of 770 resolved components. The MSident software was used to tentatively identify unknown chemicals associated to the MCR components and approximately 100 compounds were annotated, including pharmaceuticals and natural substances.

This case study highlighted the challenges faced by the ROIMCR workflow when complex data sets with a high number of ROIs are encountered: not all the resolved MCR components were chemically meaningful, based on their chromatographic profile and tandem mass spectra. Thus, by checking the reliability of the computed MCR models, a classification system was proposed with 7 classes grouped by their goodness levels (A, B and C, from high to low chemical meaningfulness). The percentage of "level A" MCR components (high quality) ranged from 15 to 48%, while the percentage raised to 65-85% if considering components belonging to level A and B (good or acceptable quality). The majority of annotated compounds were associated to good quality MCR components. If the component class assignment was automatized, it may constitute a powerful tool to evaluate MCR models' quality (in addition to the commonly used R² and lack of fit statistical parameters) and thus select the right number of components to improve the reliability of ROIMCR results.

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O-060

CLASS MODELING TOOLS IN ANALYTICAL CHEMISTRY

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Many chemometric applications, particularly in spectroscopy-related problems, involve classification tasks aimed at predicting one or more qualitative attributes of samples based on measured data. In this context, class-modeling techniques [1], which model one class at a time, are particularly well-suited for asymmetric classification problems—such as food authentication or process control—where only one category (e.g., PDO-certified products or Normal Operating Conditions) is well-characterized and adequately sampled, while the alternative class is heterogeneous and under-represented. Unlike discriminant methods that attempt to model all classes simultaneously, class modeling treats classification as an outlier detection problem: if a sample lies outside the modeled class space—typically based on a distance-to-model criterion—it is predicted as not belonging to the target class. A popular example is SIMCA [2], which combines residuals with score distances (often Mahalanobis-like) for classification. This communication illustrates the fundamentals of class modeling, presenting representative examples and highlighting the advantages over discriminant techniques, especially when the class distribution is far from normal. A novel extension is proposed where the distribution of scores is defined non-parametrically using Gaussian Mixture Models (or potential functions), offering a more flexible and accurate representation of the class space. Due to this adaptability, the approach is naturally extendable to multi-block scenarios in a mid-level data fusion framework, where multiple data matrices are used to characterize the same samples. Moreover, non-linear modeling strategies based on support vector machines and non-parametric approaches based on inter-sample distances will also be discussed and illustrated through representative examples.

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O-061**BLIND UNMIXING OF PHOTOCROMIC FLUORESCENT PROTEINS LEVERAGING THEIR PHOTOSWITCHING PROPERTIES****Gómez-Sánchez, Adrián¹; Valenta, Hana²; Vitale, Raffaele¹; Vandenberg, Wim²; Ruckebusch, Cyril¹; Dedecker, Peter².**¹Univ. Lille, CNRS, LASIRE (UMR 8516), Laboratoire Avancé de Spectroscopie pour les Interactions, la Réactivité et l'Environnement, F-59000, Lille, France; ²Department of Chemistry, KU Leuven, Leuven, Belgium.

Nowadays, smart imaging labels such as photochromic fluorescent proteins (FPs) are increasingly used in fluorescence microscopy [1]. Photochromic FPs can be illuminated under predefined patterns of light over short series of consecutive fast imaging captures, inducing reversible transitions between fluorescent and non-fluorescent states that depend on their inherent chemical kinetics. The resulting intensity variations across images produce characteristic fluorescence patterns for the fluorophores. Current methods, though, mostly rely on prior knowledge about these patterns to effectively disentangle them [2], which limits their applicability in complex real-world scenarios.

An alternative approach is to measure kinetic fluorescence decay curves at all pixels of an image and apply chemometric tools to retrieve the pure fluorescence decays and spatial distribution maps associated with each FP. However, in the context of microscopy imaging, this method is often impractical when sampling large areas or volumes due to the extended acquisition time required, which can engender phototoxicity and jeopardize sample integrity.

Here, we propose a novel approach to leverage the advantages of these two measurement procedures while circumventing their respective limitations. In a nutshell, the data collected via both methodologies are fused and simultaneously subjected to Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS), a linear unmixing technique [3-5]. The combination of both datasets allows one to perform accurate blind unmixing without prior knowledge about the fluorophore kinetic properties. Moreover, after the unmixing has been carried out, the extracted kinetic fingerprints can be readily exploited to resolve new fluorescence images collected using only the aforementioned rapid acquisition protocol of short series of images, overcoming the need for additional repeated slow kinetic measurements.

We tested our method on fixed HeLa cell samples and achieved an accurate resolution of pure fluorophore kinetic fingerprints as well as a precise identification of fluorophore spatial locations, leading to a significant improvement over existing state-of-the-art approaches.

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O-062**FROM MEASUREMENT ERROR TO HISTORICAL INSIGHTS: A NEW APPROACH FOR CLASSIFYING ZOOLOGICAL SPECIMENS USING PORTABLE NIR SENSORS AND CHEMOMETRICS****Giussani, Barbara¹; Riu, Jordi²; Monti, Manuel¹; Baruffaldi, Lorenzo¹; Campeny, Marc³; Quesada, Javier³.**

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Zoological specimens are essential for reconstructing past biodiversity, but their research value relies on metadata such as species, age, and collection context. When key information—especially location and historical period—is lost (due to war, neglect, or poor documentation e.g.), specimen utility is compromised. While biological traits can be recovered, assigning specimens to specific historical periods or collections remains challenging. Traditional research is time-consuming, and some analytical techniques are unsuitable for recent collections, highlighting the need for efficient methods of historical classification. Moreover, given the historical and archaeological value of these samples, it is essential to employ non-destructive analytical techniques that can be applied directly on-site, minimizing the risks associated with handling such valuable and fragile materials.

In this presentation, we will share our initial encouraging results using portable NIR (Near-Infrared) sensors to classify specimens - mammal skeletons of red squirrel (*Sciurus vulgaris*) - from the collections of the Natural Sciences Museum of Barcelona into historical periods. The project aimed to optimize an analytical protocol suitable for practical application in museums, taking into account the various sources of variability that contribute to spectroscopic measurement errors.

The approach focused on studying the multivariate error associated with the measurements and incorporating this information directly into visualization and into classification multivariate models to enhance their performance. The multivariate error was estimated through the analysis of error covariance and correlation matrices of the raw spectroscopic data [1, 2 and references therein].

The first aspect we optimized was the measurement protocol for the samples, which were presented as whole skulls, partially preserved skulls, mandibles, and, in some cases, additional bone elements considered fit for analysis. The study of multivariate error of the raw data proved valuable in selecting the most suitable sampling configuration and determining the optimal number of replicates to perform on each zoological sample.

Subsequently, visualization and classification models were developed using Principal Component Analysis (PCA) and Discriminant Analysis (DA), integrating the information derived from multivariate error estimation [3]. This approach allowed for effective separation of samples into classes representing modern and historical periods. The classification models were validated using external validation.

In this study, we evaluated two of the most widely used portable NIR sensors available on the market. The first, the NeoSpectra Scanner (Si-Ware), operates within the 1250-2500 nm range, while the second, the MicroNIR OnSite-W (VIAVI Solutions), spans from 908 to 1976 nm. Both sensors operate using external reflection. When combined with chemometric data analysis, both sensors were deemed suitable for the intended purpose. In both cases, incorporating multivariate error into the models improved their performance, making the visualization models clearer and more interpretable and the classification models more effective. Differences in the sources of variability between the two sensors were observed, affecting the quality of the spectroscopic signal in different ways and, consequently, the performance of the classification models. Incorporating multivariate error into the models allowed us to identify the optimal solution for each sensor, demonstrating the significant value of studying raw spectroscopic data error when working with handheld NIR devices.

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O-063

CHEMOMETRIC DATA FUSION OF METABOLOMIC AND LIPIDOMIC PROFILES FOR CHD PREDICTION: ANALYSIS IN PREGNANT AND NON-PREGNANT WOMEN STUDIES

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Congenital heart disease (CHD) remains the most prevalent congenital anomaly globally, yet its early prediction through maternal biochemical profiling is still limited. This study applies a chemometric framework integrating metabolomics and lipidomics with multivariate data analysis and data fusion to identify discriminant biomarkers and improve CHD risk classification. We present findings from two **independent case-control studies**:

(i) **Non-pregnant women** with a history of giving birth to either a CHD-affected or healthy child [1]

(ii) **Pregnant women** currently carrying either a CHD-affected or healthy fetus.

Omics data were acquired from maternal plasma using untargeted LC-MS-based metabolomics and lipidomics. To manage these high-dimensional, multi-block datasets, we applied a Sequential and Orthogonalized Covariance Selection (SO-CovSel) algorithm to identify the most informative variables. The selected features were used as input for Linear Discriminant Analysis (LDA) [2,3], providing a reduced yet highly predictive model. Alternative chemometric and machine learning approaches, including Partial Least Squares Discriminant Analysis (PLS-DA), Support Vector Machines (SVM), Random Forest, and univariate statistical comparisons, were evaluated to assess robustness across methods.

In the **non-pregnant cohort**, SO-CovSel-LDA achieved a classification accuracy about 92.00% using only five variables, with further enhancement to 94.70% when focusing on structural CHD (sCHD) subtypes.

In contrast, the **pregnant cohort** presented a more complex metabolic landscape due to gestational physiological changes, leading to comparatively lower classification performance. The best-performing model in this cohort achieved an 84.91% accuracy on the test set. So, additional dimensionality reduction strategies, such as Minimum Redundancy Maximum Relevance (MRMR) and Principal Component Analysis (PCA), were evaluated to improve variable selection. Further, machine learning classifiers were implemented and compared to chemometric models. To address the limited sample size and class imbalance in the pregnant cohort, synthetic data augmentation techniques were introduced. These strategies did not improved classification sensitivity for the CHD group. Overall, So-Covsel-LDa give the best performances and the more robust approach.

Data fusion was performed at both low-level and mid-level to preserve block-specific information while enhancing predictive synergy. Results indicate that models trained on fused datasets consistently outperformed those based on single omics layers, demonstrating the added value of a multi-block approach.

Univariate analysis confirmed several of the multivariate-selected biomarkers, revealing differential regulation of lipid subclasses and amino acid metabolites across groups. In particular, certain phospholipids and carnitine derivatives were consistently altered in both cohorts, albeit with differing patterns between the pregnant and non-pregnant states, highlighting both shared and condition-specific metabolic signatures. In both studies, the selected variables were also assessed for correlation with potential confounding parameters such as maternal age and BMI, and no significant associations were found, supporting the specificity of the discriminant features.

The multiblock approaches applied to untargeted plasma metabolomic and lipidomic analysis have facilitated the development of sensitive risk prediction models classifying mothers of children with CHD across both non-pregnant and pregnant cohorts. Each study independently demonstrated that multi-block chemometric strategies, particularly SO-CovSel-LDA, are effective in handling complex omics data to identify discriminatory biomarkers. Analyses incorporating non-isolated and genetically associated CHD, along with phenotypic subclassification of structural CHD, expanded the biological scope of the findings. Importantly, the selected variables showed no correlation with common maternal parameters such as age and BMI, enhancing their relevance as potential biomarkers. Together, these studies reinforce the utility of untargeted multi-omics and robust chemometric modelling for maternal risk profiling and provide insight into the pathophysiological mechanisms of CHD.

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O-064**ASSESSING GRAPE QUALITY AND ENVIRONMENTAL INTERACTIONS IN THE BAIRRADA REGION USING LIMM-PCA AND COMDIM**

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Viticulture and oenology are increasingly challenged by the dual pressures of climate change that impacts grape yield and composition and growing societal demand for environmentally sustainable agricultural practices. Of particular interest is the spatiotemporal interaction between autochthonous grape varieties and local climatic and vineyard conditions. This study aims to advance understanding of the adaptability and phenotypic plasticity of grape cultivars from Bairrada Appellation in Portugal, with a focus on those used in sparkling wine production, through the application of advanced chemometric tools.

Five *Vitis vinifera* L. cultivars—Arinto, Cercial, Bical, Maria Gomes, and Baga—were selected as case studies. Grapes were sampled from two representative vineyards over five consecutive harvests (2017–2021). For each cultivar, physicochemical parameters (titratable acidity, pH, and sugar content) that are used to estimate the technological maturity were measured. Aroma potential was evaluated through characterization of volatile profiles of both free and glycosidically bound fractions. Analysis was carried out using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS).

The effects of harvest year and vineyard on grape composition were examined. Since grapes were harvested at different vineyards at different years, vineyard was necessarily considered a random factor. The vineyards selected for sampling were representative of the Bairrada Appellation, thus, considering vineyard a random factor permitted to extrapolate findings across the Bairrada region. To accommodate the experimental design incorporating both fixed and random effects, a Linear Mixed Model Principal Component Analysis (LIMM-PCA), a combination of ASCA+ and linear mixed models, was employed. This approach enabled the ranking of variables by influence and provided insights into cultivar-specific adaptability, offering valuable guidance for vineyard and wine production management.

The relationship between grape composition and meteorological conditions was explored using multiblock method - Component and Specific Weight Analysis, also called ComDim [2]. The meteorological dataset included seven monthly parameters (e.g., maximum, mean, and minimum temperatures, rainfall, number of cold hours with temperatures below 7.2 °C, etc.) ComDim allowed to assess commonalities between chemical composition data sets as well as identify meteorological conditions that most affect grape composition and quality.) over the five-year study period. ComDim facilitated the identification of shared structures among the chemical datasets and highlighted the climatic variables with the greatest impact on grape quality.

The findings reveal distinct interaction patterns between each grape variety and the regional ecosystem. The approach can be used to hierarchize impact of the different factors on the grape quality and estimate adaptability of the four grape varieties serving for development of tools for efficient management and rational use of vines.

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O-065

MASS SPECTROMETRY-BASED FAECAL PROTEOMICS AND ELECTROCHEMICAL MAGNETO-IMMUNOASSAY INTEGRATED STRATEGY TO ADVANCE NON-INVASIVE DIAGNOSIS OF COLORECTAL CANCER

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Colorectal cancer (CRC) globally ranks as the third cancer for incidence rate, and as second in terms of mortality, with almost 1 million deaths estimated every year.^{1,2} Current screening approaches often yield a high rate of false positives, requiring a follow-up colonoscopy, which is an invasive procedure. This highlights the urgent demand for novel, specific and non-invasive diagnostic tools to improve screening efficiency and enable early patient stratification.³ The identification and validation of new CRC biomarkers in stool samples is a crucial step in increasing patient participation in preventive screening initiatives.

The present study integrates the high sensitivity and selectivity of mass spectrometry (MS)-based proteomics for the discovery of faecal CRC biomarkers with the development of electrochemical immunoassays for their determination in point-of-care testing. ^{4,5} A wide cohort of patients undergoing colonoscopy was stratified into four distinct groups, with at least 10 individuals per group, ranging from healthy individuals to those diagnosed with adenocarcinoma. For biomarker discovery, stool samples underwent a multi-step preparation process, including cell lysis, sonication, protein precipitation and tryptic digestion. The extracted and digested proteins were then analyzed using an untargeted MS-based shotgun proteomic approach, using nano-LC coupled to an Orbitrap Exploris 480 system equipped with High-Field Asymmetric Waveform Ion Mobility Spectrometry obtaining an average of 100 human proteins per sample. Statistical analyses allowed the identification of two novel biomarker candidates, here referred to as PROT1 and PROT2, whose upregulation along CRC progression is consistent with previously reported trends in the literature. PROT1 is involved in apoptosis, while PROT2 acts as a regulator in lysosome biogenesis and autophagy, which is consistent with their upregulation during disease progression.

The next phase of the study focused on translating these findings into an electrochemical immunoassay for the rapid and accurate determination of the identified biomarker candidates. A sandwich-type immunoassay was developed, exploiting carboxylated magnetic microbeads as a sensing platform for the immobilization of monoclonal capture antibody. The detection antibody was conjugated with horseradish peroxidase in order to generate an enzymatically amplified chronoamperometric current signal. Initially, the focus was on PROT1, for which the concentrations of both capture and detection antibodies were optimized by a full-factorial experimental design, in order to maximize the signal intensity with respect to the blank. The developed immunoassay is able to provide a result in just 10 min, exploiting the one-pot incubation of the sample and detection antibody with the capture antibody-functionalized magnetic beads. Selectivity studies were also performed by comparing the signal in presence and absence of the most abundant interfering human proteins associated with colorectal disease, such as hemoglobin, albumin and calprotectin, at the concentration expected in the worst-case conditions: no significant differences were observed ($p > 0.05$), thus evidencing the high specificity of the developed electrochemical immunoassay. No matrix effect was observed with respect to the extraction buffer (Calprest® NG extraction solution, Eurospital Diagnostics) and a detection limit at ng/mL level of antigen in faecal extract was assessed. Furthermore, a good inter-assay precision was obtained, with RSD% less than 15 % ($n=3$).

Ongoing research efforts are directed toward the study of protein extraction yield from stool samples under non-denaturing conditions. Upon completion of analytical validation in real sample matrices, the assay will undergo external validation on an independent patient cohort to assess its clinical applicability. Additionally, a similar biosensing approach will be developed for the detection of PROT2, further strengthening the potential of this diagnostic platform for CRC screening.

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O-066

DUAL-TEMPLATE MOLECULARLY IMPRINTED POLYMER STOCHASTIC SENSOR FOR THE SIMULTANEOUS ASSAY OF TNF- α AND CRP IN WHOLE BLOOD SAMPLES FROM CARDIOVASCULAR DISEASE PATIENTS

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Cardiovascular disease (CVD) comprises illnesses that impact the heart, blood vessels, and circulatory system, including myocardial infarction, stroke, coronary artery disease, and hypertension. A significant percentage of fatalities are due to cardiovascular disorders. Tumor necrosis factor-alpha (TNF- α) and C-reactive protein (CRP) are important predictive biomarkers in cardiovascular research. There is a dire need for the selective, sensitive, precise, and rapid identification of cardiac biomarkers for the early detection and assessment of the long-term risks associated with cardiovascular disease. Electrochemical sensors are preferred for biomarker analysis given their numerous advantages, including high sensitivity, ease of use, straightforward application, low cost, low limit of detection (LOD) values, and remarkable reproducibility. The application of electrochemical sensors utilizing dual-template molecularly imprinted polymers in conjunction with stochastic mode was proposed as a solution. For this reason, a novel stochastic sensor based on dual-template molecularly imprinted polymer (dual-MIP) was proposed for the simultaneous detection and quantification of TNF- α and CRP. The dual MIP/g-CD/AgNSs/GCE was prepared by electropolymerizing 8-amino-1-naphthol-3,6-disulfonic acid (8-ANDSA) as monomer in the presence of TNF- α and CRP as protein templates on the surface of the glassy carbon electrode (GCE) modified with silver nanospheres (AgNSs) and g-cyclodextrin (g-CD). Then, the templates were completely eluted, leaving imprinted cavities in the polymeric film that could effectively recognize both biomarkers in solution. The unique signatures (t_{off} values) of TNF- α and CRP were determined and utilized for the identification of their signals in the diagrams obtained after the rapid screening of biological samples, succeeded by their quantitative assessment. The proposed stochastic sensor exhibited high sensitivity and minimal determination limits. The validation was conducted utilizing real biological samples.

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O-067**AN INVESTIGATION INTO MASS SPECTROMETRY COMPATIBLE ELUENTS FOR OLIGONUCLEOTIDE IMPURITY PROFILING****Bui, Quang Dong¹; Deschrijvers, Tiny²; Noten, Bart²; Verluyten, Willy²; Eeltink, Sebastiaan¹.**¹Vrije Universiteit Brussel, Brussels, Belgium; ²Johnson and Johnson Innovative Medicine, Beerse, Belgium.

Oligonucleotides (ONs) represent an emerging and potent class of pharmaceutical agents that exert therapeutic effects through various mechanisms, including mRNA degradation and splice modulation. Challenges in ON therapeutics, such as stability against nucleases and effective delivery to target tissues, have been addressed through chemical modifications, including 2'-O-methylation of ribose, phosphorothioate incorporation, and GalNAc or lipid conjugation. Analytical characterization of oligonucleotide drugs and related products is extremely challenging due to structurally closely related impurities, resulting in a dynamic concentration range over multiple orders of magnitude.

Ion-pair reversed-phase liquid chromatography-mass spectrometry (IPRPLC-MS) is the most commonly used for ON impurity profiling. However, in this conventional method, the use of 1,1,1,3,3,3-hexafluoroisopropanol as an MS-signal-enhanced agent can raise an environmental concern due to the formation of PFAS. In this study, we investigated different carbonate-based additives on ONs separation in terms of impurity profiling in both single-stranded and double-stranded ONs. The current comprehensive analysis results will contribute to a substantial knowledge of the future application of IPRPLC-MS in the analysis of ONs.

Various additives were evaluated for their effectiveness in the separation of polydeoxythymidines (15 to 35 mers). The retention of oligonucleotides in RPLC is highly influenced by their hydrophobicity. The presence of ionic salts such as ammonium bicarbonate (NH_4HCO_3) and ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$) is proposed to sequester the hydrophobic nucleobase moieties within the molecular interior, thereby reducing the exposure of hydrophobic surface areas. Due to the slight differences in hydrophobicity among the analytes, their separation was highly sensitive to the gradient slope, necessitating a narrowly defined gradient range. Higher additive concentrations and elevated column temperatures further enhanced separation efficiency. The introduction of a hydrophobic ion-pairing agent into the bicarbonate-based mobile phase improved ON separation, as it facilitated electrostatic interactions mediated by the alkylamine group.

In the second part of the study, RPLC was employed to analyze lipid-conjugated ONs. A comparative assessment was conducted using two stationary phases with varying degrees of hydrophobicity, specifically C_{18} and C_4 columns. Notably, in RPLC, lipid-conjugated shortmers exhibited delayed elution relative to their full-length counterparts. In addition, a high-throughput separations was established within a 1-minute gradient.

In the last part of the project, the structure integrity of small interfering RNA (siRNA) was evaluated in various mobile phase additives; the high-order structure (HOS) of si-RNA exhibited partial denaturation with low and preserved at high concentrations of NH_4HCO_3 . The melting temperature was probed in different conditions, considering the effects of different additives and stationary phase interaction. Furthermore, the method was also successfully applied to separate the lipid-conjugated si-RNA from their truncated impurity, demonstrating its applicability and precision.

N/A

O-068**PAPER-BASED BIOSENSORS FOR DETECTING BACTERIA AND ANTIBIOTICS IN WATER****Yang, Zhugen¹; Pan, Yuwei¹; Hui, Qingxin².**¹*Cranfield University, Bedford, United Kingdom;* ²*Cranfield University, Bedfords, United Kingdom.*

Microbial and chemical pollutants in aquatic ecosystems pose a substantial global health hazard. The principal factors contributing to bacterial contamination of water are the inappropriate disposal of waste and insufficient water infrastructure. The incomplete removal of antibiotics during wastewater treatment, leading to their entry into aquatic systems, exacerbates the emergence of antimicrobial resistance. In this study, we engineer cost-effective, rapid, and user-friendly paper-based biosensors for the detection of bacteria and antibiotics, comprising loop-mediated isothermal amplification (LAMP)-based paper origami device and paper-based electrochemical aptamer sensor. The wax-printed paper origami device facilitated paper-based nucleic acid extraction, device-based isothermal amplification, and signal detection. A handheld syringe system was utilized for sample concentration, obviating the need for external electricity. The system achieved sample-to-answer results within 1.5 hrs, enabling multiplexed detection of bacteria with a LOD of 1 CFU/mL. The device was utilized to analyze 8 drinking water and 12 wastewater samples, with results corroborating those obtained from the gold-standard polymerase chain reaction (PCR) method. The nanocomposites were synthesized to enhance the sensitivity of the aptamer sensor, demonstrating robust stability, superior dispersibility, and exceptional electrochemical activity. Aptamers were immobilized on the fabricated paper-based electrode to facilitate high-affinity and selective target binding. The aptamer sensor exhibited a LOD of 1 nM and demonstrated exceptional repeatability, storage stability, selectivity, and anti-interference capabilities, rendering it highly suitable for antibiotic detection in bottled water, tap water, and river water. Our paper-based biosensors offer a combination of cost-effectiveness, sensitivity, specificity, accuracy, portability, and user-friendliness, providing an promising solution for point-of-use testing in water monitoring.

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O-069

STOCHASTIC SENSORS – NEW TOOLS IN THE PROGNOSIS OF CARDIOVASCULAR DISEASES IN OBESE YOUNGSTERS

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Obesity pandemics became a fact in the past years, and a public health issue. Type 2 diabetes, hypertension, and cardiovascular diseases (CVDs) are well known comorbidities. It was also demonstrated that there are changes in the epidemiology of cardiovascular diseases risk factors with the obesity pandemics. The dysfunctional adipose tissue in obese youngsters secretes pro-inflammatory proteins such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1), to mention a few. Accordingly, there is a high need for simultaneous determination of these biomarkers in biological samples. This can only be done reliably using stochastic sensors - the only class of sensors that can perform qualitative and quantitative analysis. Design and applications of stochastic sensors for simultaneous assay of biomarkers specific for CVDs will be shown.

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O-070**FROM CANNABINOIDS TO NEW PSYCHOACTIVE SUBSTANCES: ADVANCED ANALYTICAL STRATEGIES FOR DUAL CHALLENGES IN DRUG DETECTION**

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The dynamic landscape of substance use, marked by the introduction of new psychoactive substances (NPS) and increased cannabis consumption, has raised significant challenges in forensic and clinical toxicology. This presentation highlights recent advances in bioanalytical methodologies for the detection and quantification of cannabinoids and NPS using non-conventional biological matrices and novel separation strategies.

Cerumen, a non-conventional biological secretion, was explored as an alternative matrix for forensic analysis. A robust UPLC-MS/MS method was developed and validated for the simultaneous determination of six major cannabinoids commonly found in *Cannabis sativa*. The method demonstrated strong analytical performance and offers a practical, non-invasive approach for drug monitoring through earwax analysis.

The method's applicability was demonstrated through the analysis of cerumen samples collected from cannabis users. In 12 out of 14 cases, Δ^9 -THC was found to be positive, while in six cases, a few cannabinoids were quantified at concentrations ranging from 0.01–4.86 $\mu\text{g g}^{-1}$. A successful detection window was established for key analytes in cerumen, with Δ^9 -THC reaching a maximum detection frame of up to fifteen days after smoking 0.5 g of marijuana cigarette. ANOVA one-way analysis indicated that average earwax production in cannabis users significantly differed from non-users, while factors such as gender, age, and frequency of use appeared to influence cannabinoid concentrations but not the amount of cerumen produced.

In parallel, the growing availability of NPS, often synthesized via minor structural modifications of controlled substances to evade legislation, has emphasized the need for stereoselective analytical techniques. Many illicit drugs, including NPS, are chiral, and their enantiomers can exhibit distinct pharmacological and toxicological profiles. In this context, simple electrophoretic and chromatographic methods were developed for the enantioseparation of several NPS derivatives. Different types of native and derivatized β -cyclodextrin (CD) and cyclofructan (CF) chiral selectors, along with novel supramolecular deep eutectic solvents (SUPRADESs), were evaluated in regard to resolution and analysis time.

The combination of amino acid-based ionic liquids (AAILs) and deep eutectic solvents (DESs) with CD or CF chiral selectors was also investigated. While AAIL/CD or AAIL/CF systems offered limited improvements, a dual derivatized β -CD/DES system significantly enhanced chiral separation, highlighting the existence of a synergistic effect. This was not the case for the CF/DES dual system, in which the separation of amphetamines worsened, indicating an antagonistic effect. The most remarkable results were obtained using a novel SUPRADES composed of sulfated- β -CD and citric acid (S- β -CD-CA), which enabled baseline separations of all amphetamine and cathinone analogs in under 21 minutes.

O-071**ELECTROCHEMICAL DETECTION OF POLYSTYRENE IN SOFT AND SEA WATER SAMPLES****Surucu, Ozge.***Ege University, Faculty of Science, Department of Chemistry, Izmir, Turkey.*

One of the most frequently detected polymer type is polystyrene (PS) and PS is calculated as 7% for the almost two-thirds of European polymer production in 2016 and the biggest problems about microplastic are upon water products. Therefore, PS microplastic analysis in soft and sea water samples is very significant. Modern analytical methods are playing an important role in the such a critical area of microplastics. Because, these methods provide a large-scaled monitoring opportunity with a low investment and management expenses as well as high sensitivity and selectivity, easy automatization and miniaturization. Within these methods, electroanalytical methods are simple, economical and effective alternatives among other analytical methods. Surface modification is very crucial for the construction of new and specific surfaces to develop electroanalytical methods which are very restricted in the field of microplastics. Besides, nanofilms such as carbon nanotube (CNT) and graphene oxide (GO) create functional groups over the electrode surfaces, help to increase the performance of sensors with an enhanced thermal and electrical conductivity. At the same time, metal nanoparticles (NPs) like gold (Au) is widely preferred as a chemical sensing material, because its advantages such as high conductivity, superior optical feature and chemical stability.

In this presentation, the electrochemical result which is suitable for PS microplastic has been obtained by the functionalization of glassy carbon electrode (GCE) with gold AuNP, GO and CNT. The surfaces of designed electrodes have been characterized by surface imaging techniques such as Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy (SEM-EDX). PS analysis was applied in both soft and sea water samples by means of AuNP/GO/CNT/GCE. The metal and mineral analysis of soft and sea water samples which can be contaminated by microplastics has also been supported by reliable spectroscopic methods such as Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Thus, a practical and novel detection approach for PS and upcoming microplastics has been offered for aquatic ecosystem.

O-072

ASSESSING THE PRESENCE OF PERSISTENT AND MOBILE CHEMICALS IN SPANISH AQUATIC ENVIRONMENT: INSIGHTS FROM THE NEPMTUNE PROJECT

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In recent years, concern regarding Persistent, Mobile and Toxic (PMT) and very Persistent and very Mobile (vPvM) substances has grown to the point of having been included in the REACH CLP regulations with the same level of requirements as e.g. persistent, bioaccumulative and toxic substances (PBT) [1]. These substances, which include various chemical classes, are characterized by their persistence in the environment (high biodegradation half-life), mobility through water systems (low log K_{oc} , thereby low log K_{ow}), and toxicity to humans and wildlife. They are commonly introduced into ecosystems through agricultural or urban runoff, industrial discharges, and domestic wastewater since wastewater treatment plants (WWTP) often fail to remove them effectively. Consequently, due to their intrinsically high polarity, PMT/vPvM substances present a high tendency to be ubiquitous in the water cycle and can contaminate drinking water sources and disrupt aquatic ecosystems. The NEPMTUNE project aimed at: i) apply screening methods based on the use of high resolution mass spectrometry (HRMS) to detect PMT/vPvM substances in the Spanish aquatic environment; ii) provide a validated methodology to obtain quantitative data on the most frequently detected PMT/vPvM substances in the considered sampling points.

Thus, a sampling campaign was conducted in 2023, including 47 samples of river water, raw and treated wastewater, urban runoff, and landfill leachate from three different regions in Spain (Galicia, Catalonia and Community of Valencia). The samples were screened for over 1100 substances contained in an in-house database built using PMT/vPvM criteria. A freeze-drying protocol was applied to the samples previous to their injection in a liquid chromatograph (LC) coupled to (ion-mobility) quadrupole time-of-flight (QTOF) HRMS. A mixed-mode (MM) chromatographic column was used for separation. Using this methodology, a total of 85 PMT/vPvM substances were tentatively identified in all the samples, being the most frequently detected industrial chemicals, such p-cumenesulfonic acid and 1,3-di-o-tolylguanidine, as well as pharmaceuticals, such as venlafaxine and N-desmethylvenlafaxine and metformin.

Analytical standards were available for 52 of the substances identified in the screening campaign, thus a method validation was performed for the freeze-drying protocol, including a determination step based on the use of mixed-mode chromatography coupled to triple quadrupole mass spectrometry. The freeze-drying methodology consists solely of a concentration step, without any purification beyond the eventual precipitation of salts [2]. This technique provided appropriate results as a compromise solution to achieve the concentration of very polar ionizable and neutral compounds from water matrices (surface water and treated and raw wastewater) and, although an important matrix effect was observed for some compounds, method accuracy in the range of 70-130 % was obtained for most of the considered PMT/vPvM substances.

An additional sampling campaign was performed in 2024 and samples collected in both sampling campaigns (2023 and 2024) were processed using the validated methodology. The highest concentrations (up to 310 $\mu\text{g L}^{-1}$) were found for metformin and p-cumenesulfonic acid. It should be noted that the most abundant PMT/vPvM in raw wastewater are discharged in high proportions in the receiving media, being the concentrations consequently high in surface water, reinforcing their estimated persistence and mobility in the water cycle.

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O-073

PFAS SCREENING IN AGRICULTURAL FORMULATIONS USED IN RICE CULTIVATION

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The most common characterized sources of environmental perfluoroalkyl substances (PFAS), precursors and their transformation products are associated with wastewater, aqueous film-forming foams (AFFF), household utilities and single-use food contact materials [1]. However, the release of fluorinated chemicals used as additives in agricultural formulations and their impact on wildlife remains unknown. To the best of our knowledge, only one study explored these compounds in insecticides [2], which raised the attention of the US Environmental Protection Agency (EPA). Consequently, the objective of the present study was to conduct an analytical screening of 20 widely used herbicide and fungicide formulations (as Novixid®, Command®, or Seltima®) in the Ebro Delta rice fields to evaluate the presence of fluorinated chemicals and their potential threats on wild bird species. Samples were diluted as 1 mL of the formulation in 10 mL of water. These extracts were analyzed by solid phase extraction (SPE) cartridges Waters Oasis WAX 6 cc Vac Cartridge, 150 mg, 30 µm particle size. To ensure the recovery of the compounds, 8 mL of a 0.01% formic acid were added before eluting with 5 mL of 0.2% NH₄OH (aq) in methanol (MeOH) as elution solvent. These extracts were evaporated under a gentle stream of N₂ to 500 µL and stored at -21°C. Blank controls included 5 extraction blanks of MeOH subjected to the complete analytical procedure. Chromatographic separation was achieved on an Agilent C18 column (Poroshell 120 EC-C18, 2.1 mm × 100 mm, particle size 2.7 µm) with a flow rate of 0.3 mL/min at 40 °C. A 23-min gradient elution was employed with eluent A (95/5 H₂O/MeOH + 2 mM NH₄Ac) and eluent B (95/5 MeOH/H₂O + 2 mM NH₄Ac). The injection volume was 20 µL. Analyses was achieved by HPLC coupled to a quadrupole time-of-flight mass spectrometer (QTOF) in negative electrospray ionization mode (ESI⁻). MS1 and MS2 spectra were acquired using an iterative data-dependent MS2 mode (ddMS2). For prioritization of features and identification of other potential PFAS, the in-house developed open-source, Python-based software PFAScreen was used [3]. PFAS presence was confirmed from accurate mass matches with an extended suspect list of about 4.800 fluorinated compounds, where the specific properties of PFAS and their transformation products such as the chemical mass defect (m/C), homologous series based on Kendrick mass defects (MD/C), as diagnostic fragmentation patterns have been considered [4, 5]. For each sample, PFAScreen was executed individually together with the extraction blank to remove background signals. Moreover, the distribution of feature assignments to potential fluorinated structures has been considered as common mass fragment differences and assignment to homologue series (based on CF₂ repeating units). So far, LC-HRMS analysis and data evaluation by PFAScreen revealed the occurrence of hundreds of features at m/C < 30 thus, with few fluorine atoms in the structure, which will be tentatively investigated as originated from fluoropolymer additives, emulsifiers or transformation products. Additionally, the presence of fluorinated precursor compounds will be investigated through PhotoTOP (Total Oxidizable Precursors assay), a new technique based on UV/TiO₂ photocatalysis [6]. Further research is needed to discuss these findings, with more background information on the agricultural products, formulations used, and volumes and/or quantities applied to rice fields to understand the potential uptake by wild bird and other species.

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O-074

MICROPLASTIC-ZINC INTERACTIONS AND THEIR IMPACT ON *DAPHNIA MAGNA*'S METAL REMOVAL EFFICIENCY

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Water pollution is a global problem that severely impacts both the environment and human health. Microplastics (MPs) are among the emerging pollutants of concern in the aquatic media, especially because conventional water treatments are not designed for their removal, and they can also act as vectors of other contaminants. To overcome this challenge, new alternatives to traditional water treatment methods are being investigated through nature-based solutions, which are more sustainable [1-3]. Previous studies have shown the filtration capacity of *Daphnia magna* (*D. magna*) to improve the quality of reclaimed water [4]. Most of the research conducted on the impact of MPs on *D. magna* is related to their toxicological effects. In this contribution, the main goals were to provide a more in-depth understanding of the Zn adsorption process on MPs and the potential of *D. magna* for removing Zn from water in the presence of MPs under laboratory-scale conditions.

Fragments of single-use plastics are one of the most prolific types found in the environment. Therefore, in this investigation real-to-life MPs obtained by micronizing commercially available plastic devices (e.g., plastic cups) of different polymers were employed. Additionally, the produced MPs were aged for 8 weeks under UV radiation at 42°C, considering that MPs in natural environments can undergo aging processes that can lead to changes in their physicochemical properties, as well as their bioavailability and toxic effects to organisms.

In the first phase, batch adsorption studies were conducted by tumbling a Zn aqueous solution with MPs at different contact times (1, 2, 3, 4, 7 h). Real-to-life MPs of polyethylene (PE, 63-80 µm), polypropylene (PP, <63 µm and 63-80 µm), and polystyrene (PS, 63-80 µm) were used in these experiments. Afterwards, the supernatants were analyzed with inductively coupled plasma optical emission spectroscopy (ICP-OES) and the MPs were collected on filters and analyzed with energy dispersive X-ray fluorescence spectrometry. Results showed higher Zn adsorption on real-to-life PE MPs, indicating plastic material influences the adsorption process. In order to determine whether environmental degradation could modify their adsorption capacity, aged real-to-life MPs were also tested under the same experimental conditions. The Zn concentrations determined in the aqueous solutions were lower compared to non-aged MPs, demonstrating that the aging process influences their metal adsorption capacity.

In the second phase, *D. magna* individuals were exposed for 48 h to an aqueous mixture of Zn and different sized real-to-life PP MPs (63 µm and 63-80 µm). After 48 h, Zn content was determined by ICP-OES in aqueous solutions and *D. magna* individuals (after a digestion process). Zn removal efficiency from water by *D. magna* was around 81-83% for both tested MPs, indicating that the presence of different sized MPs does not affect the *D. magna* filtration capacity for Zn. These results were in agreement with the ones obtained from the accumulated Zn in *D. magna* individuals. In addition, the ingestion of MPs by *D. magna* was verified by taking microscope images. The same experiments were carried out with non-aged and aged MPs of different materials (PP, PE, PS) with a size range of 63-80 µm. Zn concentrations determined in aqueous solutions mainly showed that *D. magna* capacity to eliminate this metal is not altered by the MP material or aging (removal efficiency around 75%). However, differences were observed in Zn accumulation in *D. magna* individuals between non-aged and aged MPs (PP and PE) due to the possible interaction between aged MPs and Zn.

Overall, these findings contribute to a better understanding of how MPs can interact with aquatic organisms and Zn.

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O-075

SPATIAL VARIATION OF PER AND POLY-FLUOROALKYL SUBSTANCES AND ORGANOCHLORINE PESTICIDES IN GREAT TIT EGGS ACROSS DIFFERENT AREAS IN PADOVA, ITALY

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Birds serve as crucial bioindicators of environmental pollution, reflecting the accumulation of contaminants through the food chain (Sun et al., 2023). This study investigates the contamination of per- and polyfluoroalkyl substances (PFAS) and organochlorine pesticides (OCPs) in great tit (*Parus major*) eggs collected from woodland, urban, and agricultural areas around the city of Padua, Italy. The primary objective was to evaluate the potential of great tit eggs as bioindicators for diffuse PFAS and OCP sources and to explore the factors contributing to the observed variations in contamination levels. PFAS and OCPs are persistent contaminants with significant environmental and biological impacts, including alterations in avian reproduction. PFAS, known for their stability and wide industrial applications, have raised concerns due to their bioaccumulation and toxicity (Buck et al., 2011). OCPs, although largely banned, persist in the environment, affecting wildlife and human health (Jones, 2021). The great tit, with its well-studied ecology and cavity-nesting behavior, offers an ideal model for assessing local contamination (Van den Steen et al., 2009).

In this study, abandoned and unhatched eggs collected during nest monitoring were analyzed. The results revealed the presence of both PFAS and OCPs in all studied areas, albeit with significant variations in concentrations and contamination profiles. Perfluorooctane sulfonate (PFOS) was the most prevalent PFAS, followed by long-chain perfluorocarboxylic acids (PFCAs). Agricultural areas showed the highest PFOS concentrations, particularly at the Agri – CDM site, likely due to biosolid fertilizers (Bertanza et al., 2024). Perfluoroalkyl carboxylic acids (PFCAs) concentrations were higher in urban and woodland areas, suggesting different contamination sources and transport pathways. Notably, woodland areas exhibited higher concentrations of PFUnDA and PFTrDA, and the observed odd-even PFCA patterns across different locations highlighted the influence of atmospheric chemistry, especially the oxidative breakdown of PFCAs precursors, in determining contamination profiles. Regarding OCPs, contamination was dominated by dichlorodiphenyltrichloroethane (DDT) and its metabolites, especially p,p'-DDE. The highest OCP concentrations were found in Agri – CDM, followed by urban areas, indicating persistent residual contamination and local pollution sources (Bertanza et al., 2024). Urban areas also showed higher concentrations of DDT metabolites, reflecting historical pesticide use and limited soil disturbance, which prevents the dilution and breakdown of persistent compounds like DDT. Principal Component Analysis (PCA) showed a clear separation between the study areas based on contamination profiles. Agricultural areas were more influenced by PFOS and PFOA, likely due to local biosolid applications, while woodland and urban areas showed a higher influence of long-chain PFCAs. Elevated levels of p,p'-DDE and p,p'-DDT were detected in urban zones, which were separated from woodland areas, while agricultural regions overlapped with both.

This study highlights the use of great tit eggs as an effective tool for monitoring environmental contamination and the persistence of both PFAS and OCPs in the environment. The results reveal distinct contamination patterns across different environments, reflecting the influence of anthropogenic activities, local ecological factors, and atmospheric deposition. The identification of specific contamination sources in each area provides valuable information for environmental management and policy formulation. The persistent presence of these contaminants underscores the need for continuous monitoring and mitigation strategies to protect ecosystems and human health. Additionally, this study contributes to the understanding of contaminant dynamics in terrestrial ecosystems and highlights the importance of considering both local and long-range transport sources in environmental contamination assessments.

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O-076

ANALYSIS OF ORGANIC TOXIC COMPOUNDS IN SYNTHETIC/RECYCLED RUBBER AND ARTIFICIAL TURF USED IN PUBLIC FACILITIES: ASSESSMENT OF DERMAL BIOACCESSIBILITY

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¹Verified Market Report (2024) Global Rubber Recycling Market Size By Application (Automotive Industry, Construction Industry), By Material Type (Ground Rubber, Shredded Rubber), By End-use Industry (Automotive & Transportation, Building & Construction), By Recycling Process (Mechanical Recycling, Chemical Recycling), By Features (Energy Efficient, Compact Design), By Geographic Scope And Forecast (report ID: 867076). <https://www.verifiedmarketreports.com/product/rubber-recycling-market/> (accessed on April 10, 2025); ²ECHA (2022) Granules and mulches on sports pitches and playgrounds. <https://echa.europa.eu/hot-topics/granules-mulches-on-pitches-playgrounds> (accessed on April 10, 2025); ³O. A. Abafe et al. *Environ. Sci. Technol.* 57 (2023), 10554-10562.

Synthetic rubber materials made from waste tire crumb, along with recycled ones, are widely used in the construction of various indoor and outdoor synthetic surfaces, such as playground flooring, sports fields, and recreational areas, being also commonly employed as granules or crumb infill for artificial turf in football/soccer pitches. The use of these materials on surfaces offers several key advantages over traditional materials in terms of safety (providing cushioning and shock absorption, thereby reducing the risk of injury particularly in children's play areas), durability (high resistance to wear and weather conditions), cost-effectiveness (long lifespan and low maintenance requirements), accessibility (enhanced usability for children with disabilities), versatility (customizable in both design and appearance), and environmental sustainability (repurposing waste tires and contributing to waste reduction). Consequently, the recycled rubber market was valued at USD 100 billion in 2023 and is projected to reach USD 200 billion by 2030, with the rising demand for synthetic surfaces serving as a major driver of this growth (1). Despite these benefits, the use of synthetic and recycled rubber raises concerns regarding the release of potentially toxic compounds—such as metal(oid)s and organic compounds, including phthalate esters (PAEs) and organophosphorus flame retardants (OPFRs)—which may pollute the environment and pose health risks. Additionally, the safety of individuals exposed to these materials remains a major concern, being dermal contact the primary exposure pathway, particularly for children in playgrounds and the general public using sports and recreational facilities.

In this context, knowledge of the total content of pollutants in synthetic or recycled rubber and artificial turf may not accurately reflect the health risks they pose. A more realistic risk assessment approach would involve assessing the compounds that could be dissolved in simulated human fluids (bioaccessibility) such as dermal fluids, as these compounds would have a higher potential for absorption through the skin. The present study comprehends the development of a UPLC-MS/MS method, targeting 35 organic toxic compounds (namely 17 PAEs, 13 OPFRs and 5 further additives associated to polymer-based products), and 5 deuterium-labelled compounds used as surrogates/internal standards, to investigate the occurrence and bioaccessibility of these compounds in synthetic/recycled rubber and artificial turf used in public facilities, comprising sports fields and playground surfaces. Chromatographic separation was implemented in a ACQUITY UPLC BEH C18 (1.7 μ m, 2.1 x 100 mm) column, comprising gradient elution with two components (0.1% V/V formic acid in both methanol and water) over a 34-min runtime, using also an ACQUITY UPLC (2.1 x 50 mm) isolator column to delay contamination from the mobile phase and equipment. Sample preparation for total content included an ultrasonic-assisted solvent extraction (UASE) followed by a vortex-assisted dispersive solid phase extraction (d-SPE) clean-up; whereas compounds in bioaccessible fractions, obtained after a physiologically-based extraction test (PBET) using synthetic human sweat/sebum mixture, were extracted by vortex-assisted liquid-liquid extraction (VALLE). After validation, the procedure was successfully applied to explore the presence of targets and estimation of the dermal bioaccessibility in those materials used in different public facilities.

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O-077

LABEL-FREE HAPTOGLOBIN QUANTIFICATION USING A QCM IMMUNOSENSOR: A NOVEL TOOL FOR EARLY NEURODEGENERATIVE DISEASE SCREENING

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Neurodegenerative diseases (NDs), including Alzheimer's, Parkinson's, Huntington's disease, and Multiple Sclerosis, exhibit distinct molecular mechanisms but share common pathological hallmarks such as dopaminergic neuron degeneration, cognitive decline, and neuroinflammation [1]. These processes are closely linked to chronic inflammation and oxidative stress, which correlate with elevated levels of systemic biomarkers like haptoglobin (Hp), an acute-phase inflammatory glycoprotein. Studies indicate significantly higher serum Hp levels in ND patients compared to healthy controls, and its interaction with β -amyloid aggregates further underscores its potential as a biomarker for disease progression [2–5]. However, current diagnostic methods for Hp, such as ELISA and nephelometry, are costly, time-consuming, and lack sensitivity, while conventional ND biomarkers (e.g., tau, amyloid- β) require invasive cerebrospinal fluid (CSF) sampling [6–8]. To address these limitations, we developed the first quartz-crystal microbalance (QCM)-based immunobiosensor for rapid, non-invasive quantification of Hp in human serum.

This work introduces an in-house QCM-resonance (QCM-R) device capable of dual high-resolution measurements of resonance frequency and dissipation. The immunosensor fabrication began with functionalizing the quartz crystal surface using a self-assembled monolayer (SAM) of thiolated polyethylene glycol amine ($\text{SH}_2\text{-PEG-NH}_2$). Hyaluronic acid-coated solid lipid nanoparticles (HA-SLNs) were then immobilized via NHS-EDC chemistry - a novel application of HA-SLNs in biosensing designed to enhance surface stability, antibody loading capacity, and sensitivity. Subsequent steps included covalent attachment of anti-Hp antibodies and ethanolamine blocking to minimize nonspecific binding. The biosensor demonstrated robust analytical performance, achieving high sensitivity ($72.8 \mu\text{g mL}^{-1}$), reproducibility ($\text{CV}_{\text{av}} = 5\%$), selectivity, and exceptional limits of detection ($0.05 \mu\text{g mL}^{-1}$) and quantification ($0.19 \mu\text{g mL}^{-1}$) in both buffer and human serum. Layer-by-layer surface modifications were validated using atomic force microscopy (AFM), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and electrochemical impedance spectroscopy (EIS). The integration of HA-SLNs not only improved sensor stability but also amplified the signal response, addressing key challenges in label-free biosensing. By eliminating the need for invasive CSF sampling and complex instrumentation, this platform offers a cost-effective, user-friendly solution for early ND diagnosis and therapeutic monitoring. Its success highlights the transformative potential of QCM-R technology in clinical diagnostics, enabling point-of-care testing and personalized disease management. This study pioneers the use of piezoelectric immunosensors for Hp detection, bridging a critical gap in ND diagnostics. Existing Hp biosensors focus on veterinary applications (e.g., bovine mastitis), leaving human-focused tools underdeveloped. Our work establishes a foundation for scalable, non-invasive Hp monitoring, which could revolutionize preclinical ND detection and progression tracking. Future efforts will focus on multiplexed biomarker detection and validation in diverse clinical cohorts to further enhance diagnostic accuracy.

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O-078

DESIGN AND VALIDATION OF TEXTILE ELECTROCHEMICAL SENSORS FOR WEARABLE APPLICATIONS

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The integration of electrochemical sensors into clothing and textiles represents a frontier research topic in the context of wearable devices. Not only smart garments and bandages may offer the possibility to monitor in real time various physiological parameters, but also to reach a deeper understanding of the underlying biochemical processes within complex body conditions, as well as improve the management of some chronic diseases. In this scenario, careful selection of conformable, lightweight and biocompatible materials plays a fundamental role in the successful development of a wearable sensor. Nonetheless, the characterization of this emergent class of sensors still lacks standardized analytical guidelines, which would enable their validation in a medically relevant environment and therefore improve their scientific maturity. In this contribution, the design and characterization of smart textiles for the electrochemical sensing of moisture and ions, such as H⁺ for pH determination, is presented for the real-time monitoring of the body status (1-3). Specifically, we focus on the physico-chemical properties of the wound bed and particularly the composition of the wound exudate, which hold significant diagnostic information about the healing status of chronic and acute wounds. In order to access this information, we have optimized the manufacturing of moisture and pH sensitive medical dressings through the screen printing of commercial inks containing a biocompatible organic semiconductor, i.e. poly(3,4- ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS), directly on medical grade bandages, followed by appropriate (electro)chemical functionalization to obtain the desired selectivity. Moreover, analytical protocols for laboratory testing under real-life conditions have been developed using artificial wound exudate samples, which allow us to validate our sensors with independent analytical methods. Multisensing experiments and ex-vivo testings on human skin in a setup including portable electronics are in progress.

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O-079**A MULTI-EPITOPE NANOSWITCH PLATFORM FOR RAPID AND SELECTIVE DETECTION OF BIOAVAILABLE THERAPEUTIC MONOCLONAL ANTIBODIES****Di Lena, Denise¹; Sisti, Edoardo²; Marini, Bruna³; Porchetta, Alessandro⁴; Da Pozzo, Eleonora²; Ippodrino, Rudy³; Bertucci, Alessandro¹.**

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Therapeutic monoclonal antibodies (mAbs) have advanced the treatment of chronic diseases, but their efficacy is often compromised by unpredictable pharmacokinetics and patient-specific immune responses. Personalized therapeutic drug monitoring (TDM) tools are essential to assess the bioavailable fraction of mAbs, the active portion that remains effective in treating patients. Here, we present an innovative multi-epitope protein-ssDNA nanoswitch platform, an evolution of Ulisse BioMed's NanoHybrid system¹, specifically engineered for rapid and selective detection of bioavailable therapeutic antibodies directly in serum.² By leveraging entire protein binding domains conjugated to tailored ssDNA, the nanoswitch enables rapid, one-step quantification of mAbs such as Infliximab and Adalimumab across a clinically relevant dynamic range (2.4 to 19 µg/mL). Importantly, the platform distinguishes between total and bioavailable antibody levels, detecting significant reductions in active Infliximab in the presence of anti-drug antibodies (ADAs), a key factor often overlooked in standard assays. This adaptable, multi-epitope sensing approach provides real-time insights into biologics pharmacokinetics, offering a powerful tool for optimizing therapeutic regimens and improving patient outcomes in precision medicine.

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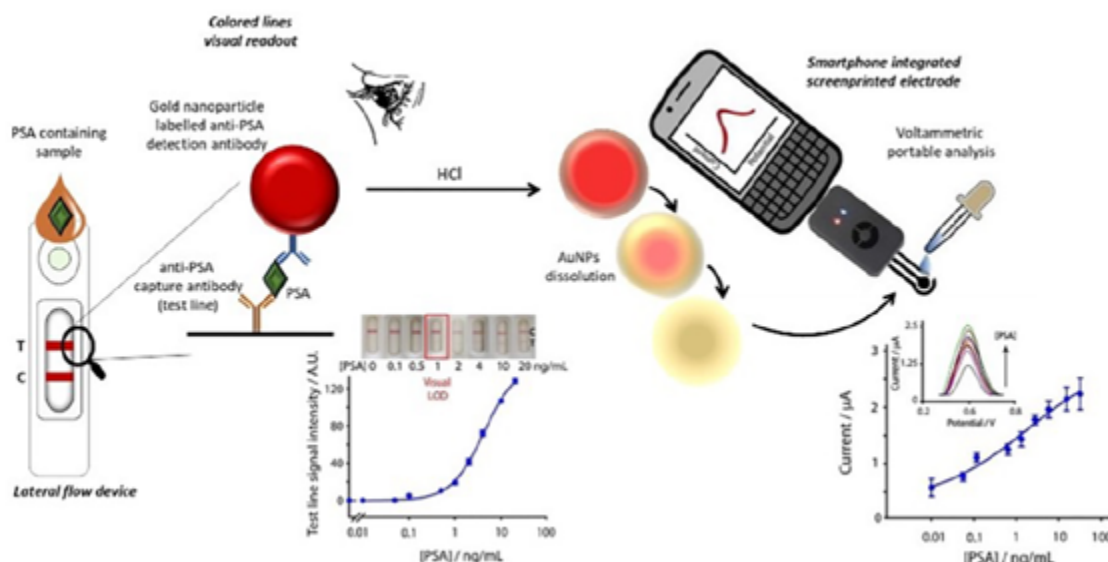
O-080

CAN ELECTROANALYSIS ENHANCE LATERAL FLOW IMMUNOASSAY? PROSTATE - SPECIFIC ANTIGEN AS THE CASE OF STUDY

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The COVID-19 pandemic underscored the importance of lateral flow immunoassay (LFIA) strips as widely used point-of-care (POC) diagnostic tools, enabling rapid and user-friendly biomarker detection by nonspecialists. However, these tests are predominantly qualitative. In contrast, electrochemical diagnostics, commonly employed in diabetes management, enable quantitative biomarker determination. The integration of electrochemical transduction into LFIA strips offers a promising advancement in POC testing, combining the simplicity, affordability, and rapidity of LFIA with the high sensitivity and quantitative capabilities of electrochemical detection [1]. In this study, an electrochemical LFIA (eLFIA) platform was developed by integrating a printed-electrochemical strip with a conventional LFIA test for the detection of prostate-specific antigen (PSA). To optimize the electrochemical response, four types of gold nanoparticles (AuNPs) with different optical properties (525, 529, 521, and 618 nm) were tested in presence of increasing concentration of PSA (0.01-20 ng/mL). Among them, AuNP-525 exhibited the best analytical performance. Electrochemical detection was based on the measurement of gold ions released from the dissolution of gold nanoparticles captured on the test line, using differential pulse voltammetry (DPV). A comparative analysis between standard LFIA and eLFIA demonstrated that eLFIA achieved a 2.5-fold lower detection limit (0.15 ng/mL vs. 0.38 ng/mL), indicating enhanced sensitivity [2]. To assess its applicability in complex biological samples, the eLFIA system was further tested in PSA-free female serum through spiked experiments in the same range of concentrations. The preliminary results confirmed the system's reliability and robustness in detecting PSA, even in complex matrices, with a limit of detection of 0.40 ng/mL and demonstrating eLFIA potential for real-world applications. Despite requiring a longer analysis time, the eLFIA approach successfully enhances the sensitivity of conventional LFIA, bridging the gap between qualitative and quantitative POC diagnostics. The strong correlation between LFIA and eLFIA results highlights the feasibility of integrating electrochemical detection into LFIA-based platforms. This work provides a comprehensive evaluation of the advantages and limitations of combining these two techniques, offering valuable insights into their potential synergy for biomarker detection in POC applications.



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O-081**HIGH-THROUGHPUT SCREENING FOR (BIO-)SENSOR DESIGN****Kuom, Magdalena T.¹; Galonska, Phillip¹; Vukovic, Lela²; Kruss, Sebastian¹.**¹Ruhr University Bochum, Bochum, Germany; ²University of Texas at El Paso, El Paso, United States.

Optical nanosensors, such as those based on single-walled carbon nanotubes (SWCNTs), allow sensitive biomolecule detection with high spatiotemporal resolution. SWCNTs fluoresce in the beneficial near infrared (NIR) tissue transparency window, which decreases background noise and enables sensing applications *in vitro* with different cells and even *in vivo*. Compared to fluorescent dyes emitting in the visible spectrum they do not bleach. Sensor design often involves screening of different types of surface chemistry including modification with DNA. Furthermore, it is slowed down by time consuming preparation steps. Hence, even though the DNA sequence space promises tuneable sensor design, there is a need for higher throughput and predictability.

Recent advances in covalent DNA-chemistry on carbon nanotubes allow for such a drastic increase in synthesis throughput. In our approach, we bind DNA sequences to SWCNTs covalently ("anchors") and then recruit ssDNA-recognition motifs via hybridisation to bypass the laborious dispersion process of traditional sensor fabrication. In combination with fully automated pipetting and spectroscopy, sensor synthesis speed is drastically enhanced. Combining 60 recognition sequences with three anchors and three analytes each makes 180 sensors and 540 conditions accessible in only three working days. We demonstrate improvements in selectivity and sensitivity of the sensors for the neurotransmitter dopamine in complex media. Additionally, we show how machine learning (ML) methods can be used for an iterative process. Overall, we show how high throughput preparation of sensor candidates creates data that can be used to train a machine learning model to predict new recognition sequences to ameliorate sensor performance.

O-082

FOLDING PAPER-BASED ANALYTICAL DEVICE FOR SIMULTANEOUS QUANTIFICATION OF CANCER BIOMARKERS EXPLOITING QUANTUM DOTS AS ULTRASENSITIVE ELECTROCHEMICAL LABELS

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Cancer is a leading cause of death worldwide, accounting for almost 10 million deaths in 2020 [1]. Various tumor biomarkers can be used in clinical applications to identify specific types of cancer. Simultaneous detection of different cancer biomarkers in a single assay is crucial for early screening of cancer, evaluating the effectiveness of treatment and improving the survival rates [2].

Cancer antigen 125 (CA 125) is a naturally-produced glycoprotein which is present on the surface of cancer cells in the ovary and is widely used as a cancer biomarker. The diagnostic cut-off concentration of CA125 in human serum is typically around 35 U mL⁻¹, while higher levels are considered an early sign of ovarian cancer [3].

Carcinoembryonic antigen (CEA), a high-molecular-weight glycoprotein (>180 kDa), is a well-known tumor marker that has been correlated in the last three decades with certain types of cancer, for example colorectal, breast, lung, or ovarian cancer, with the cut-off value of CEA having been set to 5 ng mL⁻¹ [4].

The concurrent determination of two, rather than one, tumor markers improves diagnostic accuracy and is more cost-effective, but also increases the demand on resources, and therefore low-cost, rapid, easy-to-use, multiplexed and sensitive analytical techniques are necessary for the effective point-of-care (POC) diagnosis of [5].

Paper-based analytical devices (PADs) exploit inexpensive cellulose paper for (bio)chemical analysis and are promising tools as portable bioanalytical platforms for POC [6].

In this work, we describe a paper-based immunosensor for simultaneous quantification of CA125 and CEA in human serum with electrochemical detection. To fabricate the paper-based immunodevice, fluidic channels were formed on the paper substrate using an inexpensive computer-controlled x-y plotter and a commercial hydrophobic marker pen. Then, capture anti-CA125 and anti-CEA were immobilized on two separate assay zones of the PAD. The sample was added at the device and CA125 and CEA were selectively retained on detection zones. Sandwich-type bioconjugates were formed by further adding anti-CA125 and anti-CEA conjugate probes labeled with Pb-based and Cd-based quantum dots, respectively. Then, the QDs in each assay zone were dissolved in acid releasing Pb(II) and Cd(II) cations. Then, a three-electrode electrochemical PAD (ePAD), fabricated by a combination of x-y plotting and screen printing, was attached to the PAD and the two assay zones were folded over the ePAD. Detection was performed by measuring the Pb(II) and Cd(II) by anodic stripping voltammetry. The fabrication of the device, the development of the assay and the analytical features of the method are discussed. The biosensor can detect CA125 and CEA at concentrations <0.03 U mL⁻¹ and <0.005 ng mL⁻¹, respectively, and is suitable for low-cost multiplexed PoC applications.

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O-083

RAPID AND ACCURATE ETHANOL DETERMINATION USING MICROPLASMA EMISSION SPECTROMETRY

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Molecular emission spectrometry (MES) using a microplasma emission source, specifically solution anode glow discharge (SAGD), was successfully applied to ethanol (C_2H_5OH) determination in alcoholic beverages. The sample solution was introduced into a microplasma system, where organic species underwent evaporation, fragmentation, and excitation. This process generated an intense CO molecular band in the emission spectrum, which served as the basis for ethanol quantification. Compared to commonly used methods, the developed approach eliminated the need for sample distillation, significantly reducing analysis time. The novel method exhibited a measurement precision ranging from 1% to 3%, with a detection limit as low as 40 mg L^{-1} (equivalent to 0.005% alcohol by volume). SAGD MES was successfully applied to analyzing commercially available alcoholic beverages, including high-proof spirits, wines, and beers. The results were validated against the reference method, showing high accuracy.

O-084

A SPECTROSCOPIC STUDY OF TERNARY GAS DIFFUSION

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The interdiffusion of multicomponent gases is a relevant topic in Chemical Sciences and in many industrial applications such as absorption and stripping columns, distillation and sorption in porous and dense solid materials. The diffusion of binary gas mixtures has been generally studied in the literature while only a few experiments have been presented so far for ternary gas mixtures [1]. The latter confirmed that osmotic, reverse and barrier diffusion can be produced when the mutual diffusivities of the binary sub-systems are much different in magnitude from each other. These phenomena have been theoretically described with the Maxwell-Stefan theory of multicomponent gas diffusion [2].

Recently, a new approach based on FTIR Spectroscopy in the transmission mode was introduced to investigate the mutual diffusion of binary gas mixtures [3]. In this work, the same method is successfully extended to the case of ternary gas mixtures [4]. The experimental setup consists of two closed volumes interconnected with a shut-off valve (Figure A) and the analysed gases are carbon dioxide, methane, ethane and propane. The study is divided in three consecutive steps. First, unary gases and binary gas mixtures of known composition are prepared and stored in the apparatus and are analysed spectroscopically. Analytical IR signals are identified for each component. Second, binary diffusion tests are conducted at 308.15 K and 0.1 MPa for the gas pairs C_3H_8/CO_2 , CH_4/CO_2 , CH_4/C_2H_6 , CH_4/C_3H_8 (Figure B) and C_2H_6/C_3H_8 . Despite the complex geometry of the experimental setup, an analogy is introduced with the classical *two-bulb* system and the geometry of this theoretical setup is calibrated from the diffusion experiment of C_3H_8 in CO_2 . Afterwards, the mutual diffusivities of the other gas pairs are evaluated by resorting to the classical Fick's law of binary diffusion and the results are compared with the literature. Last, the ternary diffusion of $CH_4(\alpha) - CO_2(\beta) - C_3H_8(\gamma)$ is measured at 308.15 K, 0.1 MPa and gas mixture composition at equilibrium equal $tox_{\alpha,\infty}=0.333$ and $x_{\beta,\infty}=0.363$ (Figure C). The diffusion kinetics are efficiently interpreted with the Maxwell – Stefan theory of multicomponent diffusion: specifically, osmotic, reverse and barrier diffusion are observed and predicted for methane before reaching thermodynamic equilibrium.

The whole unsteady diffusion kinetics is monitored during the experiment *in situ* and the analytical technique is described in detail. It is also experimentally demonstrated the occurrence of equimolar diffusion during binary and ternary gas diffusion. The present study shows that FTIR Spectroscopy is a useful and versatile tool for describing exhaustively the interdiffusion of non-reacting heteronuclear gas mixtures.

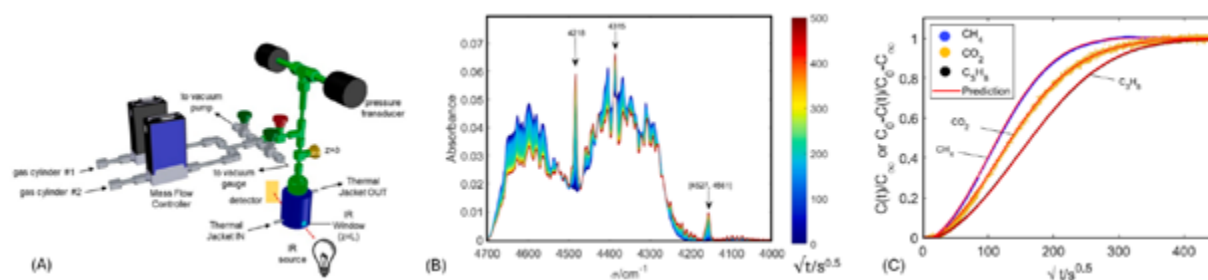


Figure (A) Experimental setup. (B) Time evolution of the IR spectrum during binary diffusion of C_3H_8 in CH_4 . (C) Ternary gas diffusion kinetics: experiment and modelling.

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O-085**HOW WE CAN GAIN STRUCTURAL INFORMATION ABOUT INDIVIDUAL COMPOUNDS IN COMPLEX ENERGY MIXTURES: THEORETICAL CALCULATIONS SUPPORT ANALYTICAL DATA AND LEAD TO FASCINATING RESULTS****Schrader, Wolfgang; Dreschmann, Jens; Vetere, Alessandro; Pfänder, Norbert; Lehmann, Christian W.; Poidevin, Correntin; Auer, Alexander.***Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany.*

Complex mixture analysis is kind of the gold standard for analytical methods. Discrimination and suppression effects are not the exception but the rule, as not one single analytical method is able to fully describe a complex system with some hundred thousand compounds present. Here, individual methods need to be used to focus on individual questions. One of the most complex analytical system are liquid fuels, regardless of them being from natural/fossil source or man-made biofuels.

Liquid fuel systems contain a mixture of different hydrocarbon-type compounds that are produced from a carbon source. Raw materials for fossil fuels are formed under geological conditions over large time frames, while biofuels are being made from a precursor source in different ways, like Fischer-Tropsch fuels. To better understand the formation pathways it is imminent to understand how complex these mixtures are and what type of compounds are being formed under which conditions. Here, we have combined sophisticated analytical methods such as ultrahigh resolution mass spectrometry with different types of ionization methods and high resolution electron microscopy (STEM) with different types of theoretical calculations on both the DFT- and coupled cluster level of theory to understand the structures of individual compounds in complex systems. High-resolution mass spectrometry allows the parallel detection of a vast range of different compounds, which can result in some 100,000 exactly measured signals that correspond to individual elemental compositions. Theoretical calculations allow finding the thermodynamically most stable structures of those compounds. Here, fascinating and unforeseen results from heavy fossil fuels are reported that show a surprising detection of carbon-only species. In crude oil, the most complex natural sample, we found naturally formed fullerenes and their building blocks, compounds that are interesting for a vast variety of applications. Especially interesting is the presence of non-IPR Fullerenes, which are considered to be thermodynamically unstable compounds. Non-IPR Fullerenes are compounds with adjacent 5-membered rings attached and are less stable than compounds like the C_{60} Buckminsterfullerene. Here, we report that in crude oil asphaltene these compounds ranging from C_{30} to C_{60} are even present in higher abundance and DFT calculations using the B3LYP functional along with D3 dispersion correction as well as Becke-Johnson (BJ) damping together with a geometry optimizations support these findings. For the first time, a natural sample is available to study their presence and formation pathways.

Fullerene synthesis happens under high-energy conditions, mostly laser ablation or high-voltage discharge is being used, while nature forms these compounds under geological conditions (ca. 150 °C and pressure).

Our results show that nature forms a large variety of fullerenes and fullertubes, which can be detected up to C_{148} , using ultrahigh resolution mass spectrometry while, additionally, electron microscopy allows a view into large multi-walled carbon nanotubes, which can also be detected. In all these findings, theoretical calculations support the results. These findings could open up a new interest in making different types of Fullerenes and functional carbon species in very different synthetic ways than before.

O-086**SPECTRAL SIGNATURES AND DEGRADATION: A MULTIVARIATE APPROACH TO MICROPLASTIC ANALYSIS BY MICRO-FTIR SPECTROSCOPY**

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Understanding microplastic (MP) origins and assessing plastic pollution impacts require precise chemical characterization. A major challenge arises from photoaging, which induces chemical changes in MPs, reducing the accuracy of automated spectroscopic identification. Micro-FTIR is widely used for MP screening, combining optical imaging with chemical analysis. However, reflectance micro-FTIR's sensitivity to particle thickness and photodegradation can hinder automated spectral matching.

Natural microplastic samples often require chemical pretreatment to remove contaminants, and such treatments may alter their composition. This study develops a method for identifying microplastics and determining their degree of photo-oxidation using multivariate analysis of micro-FTIR data [1], with potential for automated mapping. The approach is also extended to assess the effects of acidic, basic, and oxidative digestion on microplastics.

Six polymer types, sourced from commercial products, were studied: four conventional (PE, PP, PET, PS) and two compostable (PLA, PBAT). These polymers underwent artificial photoaging and/or chemical digestion before analysis via micro-FTIR in the mid-infrared range. Principal component analysis (PCA) successfully classified polymers and tracked degradation trends, detecting even subtle spectral changes. Special emphasis was placed on data preprocessing, which proved critical for reliable analysis. Various preprocessing strategies were tested and evaluated. PCA results were validated for polymer classification using a test set and compared with the carboxyl index (CI) method for degradation assessment. While CI is commonly used, PCA, which analyzes entire spectra without relying on manual peak integration, offers a more robust evaluation of multiple degradation mechanisms. Additionally, the study addressed spectral artifacts caused by particles thicker than 100 μm , which often interfere with polymer recognition through spectral. PCA successfully separated these particles, extracting information on particle thickness that conventional spectroscopic analysis could not access.

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O-087

IDENTIFICATION AND QUANTIFICATION OF MICROPLASTICS – A NEW APPROACH FOR INTERLABORATORY COMPARISON TESTS AND ANALYSIS IN FOOD SAMPLES BY FTIR, O-PTIR AND RAMAN MICROSCOPY

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The analysis of microplastic particles (MP) is, especially in the range below 10 µm, a complex multi-step process that includes sampling, sample treatment, measurement and data analysis. For all these steps exist numerous standard operation protocols (SOPs), however mandatory SOPs do not yet exist. We focus in this presentation to measurement and data analysis. For the measurement of MP, two different methods are available, Pyrolysis-gas chromatography/Mass spectrometry for mass-based results and spectroscopic methods for particle-based results. We focus in this presentation to the spectroscopic methods FTIR and Raman in combination with optical microscopy for the identification of the MP particles. For the data analysis, we use the open-source program GEPARD [1, 2] for an automated analysis. For the assignment of the spectra, a common method is an automated library search that uses comparison algorithms to assign the spectrum with the best match. Hence, the results often require a manual follow-up control, which is time-consuming and prone to introducing human bias. As an alternative, we developed a machine learning-based approach for the assignment of the spectra. We trained a model to identify several of the most common polymer types and compare its performance to the routinely used spectral database analysis. We present first results to a comparison of both evaluation methods.

However, comparability of results between different methods and different laboratories is an important issue. For that an interlaboratory comparison (ILC) test is necessary to compare the results of different methods and laboratories. We developed a new approach for an ILC test sample by fixing defined particles in a defined area on a filter which can be sent from laboratory to laboratory [3]. Results of an ILC will be presented [4]. The assignment of the spectra is done by automated library search and by machine learning-based approaches. Furthermore, we show statistical subsampling strategies and their error limits [5].

In the second part of the presentation, we show results for the identification and quantification of MP in food samples. These are the determination of MP in yoghurt cups and beer using O-PTIR and Raman and the investigation of different mineral waters by inspection along a production line from the source water over the process water to the final mineral water in the containers. Here we compare single use and reusable PET bottles, glass bottles and carton-based packaging. We inspected also commercial products from the supermarket, like rock salt and sugar, by Raman and show the results.

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O-088**ASSESSMENT OF METAL VARIABILITY IN SEAWEED EXPOSED TO SILVER AND TITANIUM DIOXIDE NANOPARTICLES****López Mayán, Juan José¹; Rodríguez-Novoa, Laura²; González-Barcia, Sheila²; Barciela-Alonso, María-Carmen²; Peña-Vázquez, Elena²; Moreda-Piñeiro, Antonio².**

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Seaweeds are known for their beneficial properties in the diet, which has led to an increase in their consumption worldwide. However, they are also organisms that can concentrate contaminants. They can bioaccumulate heavy metals and even nanoparticles. Finally, any contaminants present in the seaweed could be transferred to the food chain. While heavy metals are recognized to be toxic to seaweeds, there are discrepancies regarding the potential toxicity of nanoparticles. Two nanoparticles that stand out for their industrial use due to their applications are silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO₂NPs). Previous studies on bioaccumulation [1,2] and bioavailability [3] have demonstrated the ability of these particles to accumulate, and be internalised by seaweeds, as well as their presence in the human gastrointestinal tract.

This study aims to investigate potential correlations between the bioaccumulation of nanoparticles and the change in elemental content. The bioaccumulation tests involved green (*Ulva* sp.) and red (*Palmaria palmata*) macroalgae exposed to 0.1 and 1.0 mg L⁻¹ of 15 nm PVP-AgNPs and 5 nm citrate-TiO₂NPs, for 28 days. The exposed seaweeds were microwave digested and analysed by ICP-MS to determine the content of Ag and Ti, as well as the elemental concentration variations.

The elements (Cu, Co, Cr, Mo, Ni, Rb, V, Zn, As, Cd, Ce, Sb, Se, and Pb) were determined at different exposure times (0, 7, 14, 21, and 28 days) for both nominal nanoparticle concentrations (0.1 and 1.0 mg L⁻¹). The selection of the analytical isotope for each element was performed studying the accuracy in the analysis of BCR[®]-279 and ERM[®]-CD281 certified reference materials and the UNE-EN ISO 17294-1: 2004 norm. The analytical performance of the methodology was assessed, as the use of standard addition, LODs and LOQs were calculated, precision was evaluated through repeatability, and accuracy using certified reference materials and analytical recoveries.

The results for *Ulva* sp. and *Palmaria palmata* exposed to silver showed high variability depending on the type of seaweed and element, with some of them below the LOQ, such as Se, Zn, As, and Sb. On the other hand, the results for *Ulva* sp. exposed to titanium showed values below the LOQ for Pb, Se, and Sb, but an increase in metal content with higher Ti concentrations was observed, except for Zn.

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O-089**BOTTOM-UP PROTEOMICS IDENTIFIES AND QUANTIFIES ALLERGENIC PROTEINS IN THE NOVEL FOOD SPIRULINA****Mariachiara Bianco^{1,2}, Giovanni Ventura^{1,2}, Cosima Damiana Calvano^{1,2}, Ilario Losito^{1,2}, Tommaso Cataldi^{1,2}**¹Department of Chemistry, University of Bari Aldo Moro, Bari, Italy; ²SMART centre, University of Bari Aldo Moro, Bari, Italy

In recent years, novel foods have emerged as sustainable alternatives to traditional meat-based protein sources. Within the European Union (EU), novel foods are defined as those not widely consumed before May 1997 [1]. A prominent example is *Arthrospira platensis* (spirulina), a nutrient-rich microalgae lauded for its high protein content (>60% dry weight), antihypertensive peptides, polyunsaturated fatty acids (ω -3 and ω -6), and essential vitamins [2]. Despite its nutritional benefits, the allergenic potential of spirulina remains unexplored [3]. This study employed a bottom-up proteomic approach, combining untargeted and targeted mass spectrometry (MS), to identify and quantify known and putative allergenic proteins in spirulina, along with their marker peptides. First, high-resolution LC-MS/MS was used to characterize the spirulina proteome [4]. Following FAO/WHO guidelines and leveraging the Allergen Online database, we assessed the similarity between spirulina proteins and known allergens. Six proteins were flagged as known or potential allergens, including thioredoxins, superoxide dismutase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, and the C-phycoerythrin beta subunit [5]. Next, a targeted MS approach was applied to analyse marker peptides from these allergenic proteins. Peptides were preselected via in-silico screening, considering criteria such as length and uniqueness, and their stability was experimentally validated in spirulina-enriched food matrices (cookies, bread, and fruit juice). Three marker peptides from the C-phycoerythrin beta subunit were identified: ETYLALGTPGSSVAVGVGK and YVTYAVFAGDASVLEDR (qualifier peptides), and ITSNASTIVSNAAR (quantifier peptide). The method was rigorously validated, assessing linearity, limits of detection (LOD) and quantification (LOQ), recovery, and matrix/processing effects [6].

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O-090**SIMULTANEOUS QUANTIFICATION OF MULTIFAMILY PLANT GROWTH REGULATORS IN GRAPES AND AVOCADOS BY QuEChERS-LC-MS/MS****Bolcato, Leonardo¹; Berli, Federico²; Fontana, Ariel³.**

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Plant growth regulators (PGRs) are involved in several life cycle processes of plants such as cell division and differentiation, growth, sprouting, and maturity, as well as in protective responses. Every plant produces a number of PGRs, such as auxins, gibberellins, cytokinins, and abscisic acid (ABA), with levels varying according to the growth stage. In addition, a number of synthetic PGRs are also available and regularly used in agriculture including cytokinins such as 6-benzyl aminopurine (6-BA) and forchlorfenuron (CPPU). The quantification of PGRs in fruits is relevant not only to evaluate specific biochemical processes associated to their level's variation but also because a common agricultural practice is the spraying of crops to optimize fruits growth and quality.

In this work, a rapid and simple analytical method for the simultaneous quantification of 14 multifamily PGRs namely, gibberellins (gibberellic acid (GA3), gibberellin 4 (GA4), gibberellin 5 (GA5)), auxins (indol-3-acetic acid (IAA), indol-3-butyric acid (IBA)), cytokinins (zeatin (ZT), kinetin (KT) and 6-benzyladenine (6-BA)), abscisic acid (ABA), salicylic acid (SA), nicotinic acid (NA), methyl jasmonate (MJ) and CPPU within a single experiment is presented. The method is based on a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) sample preparation approach coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). After optimization, the sample preparation method involved the extraction of fruit material (acidified with 1 % formic acid) with an acetonitrile-ethyl acetate mixture (1:1) for white and red grapes; and with acetonitrile for avocado by homogenization for 2 min by hands-shaking. The pH of the extract was enhanced by adding ammonium acetate and salting-out performed with 1 g of MgSO₄, followed by homogenization and centrifugation. The supernatant extract was cleaned by dispersive solid phase extraction (d-SPE) using a combination of PSA and C₁₈ sorbents (50 mg of each, per mL extract). To achieve high-throughput screening of tested samples, the final extract was analyzed directly by LC-MS/MS in positive and negative ion switching mode of electrospray ionization (ESI). The NA, IAA, IBA, KT, ZT, MJ, 6-BA and CPPU PGRs were monitored in positive ESI, whereas SA, ABA, GA3, GA4 and GA5 in negative ESI mode. Quantification was performed by scheduled multiple reaction monitoring (sMRM). All compounds were analyzed by a single chromatographic run of 18 min by using gradient chromatographic conditions. Significant matrix induced signal suppression for white grapes (9-28 %), red grapes (57-98 %) and avocado (12-66 %) extracts were observed when the responses for pre- and post-extraction addition of analytes were compared. This could be resolved by the use of matrix-matched calibration standards. The proposed method could be successfully applied in the analysis of natural and exogen incurred samples. Therefore, it is useful not only to determine the endogenous levels of PGRs to understand biochemical processes, but also in the evaluation of exogenous applications of PGRs on the basis of measured endogenous levels.

O-091**SEMI-UNTARGETED UHPLC-MS/MS ALKALOID FINGERPRINTING FOR RAW LUPIN TRACEABILITY****Eugelio, Fabiola¹; Palmieri, Sara¹; Mascini, Marcello¹; Fanti, Federico¹; Della Valle, Francesco¹; Del Carlo, Michele¹; Compagnone, Dario¹; Sergi, Manuel².**¹University of Teramo, Teramo, Italy; ²Sapienza University of Rome, Roma, Italy.

Lupins (Fabaceae family) are gaining global attention as a cost-effective alternative to other legumes due to their rich nutrient and phytochemical composition, offering numerous health benefits. However, they also contain quinolizidine alkaloids (QAs), neurotoxic metabolites derived from L-lysine (C₅NC₄ skeleton), acting as a defence mechanism against pathogens and herbivores [1]. Nearly 70 different QAs have been identified across various lupin species, whose levels and combinations vary according to several environmental factors. They impart bitterness to the seeds and can cause poisoning in humans; for this reason, a proper debittering process is essential for safe consumption. According to this, International Regulations require compliance with a maximum level of 200 mg kg⁻¹ of alkaloids in lupin flours and in seeds themselves [2].

To our knowledge, few studies using liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been developed for QAs detection, and none have comprehensively characterized QA profiles or confirmed a geographical classification by multivariate regression models.

This study presents the development of a targeted and semi-untargeted approach by ultra-high performance LC coupled with triple quadrupole-linear ionic trap-MS/MS (UHPLC-QqQ-LIT-MS/MS) combined with *in silico* experiments and multivariate analysis. The method was applied to raw *Lupinus albus* L. samples originating from different southern-center Italy regions; based on their QAs content, the origin classification of lupins was explored [3].

The samples were extracted and subjected to a suitable clean-up step by Solid Phase Extraction [4], followed by a predictive multi-experiment approach for QAs analysis, exploiting the combination of multiple reaction monitoring (MRM), information-dependent acquisition (IDA), and enhanced product ion (EPI) techniques. The analysis enabled the exploration of the QA profile of all samples, revealing the simultaneous quantification of 6 targeted QAs and a putative determination of 21 additional alkaloids, including several with previously unreported fragmentation spectra. The putative identification of these compounds was further supported by matching dependent MS/MS spectra acquired in EPI mode with in-house databases, built using *in silico* software Competitive Fragmentation Modeling for Metabolite Identification (CFM-ID).

Subsequently, an unsupervised Hierarchical Cluster Analysis (HCA) with heatmap, followed by a supervised Partial Least Squares Discriminant Analysis (PLS-DA), was applied. Remarkably, the PLS-DA model allowed a successful and accurate classification of these samples according to their origin, demonstrating the potential of QA profiling as a marker for authenticity and traceability.

This method provides a valuable tool for investigating intra-species QA variability and verifying the authenticity and traceability of lupin-based products.

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O-092

ADVANCED ANALYTICAL ASSESSMENT OF PHENOLIC COMPOUNDS FROM TANNAT GRAPE POMACE: BIOACCESSIBILITY AND METABOLITE PROFILING AFTER SIMULATED DIGESTION

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Grapes of the *Vitis vinifera* L. cv Tannat variety are known for their exceptionally high content of phenolic compounds, particularly procyanidins and anthocyanidins, which are highly valued for their health-promoting properties. One of the main by-products generated during the winemaking process is pomace, a mixture of grape seeds, skins, and pulp, that still contains significant amounts of these beneficial bioactive compounds. These molecules exhibit antioxidant properties that may help protect the brain from age-related oxidative damage, offering potential neuroprotective effects. However, the effectiveness of phenolic compounds *in vivo* depends on factors such as their bioaccessibility, bioavailability, and capacity to cross the blood-brain barrier. As such, gaining insight into these factors is essential to fully understand the health benefits these compounds may provide.

The objective of this study was to investigate how gastrointestinal digestion and metabolism influence the bioactive compounds present in a Tannat grape pomace extract. This was achieved by simulating intestinal digestion and colonic fermentation. Additionally, the study aimed to evaluate the ability of the bioaccessible metabolites to cross the blood-brain barrier and assess their potential neuroprotective properties.

Pressurized Liquid Extraction (PLE) was used as an environmentally friendly method to obtain a phenolic compound-rich extract. This extract underwent sequential static gastrointestinal and colonic digestion using a dynamic gastrointestinal simulation system (simgi®). Following colonic fermentation, the bioaccessible portion was analyzed to identify digestion-derived metabolites through HPLC-MS/MS and 2DLC-MS/MS techniques.

The results of this study highlight the significant influence of simulated gastrointestinal digestion and microbial activity on both the bioaccessibility and neuroprotective potential of phenolic compounds from Tannat grape pomace extract.

O-093**EVALUATION OF MATRIX ISSUES IN THE APPLICABILITY OF LC-MS ON THE DETECTION OF CIGUATOXINS IN FISH SAMPLES****Sanchez-Henao, Andres¹; Flores, Cintia²; Real, Fernando¹; Darias-Dágfeel, Yefermin¹; García-Álvarez, Natalia¹; Diògene, Jorge³; Rambla-Alegre, Maria³.***¹University Institute of Animal health and food safety-University of las Palmas de Gran Canaria, Arucas, Spain; ²CSIC-IDAEA, Barcelona, Spain; ³IRTA, Marine and Continental Waters Program, La Ràpita, Spain.*

For liquid chromatography-mass spectrometry (LC-MS) analysis of ciguatoxins (CTXs), the marine biotoxins accumulated in fish tissues responsible of causing Ciguatera Poisoning (CP), is almost mandatory the use of purification protocols given the complexity of the matrix that contains them, the initial raw extract usually presents co-extractives (mainly lipophilic) that interfere with the analysis of these compounds. When this interference promotes signal enhancement is less problematic than ion suppression, this inconvenience lies in low concentrations presented in fish samples resulting in CP. To date, more than 30 analogues of these compounds have been described, and they are chemically classified into 4 groups: CTX4A, CTX3C, C-CTX and I-CTX. Their differences give them unique properties that cause different LC-MS behaviour to differ between chemical groups, influenced in turn by the starting matrix, the resulting co-extractives and even the extraction method, among other variables such as the chromatographic separation conditions chosen. Consequently, the laboratories responsible for the study of these molecules employ the purification protocol that is most suitable for the CTX analogues typically encountered in their geographical region and their analytical equipment. In this regard, as there is no regulation on the levels of CTXs allowed in fish for consumption in Europe, and the sale of fish containing CTXs is prohibited, the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) from USA have set a guidance levels for safety consumption at 0.01 ng CTX1B/g fish flesh. But in addition, there is no standardized and validated method for the determination of CTXs. Moreover, only a few of CTXs are commercially available. These circumstances pose an analytical challenge for the study of these compounds in naturally contaminated matrices, when the aim is to maintain consumer safety.

In the present work, we compare a series of Solid Phase Extraction (SPE) in two study phases. Firstly, 10 different SPEs commonly used by CTX international specialist research groups (Japan, Spain, USA, France and Germany), were applied at the same fish matrix naturally contaminated with C-CTX1, in order to evaluate the performance of this CTX analogue in all purified extract analysed by Low Resolution and High-Resolution Mass Spectrometry (LC-MS/MS and HRMS) after each purification process. Then, the 6 most promising ones, were chosen for a more exhaustive comparison involving at least one CTX of each group: CTX1B from CTX4A group, CTX3C, and C-CTX1 (I-CTX group was not studied because contaminated matrices are hard to obtain). This new comparison focused on recovery, matrix effect and efficiency of each SPE using commercial standards of CTXs (CTX1B and CTX3C), spiked in a naturally contaminated fish sample with a low concentration of C-CTX1. The assessment for this second comparison was conducted by LC-MS/MS given the low concentration of C-CTX1 in the sample chosen.

The results of the first comparison demonstrated that not all SPEs were suitable for evidence the presence of C-CTX1 in the mass spectrometry assessment, this could be interpreted as no recovery of C-CTX1 at all or a total ion suppression. Another aspect that could influence this result is that most of the SPEs were designed for CTX4A and CTX3C group analogues, not for C-CTXs.

In the second comparison, the results obtained demonstrated that all cleaned-up extracts recovered the 3 analogues of CTXs, however, the recoveries and the matrix effects varied substantially, but also, the behaviour of analogues with similar polarity was even different depending on the SPE column, resulting in different efficiencies. Despite these differences, two purification protocols stood out from the others due to their efficiencies in CTX1B and CTX3C, but also in C-CTX1 signal recoveries.

O-094**Mini-SPME/GC-MS FOR THE QUANTIFICATION OF PESTICIDE RESIDUES IN GRAPES: GREENNESS ASSESSMENT VIA GAPI AND AGREEprep****Syrgabek, Yerkanat; Alimzhanova, Mereke.***Al Farabi Kazakh National University, Center of Physical-Chemical Methods of Research and Analysis, Almaty, Kazakhstan.*

Solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS) offers a solvent-free and efficient approach for the quantification of pesticide residues in various matrices [1–3]. However, conventional SPME approaches often require relatively large sample volumes and longer equilibration times, which can limit their applicability for rapid or resource-conscious analysis. To address these limitations, a miniaturized headspace solid-phase microextraction (Mini-HS-SPME) method combined with GC-MS was developed in this study for the identification and quantification of ten pesticides (atrazine, simazine, prometryn, metribuzin, cyanazine, fluroxypyr-meptyl, tebuconazole, epoxiconazole, quizalofop-p-methyl, and boscalid) in grape samples. Key extraction parameters such as extraction temperature, extraction time, selection of the optimal fiber, incubation time, effect of salt, volume of sample and pH were optimized.

The optimal method parameters were obtained, such as extraction temperature of 90°C, extraction time of 30 min, sample volume of 200 μL , pH 6.56, incubation time of 10 min and commercial PDMS 100 μm polymer fiber. The method was validated in terms of linearity, precision, recovery, and sensitivity. The limits of detection (LODs) and limits of quantification (LOQs) for the target pesticides were determined to be in the ranges of 0.0009–0.05 $\mu\text{g mL}^{-1}$ and 0.003–0.16 $\mu\text{g mL}^{-1}$, respectively. The optimized procedure was successfully applied to the analysis of real grape samples.

To evaluate the environmental impact of the analytical workflow, greenness assessments were carried out using the Green Analytical Procedure Index (GAPI) and AGREEprep tool [4,5]. Evaluations using aforementioned tools highlighted that Mini-SPME has a lower environmental impact with 0,77 points according to AGREEprep results and is environmentally friendly according to the GAPI tool. This work highlights the effectiveness of Mini-SPME/GC-MS as a green, reliable, and sensitive technique for routine pesticide monitoring in grapes.

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O-095**AMBIENT MASS SPECTROMETRY FOR FOOD ALLERGEN SWABBING TESTS: AN EXTENDED FRAMEWORK FOR SWAB TOUCH SPRAY-MASS SPECTROMETRY TECHNIQUE****Toma, Lorenzo¹; Piergiovanni, Maurizio¹; Gentili, Silvia¹; Mattarozzi, Monica¹; Careri, Maria¹; Moyano, Encarnación².**¹*Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy;*²*Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain.*

Swab Touch Spray-Mass Spectrometry (Swab TS-MS) falls within the Ambient Mass Spectrometry techniques that allow direct, in-situ and high-throughput analysis, as well as being in line with the green analytical chemistry principles. Swab TS-MS involves the generation of gas-phase ions from the tip of a medical swab which acts both as a sampling tool to collect analytes from a surface/tissue and as an electrospray probe.¹ So far, the applicability of Swab TS-MS has been investigated only for small molecules, especially in forensic and medical fields, leaving the analysis of peptides and large biomolecules still unexplored. The analysis of food allergens has received considerable and increasing interest in the recent decades to safeguard sensitized individuals.² Food allergens can be hidden by cross-contamination from food-contact surfaces in restaurants, canteens, and industrial production lines where different ingredients are processed. This requires the implementation of proper cleaning procedures, the effectiveness of which should be monitored by swab tests that are commonly based on commercially available kits for rapid and easy-to-use colorimetric or immunochemical assays.

The great promise of the Swab TS-MS technique lies in swab sampling and direct MS analysis, thus for the first time the present study aimed to combine swab sampling of allergenic proteins from food preparation surfaces with Swab TS-MS analysis.³ Egg white lysozyme was selected as a case study of an allergenic protein, taking into account that egg belongs to the eight major allergenic foods, for which the eliciting dose ED05 - adopted by the Voluntary Incidental Trace Allergen Labelling (VITAL 4.0) as reference dose for determining precautionary allergen labelling - is set at 2 mg of total egg protein.

The development of Swab TS-MS method required proper setting of geometrical and MS-tune parameters, as well as the selection of the solvent delivered to the swab tip by the syringe pump. Since none of the experimental conditions allowed the detection of intact lysozyme, a bottom-up procedure based on tryptic digestion for lysozyme detection by Swab TS-MS, operating in MS/high resolution (HR)MS tandem mode, was investigated and the surface swabbing was designed to include tryptic digestion as well. Liquid chromatography-tandem mass spectrometry was used to develop and characterize an in-situ digestion/swabbing strategy, allowing to reach a LOD value of 0.003 $\mu\text{g}/\text{cm}^2$ of egg white powder consistent with that of lateral flow immunoassay technique. Finally, the developed in-situ digestion/swabbing procedure was coupled to the Swab TS-MS/HRMS method, allowing the direct analysis of egg white powder residues on stainless steel surfaces, reaching a LOD of 68 $\mu\text{g}/\text{cm}^2$ egg white powder, corresponding to 2.4 $\mu\text{g}/\text{cm}^2$ of lysozyme. This result is successful if we consider that in the target protein is lysozyme, i.e. the least abundant allergenic protein in egg white. Furthermore, the method can be applied to more abundant egg proteins and it can be improved by evaluating other swab materials and surface properties to enhance swabbing recovery.

This study should be intended as a first step for the application of Swab TS-MS technique in protein analysis: the developed in-situ digestion and sampling strategy suitable for Swab TS-MS direct analysis of food allergens could be particularly useful for the development of effective cleaning procedures, taking advantages of the multiplexing capability of MS detection and overcoming cross-reactivity issues.

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O-096**LIQUID SCINTILLATION SPECTRA DECONVOLUTION: AN APPROACH FOR DEVELOPMENT OF RAPID AND LOW-WASTE METHODS****Fons-Castells, Jordi; Tent-Petrus, Joana; Llauredó, Montserrat; Rigol, Anna.***University of Barcelona, Barcelona, Spain.*

Measurement of radioactivity is crucial in various scientific and industrial fields, including environmental monitoring, medical applications and nuclear energy. One of the most significant areas where radioactivity measurements will play a vital role in the next few years is in the decommissioning of nuclear power plants. Ensuring accurate and reliable measurement techniques is essential for assessing contamination levels, classifying waste, and ensuring safety during dismantling operations. Furthermore, in emergency situations, rapid methods are critical to quickly assess contamination levels and guide effective response actions.

There are two main approaches to measuring radioisotopes: radiometric and mass spectrometry-based techniques. Radiometric methods, such as gamma spectrometry, alpha spectrometry, or liquid scintillation counting (LSC), are based on detecting the radiation emitted by radioactive isotopes. Mass spectrometry techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) and accelerator mass spectrometry (AMS), on contrary, do not rely on radioactive decay but rather on detecting the nuclide. While mass spectrometry-based approaches offer advantages in terms of isotopic discrimination, for radionuclides with short half-lives (less than 10 years), radiometric techniques present better detection limits [1].

LSC is a widely used technique for measuring alpha and beta emitters which involves dissolving the radioactive sample in a scintillation cocktail, where radiation interacts with the scintillator molecules to produce light pulses. These pulses are then detected and quantified to determine the activity of the sample.

One of the main advantages of LSC is its high efficiency in detecting low-energy beta emitters, which are often challenging to measure using other techniques. However, a significant challenge arises from the continuous nature of beta spectra, which can lead to spectral overlap between different beta-emitters and hinder the quantification of individual isotopes. To solve this issue, chemical separation techniques are often employed to isolate specific isotopes before measurement. Methods such as ion exchange chromatography or solvent extraction allow the selective separation of target isotopes from interfering species. While effective, these procedures are time-consuming and generate significant amounts of chemical waste, increasing the overall environmental impact of the analysis.

Spectral deconvolution techniques have proven to be effective in overcoming the challenges associated with beta spectra overlap. By using mathematical models and computational algorithms, spectral deconvolution can separate overlapped spectral components, improving isotope identification and quantification. This approach reduces the need for extensive chemical separation, leading to faster analytical methods with reduced waste generation.

In this communication we discuss the advantages of LS spectra deconvolution by means of partial least squares regression (LSS-PLS) in three scenarios: mixture of artificial alpha and beta emitters in nuclear facilities [2], tritium determination in environmental samples, and separation of isotopes of the same element, such as ^{89}Sr and ^{90}Sr [3].

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O-097

USING LIFE CYCLE ANALYSIS TO ENHANCE ANALYTICAL SUSTAINABILITY

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Analytical laboratories are working to minimize their environmental footprint and promote a more sustainable model. In this context, the insights provided by quantified data are crucial for informed decision-making. Life cycle analysis (LCA), a standardized method for evaluating environmental impacts by categories, provides a multi-criteria and quantified approach. An LCA was carried out on a method dedicated to natural ingredients using liquid chromatography (UPLC) coupled with diode array (DAD) and charged aerosol (CAD) detectors, as well as high-resolution mass spectrometry (HRMS). This method was selected due to its frequent use and because it offers the possibility of extending the conclusions to large set of analytical equipment. Objectives were to identify environmental hotspots and evaluate their potential reduction.

After defining the goals and scope of the study, the Life Cycle Inventory Analysis (LCIA) was conducted using the ecoinvent 3.10 database, and the Environmental Impacts Assessment was performed with the Product Environmental Footprint (PEF) method 3.0 of European Commission.

The LCA revealed that equipment depreciation, particularly of the computer, and its energy consumption during the two days of data processing stage contribute substantially to this impact in this context (i.e. conducting the analytical method in a French laboratory).

Key optimization strategies for reducing our environmental footprint include automating processes to shorten data processing time and utilizing refurbished computers. Optimizing equipment use offers even greater environmental benefits compared to reducing or substituting consumables. It is important to note that the dominant impact factors and most effective mitigation strategies may vary significantly in other regions due to differences in energy sources, recycling infrastructure, and other local conditions.

The environmental impact of this method is 0.40 kgCO₂eq (climate change indicator) per injection. For an analysis of natural ingredient, includes on average 20 samples per sequence and 2 days of data processing, the impact is 8 kgCO₂eq.

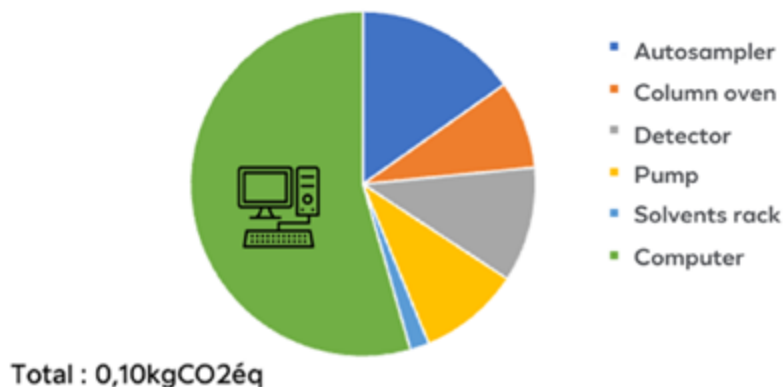


Figure 2: Contribution of each piece of equipment to the Climate Change indicator (20 samples per sequence and 2 days of data processing)

To further refine our understanding of sustainable analytical practices, a comparative LCA is currently underway, evaluating the environmental impact of different analytical techniques—HPLC, UPLC, and GC—for the analysis of the same compound. This comparative analysis will provide a more comprehensive assessment of the environmental trade-offs associated with each technique, allowing for more informed decisions on method selection for sustainable laboratory practices. Results of this comparative study will be available shortly and will be presented at the congress.

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O-098**SUSPECT SCREENING OF MICROPLASTIC-ASSOCIATED EMERGING CONTAMINANTS IN MEDITERRANEAN FISH USING SUPRAS-LC-HRMS****Saber, Ayman; Caballero-Casero, Noelia; Rubio, Soledad.***Department of Analytical Chemistry, Institute of Chemistry for Energy and the Environment, University of Córdoba, CORDOBA, Spain.*

Microplastics (MPs) in marine ecosystems are recognized vectors for emerging contaminants (ECs), including plastic additives, pesticides, pharmaceuticals, and industrial chemicals. These pollutants and their metabolites and degradation products can adsorb to MPs and accumulate in fish tissues through ingestion, raising concerns for seafood safety and human exposure.

This study applies a suspect screening workflow combining supramolecular solvent (SUPRAS) extraction with liquid chromatography–high-resolution mass spectrometry (LC-HRMS) to comprehensively identify ECs and their transformation products in Mediterranean fish. A broad suspect list of 10,000 compounds was used, encompassing parent chemicals, metabolites, and degradation products associated with microplastics.

Fish samples of *Merluccius merluccius* (European hake) and Horse Mackerel, collected from Mediterranean markets, were analyzed for ECs in fish tissues, with a focus on compounds known to adsorb onto microplastics (MPs). A SUPRAS, composed of 1-decanol and tetrahydrofuran, was employed for extraction. This amphiphilic system enabled efficient recovery of hydrophobic, semi-polar, and polar ECs from complex biological matrices, including plastic additives, PFAS, pesticides, and other trace-level pollutants potentially linked to MP exposure. Without isolating MPs, the method allowed indirect chemical evidence of MP-associated contamination to be captured from fish tissues. Compared to conventional solvents, SUPRAS offered enhanced selectivity, broad polarity coverage, and strong matrix tolerance, providing a single-step approach suitable for high-throughput screening.

Extracts were analyzed using LC-HRMS with a C18 column under a water/methanol gradient in both positive (ESI⁺) and negative (ESI[−]) ionization modes. To improve ionization efficiency across diverse compound classes, 0.5 mM ammonium fluoride was added to both mobile phases. High-resolution full-scan MS combined with data-dependent MS/MS enabled structural elucidation. Features above 1,000 counts (ESI⁺) and 500 counts (ESI[−]) were annotated using exact mass (± 5 ppm), MS/MS spectral similarity ($\geq 75\%$), and in silico fragmentation (SIRIUS), with all identifications manually verified. This SUPRAS-LC-HRMS workflow allowed untargeted suspect screening of over 10,000 compounds, supporting comprehensive chemical characterization of ECs in marine fish tissues.

A total of 33 compounds were detected in *Merluccius merluccius* and 53 in Horse Mackerel. Identified chemicals included bisphenols (BPS), plastic additives (e.g., Tri(2,4-di-*t*-butylphenyl) phosphate, erucamide), antioxidants, surfactants, and pesticide residues such as dimethrin and pyrethrin derivatives. This broad chemical profile highlights the role of MPs as multi-functional vectors for both regulated and unregulated ECs.

Sixteen compounds were shared between both species—including BPS, 2-acetylacetophenone, piperonyl butoxide, and 2-(*p*-nonylphenoxy) ethanol—despite differences in their ecological niches and feeding behavior. This overlap indicates widespread environmental distribution and the systemic nature of MP-associated contamination in the marine food web.

Transformation products were frequently co-detected with their parent compounds, confirming in situ or biological transformation. For example, methylated derivatives of pyrethroids, phosphates, and antioxidants occurred alongside their respective parent molecules. Detection of synthetic flavorants such as 4-methyl-5-thiazoleethanol—commonly associated with packaging—points to additional contamination pathways from coastal activity and food industry discharges.

Although concentrations were not determined, the co-occurrence of multiple contaminants and their derivatives suggests a potential for cumulative dietary exposure. Importantly, this study demonstrates that SUPRAS-LC-HRMS enables the untargeted detection of transformation products and polymer degradation chemicals, offering a powerful, high-coverage tool for chemical surveillance in marine organisms.

These findings underscore the need for routine monitoring of MPs and associated ECs in commercial seafood species, including transformation products typically excluded from regulatory evaluations. Risk assessment frameworks should be revised to account for the full chemical burden carried on by MPs. Future research must prioritize species-specific uptake patterns, bioavailability of transformation products, and combined toxicological impacts to improve seafood safety assessments and guide policy on plastic-associated pollution in marine environments.

Acknowledgements

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O-099**SEMI-TARGETED STRATEGIES INTEGRATING GREEN ANALYTICAL CHEMISTRY PRINCIPLES INTO FOOD LIPID ANALYSIS****Donato, Paola¹; Bonaccorsi, Ivana Lidia¹; Battaglia, Pietro²; Rigano, Francesca¹; Reale, Cristian¹; Dugo, Paola¹; Mondello, Luigi¹.***¹University of Messina, Messina, Italy; ²Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Messina, Italy.*

The need for greening all aspects related to chemistry has been favourably received within the scientific community, and thus, much effort is being put into developing more sustainable practices. Concepts such as safety, waste and energy reduction, and low environmental impact encompass all branches of chemistry, including analytical determinations. Alongside well-established high-performance liquid chromatography (HPLC) and gas chromatography (GC) techniques, in recent years the use of supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) have attracted renewed interest from researchers in the food sector, including food lipidomics.

This research focused on the development of greener analytical methods for the analysis of lipids in foods of plant and animal origin, aiming to assess their nutritional value (triacylglycerols, polyunsaturated fatty acids), authenticity and traceability.

Packed-column SFC was performed on silica-based chemically bonded sorbents, with solvent-modified fluids of low compressibility and isocratic elution. A wider range of experimental variables was available for optimization, relative to HPLC, including the temperature, the type and concentration of modifiers in the carbon dioxide mobile phase and its density. Compared with HPLC, SFC offered complementary selectivity, higher separation efficiency, and shorter analysis time. Moreover, the lower organic solvent consumption (and waste) alongside the employment of non-toxic mobile phases make SFC also a valid means for developing greener methods for routine food analysis. The twelve input variables of the AGREE metric tool were finally compared for each procedure to show the overall impact of the greenness improvement.

To this regard, also searching for green and ecofriendly solvents to replace classical solvents for industrial-scale separation and extraction of lipids is of great interest.

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O-100**THE SPECTRUM OF YOUR ANALYTICAL METHOD: IS IT GREEN, BLUE, RED, OR VIOLET?**

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In recent years, analytical chemistry has evolved significantly, driven by increasing demands for sustainable, efficient, and high-performing analytical methodologies. This evolution has led to the development of numerous assessment tools and metrics designed to evaluate essential aspects such as eco-friendliness, practicality, and analytical performance. Collectively, these aspects form the basis of White Analytical Chemistry (WAC), a comprehensive framework that extends the principles of Green Analytical Chemistry (GAC) using a Red-Green-Blue (RGB) model. Within this model, methods that integrate sustainability (green), practicality (blue), and performance (red) are considered "white," representing the ideal analytical methodology. Some key metrics developed to assess these individual aspects include the Green Analytical Procedure Index (GAPI) and the Analytical Greenness Metric (AGREE), both focused explicitly on sustainability. GAPI provides a visual color-coded scale highlighting the ecological footprint of analytical methods, while AGREE quantifies environmental friendliness using a comprehensive scoring system. Advanced holistic green assessment tools, including Complex Modified GAPI (ComplexMoGAPI), integrate multiple dimensions into a unified evaluation, allowing analysts to visualize method characteristics comprehensively. Conversely, the Blue Applicability Grade Index (BAGI) evaluates method practicality, emphasizing ease of implementation, resource accessibility, and overall convenience for analysts. The Red Analytical Performance Index (RAPI) is a tool for evaluating analytical methods in terms of their performance (repeatability, precision, reproducibility, trueness, recovery, etc.). Recently, the Violet Innovation Grade Index (VIGI) was introduced as an innovative metric specifically designed to quantify the novelty and originality of analytical methods. This survey-based tool addresses the need to recognize and promote innovation within analytical chemistry explicitly. The integration of these metrics provides analysts with robust visual representations, identifying strengths, weaknesses, and potential improvements in methodologies. Such assessments are critical for informed decision-making, method development, and benchmarking comparisons between analytical techniques. Practical implementation highlights the importance of harmonizing these tools, comprehensive user training, and their inclusion in analytical chemistry curricula to ensure standardized and effective evaluations. The future of analytical chemistry assessment tools appears promising, particularly given advancements in technology, increasing regulatory standards, and the global emphasis on sustainability. Continued refinement and harmonization of these evaluation metrics will further enhance the capability of analytical chemists to select and develop methods that are sustainable, practical, and high performing, effectively capturing the full spectrum of analytical method characteristics.

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WHERE DID ALL THE SULFUR GO? - A DETAILED EXAMINATION OF CRUDE OIL HYDRODESULFURIZATION BY FTMS AND QUANTITATIVE LEC-ICP-MS/MS

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Hydrodesulfurization (HDS) of crude oil fractions is one of the most frequently used catalytic reactions in the world. Additionally, it is one of the most important industrial reactions for the environment because it helps removing sulfur from fuels to avoid effects such as acid rain. Here, we investigate different products from an industrial HDS stream and their untreated feed stream to evaluate sinks and sources of various compound classes. The process involves different heterogeneous catalysts. A common feature of which is their ability to catalyze the removal of sulfur (and other heteroatoms) from organic compounds in the form of H₂S (or analogous), when treated with hydrogen under pressure and at elevated temperatures. Depending on the nature of sulfur containing compounds present, the removal of sulfur is more or less hindered. In such cases harsher conditions are needed, which results in the undesired hydrogenation of aromatic hydrocarbons.

Products from an industrial HDS stream and the corresponding feed were analyzed in this study. Qualitative analyses on intact molecules were performed as direct injection experiments on a 7 T FT-ICR (Thermo Fisher Scientific, Bremen, Germany), additionally equipped with an FTMS Booster X3 (Spectroswiss Sarl, Lausanne, Switzerland) to enable better mass resolution by using absorption mode Fourier Transform. Increased sensitivity was obtained by segmenting the observed mass range into small compartments for measurement, before recombination into a complete mass spectrum.[1] For ionization of the constituents of this rather non-polar sample Atmospheric Pressure Photoionization (APPI) was used.

Quantitative analyses of the sulfur content of the samples was performed by HPLC-ICP-MS/MS using a lab-made Pd(II) stationary phase (2x150 mm, 5µm spherical particles).[2] By this, sulfur containing compounds can be separated into three different compound classes and quantified separately. Element selective analysis was performed on an ICP-MS/MS system (8800 Triple Quadrupole ICP-MS, Agilent Technologies, Santa Clara, CA, USA).

Understanding the behaviour of PAH and especially sulfur containing PAH in such complex systems is important for optimizing the process. The results indicate that all HDS reaction products studied here still show a reasonable amount of sulfur, which is desirable for the evaluation of sinks and sources of different compound groups. The results show how different compounds are more or less efficiently removed, with help of the different catalysts, while for others, an increase is observed in the corresponding mass and double bond equivalent range.

On a broader scale the different processes behaved quite uniformly in case of the desired sulfur removal reaction. Only small alkylated dibenzothiophenes showed a higher recalcitrance against desulfurization, as expected. These are known to remain present even in so-called "sulfur-free" fuels and thus contribute to the environmental impact of fossil fuel consumption. The data show slight deviations in the preference of dearomatization over desulfurization.

While on the other hand hydrocarbons are being produced by the processes over a broad compound scale, some unforeseen reactivity was observed for highly aromatic hydrocarbons near the real planar limit.[3]

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CONTINUOUS PROTEIN SENSING FOR CELL DEATH MONITORING IN BIOPROCESSING

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Cell death plays a critical role in biological and biotechnological systems, ranging from living tissues and organs to engineered in vitro platforms and bioreactors¹. In bioprocessing, uncontrolled cell death can reduce the production yield and compromise the product quality, making real-time monitoring of cell death essential for early detection of cellular damage and timely intervention to minimize its impact².

Cell death can be monitored by detecting the release of intracellular components, such as proteins, into the surrounding environment of the dying cells³. Among these proteins, lactate dehydrogenase (LDH) is a widely used cell death marker due to its presence in all cell types and its rapid release upon rupture of the cell membrane⁴. Conventionally, LDH levels are determined through laboratory assays that analyze batches of samples collected over time⁵. However, this methodology has long turnaround times, typically several hours or more, which does not provide real-time data on cell death and rapid feedback to the bioprocess. To enable novel measurement-and-control strategies for timely cell death management, continuous LDH sensing technologies with fast response times are needed⁶.

Here, we report the development of a technology for the continuous real-time sensing of LDH for applications in cell death monitoring. The technology is based on Biosensing by Particle Motion (BPM), which relies on tracking the motion of hundreds of individual biofunctionalized particles over a biofunctionalized surface^{7,8}. The particles undergo reversible single-molecule interactions with the surface, exhibiting transitions between bound and unbound states that dynamically reflect the concentration of analyte molecules in solution. We will discuss the sensing principle and biofunctionalization strategies for sensing LDH and present the results of our experimental studies, including investigations of sensitivity, reversibility, binding kinetics, and selectivity. We will demonstrate how sensor designs can be tailored for LDH detection with tunable sensitivity ranges and selectivity, making the sensor adaptable to different conditions and settings. Furthermore, we will show the ability of the sensor to reversibly and continuously measure fluctuating LDH profiles over several hours, in both buffer and cell medium. Finally, we will discuss further research directions and ways to integrate LDH sensing into bioprocessing to achieve real-time monitoring and control of cell-based production.

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SCALABLE, NON-INVASIVE, IN-LINE, NANOPARTICLE SIZE MONITORING FOR PHARMACEUTICAL DEVELOPMENT AND MANUFACTURING

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Nanoparticles are widespread in industry as either intermediate or end-products in food, cosmetics, pharmaceutical and various chemical industries. Both the mean nanoparticle size and the dispersity of sizes are critical process parameters for these products.

Current particle size process and quality control is heavily reliant on manual sampling, often causing delays and increased logistic and analytic complexity. These difficulties have been largely associated with the lack of PAT tools for non-invasive inline nanosizing. As a result, process control and -as ultimate consequence- development of a 'quality attribute-based Design Space' in which process parameters are automatically tuned to ensure the required nanoparticle size characteristics, has been lacking.

In this talk, we discuss using Spatially Resolved Dynamic Light Scattering (SR-DLS) to measure nanoparticle size non-invasively in process conditions [1]. Our technique allows direct inline monitoring of the size (distribution) of nanodroplets and particles during processing, enabling real-time PAT for inline characterization at industrially relevant process turbidities and flowrates. In this contribution, we describe how a state-of-the-art instrument based on SR-DLS has been used to measure the particle size of various pharmaceutically relevant nanoparticles. We will go into relevant industry-led use cases that include: lipid nanoparticles, liposomes, transfection complexes, nanoemulsions, milled nanosuspensions as well as integration into separation techniques such as AF4 and chromatography. Importantly, using this technique it is possible to not only monitor the size characteristics during the process, but also to actively control the particle size during manufacturing by employing the measured characteristics, to tune process settings to achieve 'on target' production for nanoparticle formation strategies such as High pressure homogenizers and bottom up synthesis. Finally, we will describe and discuss several aspects relevant to the successful implementation of such nano-manufacturing process control.

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O-104**SOLVENT SUBSTITUTION FOR USP COMPENDIAL HPLC APPLICATIONS****Zhao, Weidong; Ambrose, Michael.***US Pharmacopeia, Rockville, MD, United States.*

Hazardous solvents such as dichloromethane (DCM), hexane, and acetonitrile (ACN) are widely used in high-performance liquid chromatography (HPLC) methods including compendial applications for sample preparation and testing. They pose significant health and environmental risks and raise concerns over safety and waste management.

Although the idea of substituting HPLC hazardous solvents with less hazardous solvents, i.e., greener solvents is not new, we wanted to evaluate the impact of compendial applications specifically. We hypothesized that most compendial HPLC applications could use greener solvents to achieve the same analytical performance. We initiated a project to evaluate the substitution of high-impact, hazardous solvents. We reviewed various solvent selection guidelines including USP General Chapter <467> Residual Solvents and the American Chemical Society (ACS) GCI pharmaceutical roundtable guide, and identified six solvents that were widely used in USP-NF HPLC applications, i.e., ACN, n-hexane, perchloric acid, DCM, tetrahydrofuran (THF), dimethylformamide (DMF). We selected two representative monographs that use each solvent as model applications and investigated substitution strategies for each case in this project. The system suitability criteria were used as performance indicators to compare the monograph method and the alternative method. Except for solvents, we kept monograph parameters, such as the HPLC column, the flow rate, and the run time, unchanged in each case study, so the observed effect is primarily contributed by the substituting solvents.

All twelve substitution cases achieved performance equivalency to the monograph methods. Certain solvents, such as n-hexane, perchloric acid, THF, and DMF, can be readily substituted with a greener alternative without the need for additional method changes or development. However, acetonitrile and DCM cases required adjustment in mobile phase composition with their respective alternative solvents to meet the system suitability requirements, suggesting a need for further method development and test parameter considerations for certain substitution cases.

We used the Analytical Greenness (AGREE) metric score to quantify the improvements and found that the alternative solvents improved greenness between 18% to 65% in 10 of 12 applications evaluated, while the two n-hexane applications using n-heptane instead showed only a 6% improvement. We further evaluated replacing n-hexane with supercritical CO₂ (scCO₂) as the main mobile phase using supercritical fluid chromatography (SFC) and found over 40% improvement in greenness with scCO₂ in these applications. This study suggests reverse-phase HPLC solvents can be substituted using greener solvents in compendial applications, while normal-phase HPLC may require a change in testing platforms, such as SFC, and some degree of method development to see significant greenness improvements.

USP General Chapter <467> Residual Solvents, https://doi.org/10.31003/USPNF_M99226_08_01

Collaboration to Deliver a Solvent Selection Guide for the Pharmaceutical Industry Cairen R. Hargreaves, AstraZeneca Julie B. Manley, ACS Green Chemistry Institute® Website: www.acs.org/gcipharmaroundtable E

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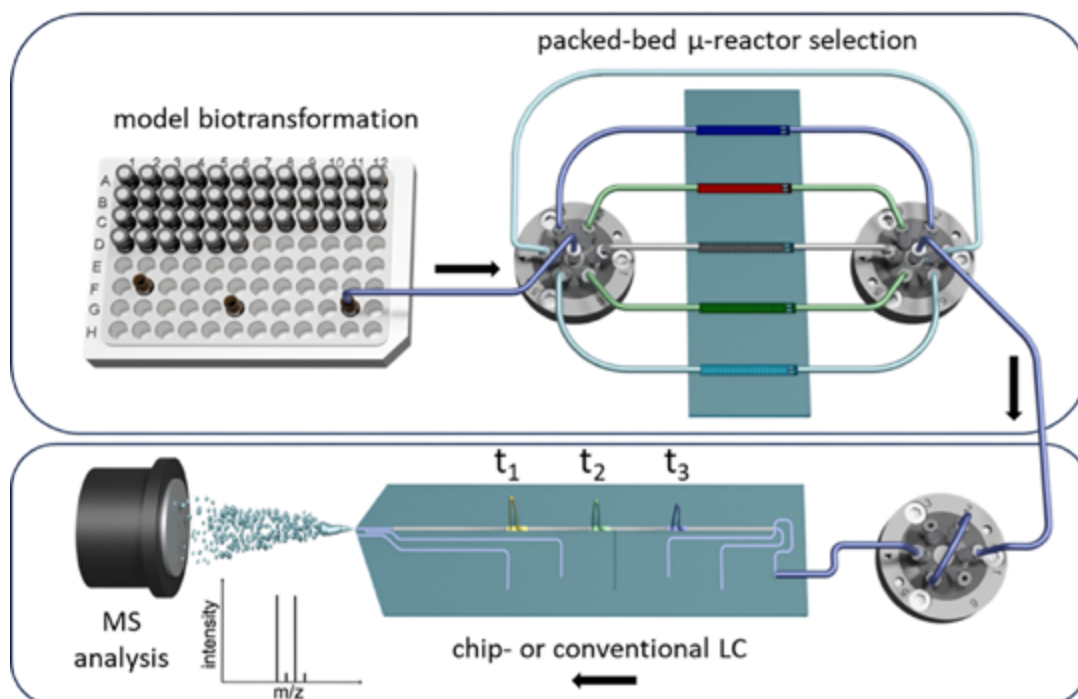
AN AUTOMATED MICROFLOW MULTI-REACTOR SCREENING PLATFORM FOR BIOCATALYST OPTIMIZATION

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The efficient use of biocatalysts, such as enzymes, plays a crucial role in the integration of sustainable chemistry into industrial processes, as they can enable highly selective reactions under milder and greener conditions. Incorporating these enzymes into miniaturized continuous flow systems, such as MIERs (microfluidic immobilized enzyme reactors), enhances stability while significantly reducing the use of solvents, reagents and most importantly, valuable biocatalytic materials. However, the successful operation of such reactors, requires a suitable analytical method to continuously investigate their performance. Online LC-MS provides an ideal solution by enabling high-frequency analysis of reaction streams and delivering reliable, high-quality qualitative and quantitative data. The integration of online LC-MS analysis, offers unparalleled data richness, yielding detailed insights into the molecular composition, structure, and concentration of biocatalytic transformations.

Therefore, we present a novel microfluidic screening setup combining microreactor technology, multi-reactor integration, and online LC-MS analysis in a sequential automated workflow, allowing for the generation of high-quality data sets on the performance of immobilized enzymes in continuous flow.¹ An automated sample feed was employed facilitating the evaluation of various substrates and reaction conditions, while contamination-free switching between several microreactor positions – each connected to an individual MIER – was achieved through external valve control. Downstream of the biocatalysis reactor module, the reaction effluent was continuously monitored via an interconnected LC-ESI-MS unit. The overall sampling frequency is constrained by the chromatographic separation time required for the components of the resulting reaction mixture. To address this, a miniaturized, modular chipLC system was also assessed, reducing the separation time to just 2 minutes and cutting total solvent consumption by over 80%. Hence, providing a faster, efficient screening platform for enzymatic reactions that minimizes biocatalyst usage while delivering reliable, high-quality LC-MS data. Beyond biocatalyst screening, the presented analytical platform is broadly applicable to the low-consumption investigation of most heterogeneous catalysts. Furthermore, the insights obtained through this approach can potentially be used to upscale or number up continuous biocatalytic processes.



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DEVELOPMENT OF EXTRACTION CHROMATOGRAPHY METHODS FOR PURIFICATION OF EMERGING MEDICAL RADIONUCLIDE STANDARDS AT THE UK NATIONAL PHYSICAL LABORATORY

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Radionuclides play a key role in nuclear medicine and are broadly applied for medical imaging, diagnosis and treatment of cancer and various other medical conditions. Radionuclides for health applications are normally produced in charged particles induced nuclear reactions using accelerator facilities or by neutron activation at high-flux research reactors. There are stringent requirements on the chemical and radiological purity of radionuclides for medical use and therefore highly efficient radiochemical purification procedures have to be developed for the removal of radioactive or stable impurities as well as decay progeny. In part, this allows for the creation of near-isotopically pure calibration sources from which the precise nuclear decay parameters and signatures can be extracted. These include precision measurements of radioactive decay half-life and gamma-ray emission probabilities which underpin all traceability chains and standardisations for such pre-clinical radioisotopes [1,2,3]. An overview will be presented of the development, validation, and application of radiochemical separation procedures for the purification of emerging medical radionuclides, carried at the UK National Physical Laboratory within the framework of the UK Medical Radionuclide Innovation Programme, the European medical radionuclide programme on the production of high purity radionuclides by mass separation (PRISMAP), and CERN-MEDICIS project for producing novel radioisotopes. As part of these efforts, a radiochemical separation method based on the use of highly selective extraction chromatography resins has been developed for purification of ^{225}Ac , produced by proton and deuteron irradiations of electroplated ^{226}Ra targets at the MC40 cyclotron at the University of Birmingham. Furthermore, the method has been optimised for extraction of ^{227}Ac from nuclear legacy material allowing its use as a precursor and generator of ^{223}Ra and ^{227}Th for cancer treatment applications. Terbium-155 (^{155}Tb) offers promise as an imaging tracer in single photon emission computed tomography (SPECT), with initial pre-clinical studies indicating excellent image quality even at low doses. The administration of ^{155}Tb prior to a therapeutic terbium isotope would give a theragnostic pair with identical chemical properties; this is particularly advantageous as it facilitates the application of personalised medicine. ^{155}Tb prepared by high-energy proton spallation at the CERN ISOLDE and MEDICIS facilities contains radioactive $^{139}\text{Ce}^{16}\text{O}$ following on-line mass separation, in addition to stable zinc and gold impurities from the collection foil. A highly efficient radiochemical purification method has been developed using ion-exchange and extraction chromatography resins in two column separation steps to successfully isolate ^{155}Tb with a chemical recovery of 95 % and an isotopic purity exceeding 99.9% [1] Other examples which will be presented include Erbium-165 (^{165}Er) which is another promising medical radio-lanthanide, proposed for use in Auger therapy and Single-photon emission computed tomography (SPECT) imaging.

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O-107

DERMAL ABSORPTION OF POLYCYCLIC AROMATIC HYDROCARBONS AND METAL ALLERGENS

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Polycyclic aromatic hydrocarbons (PAH) are persistent environmental pollutants, which occasionally also appear as contaminants in consumer products, particularly those containing carbon black.^[1] Upon dermal contact to such articles, transfer of PAH into the *stratum corneum* and migration through the skin may occur, resulting in this class of toxic compounds to become bioavailable.

Dermal penetration through human and porcine skin of 24 different PAH was evaluated *via* Franz diffusion cell *in vitro* assays combined with PAH quantification based on gas chromatography tandem mass spectrometry (GC-MS/MS).^[2] More lipophilic and potentially more toxic PAH were characterized by decreased permeation rates through the rather lipophilic *stratum corneum* into the more hydrophilic viable (epi-)dermis. Small PAH permeate the skin quickly and were detected in the receptor fluid after 2 h, whereas large PAH did not fully permeate the skin up to 48 h, indicating lower bioavailability upon dermal uptake for the latter. Furthermore, human skin was less permeable than pigskin, retaining a greater share of smaller PAH in particular.

Moreover, dermal uptake of PAH from squalene, a commonly used surrogate for aliphatic polymers, was investigated under quasi-infinite dose conditions.^[3] Fitting of measured intradermal PAH distributions obtained by tape stripping^[4] and analysis by GC-MS/MS to solutions of Fick's second law revealed partition ($K_{s.c./squalene}$) and diffusion ($D_{s.c.}$) coefficients for the *stratum corneum*. $K_{s.c./squalene}$ show a trend to higher values for larger PAH, whereas $D_{s.c.}$ is similar for larger PAH but lower for naphthalene. The *stratum corneum* / viable epidermis boundary layer probably represents the most relevant barrier for the skin penetration of higher molecular mass PAH.

Finally, skin penetration of the metal allergens nickel, cobalt and palladium from commonly used diagnostic patch test preparations in petrolatum and from aqueous metal salt solutions was investigated by means of inductively coupled plasma mass spectrometry (ICP-MS).^[5] The metal ions were mainly retained in the upper layers of the *stratum corneum*. However, from both applied matrices, nickel and cobalt penetrated the skin more efficiently than palladium and may thus sensitize and elicit allergic contact dermatitis (ACD) more easily. This may partly explain why palladium-induced ACD is relatively rare.

This work characterizes the skin penetration behavior of several polycyclic aromatic hydrocarbons (PAH) and metal allergens by means of Franz diffusion cell *in vitro* assays combined with GC-MS/MS and ICP-MS analyses. The obtained results contribute to a better understanding of the barrier function of the skin and are relevant in the context of exposure assessment.

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O-108

SEASONABLE AND LONGITUDINAL VARIATIONS IN DISINFECTION BY-PRODUCTS FROM SOURCE TO TAP AND TO SWIMMING POOL WATER : MULTI-PARAMETERS ANALYSIS OF DPB AND THEIR PRECURSORS

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Disinfection byproducts (DBP), formed by the reactions between natural organic matter and chlorinated disinfectants during water treatment, have been a major health problem for several decades. To date, nearly 700 DBP have been identified (Richardson & Ternes, 2022), and according to their chemical structures and halide content, they have more or less pronounced cytotoxic, mutagenic and/or carcinogenic effects (Neguez & Laky, 2023; Plewa et al., 2008).

Climate change events (such as heavy precipitations, rising seawater levels, and wildfires) exacerbate DBP formation. Studies indicate that brominated, iodinated and nitrogenated DBP – known to be more toxic than their chlorinated homologues – are increasing under these conditions (Chowdhury, 2022; Delpla et al., 2023; Hohner et al., 2016). However, drinking water regulations monitor only two families: Trihalomethanes (chloroform, bromoform, chlorodibromomethane and dichlorobromomethane = THM4) and Haloacetic acids (most of the time, only five of them: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid = HAA5) which do not represent all the DBP, or the toxicity induced by all these DBP. To better assess the health risks associated with DBP in drinking water, a more insight into nature and level of other DBP is needed (Richardson & Plewa, 2020).

This presentation will focus on a longitudinal monitoring study, conducted in a large urban area in north-eastern France. We monitored water quality from source to tap and to swimming pool, during four seasonal campaigns over one year.

Analyses included measurements of global parameters (TOC/N, TKN, UV absorbance), physico-chemical parameters (conductivity, pH), specific parameters (NH_4^+ , NO_2^- , NO_3^-), oxihalides by IC-MS, and a large range of DBP families, analyzed globally (adsorbable organohalogens (AOX), non-purgeable organohalogens (NPOX)), and individually by GC-ECD allowing analyses of THM4, iodo-THM, 16 HAA, Halonitromethanes (HNM), Haloacetonitriles (HAN), Haloacetaldehydes, and Haloketones. On-site measurements also included free chlorine and combined chlorine.

Besides optimization of GC-ECD analytical features, the presentation will focus on the development of the AOX method used for its application to natural, treated, and swimming pool water. The limit of quantification was determined to be 25 $\mu\text{g Cl/L}$, with a calibration curve showing excellent linearity ($R^2 = 0.99$), whatever the aqueous matrix.

As regards the analytical results obtained during the first two campaigns, we observed a decrease in AOX values between June and September at the outlet of the treatment plant and in the drinking water distribution network (120 $\mu\text{gCl/L}$ to 40 $\mu\text{gCl/L}$). However, sums of individual DBP explaining the AOX values increased from 10 to 30% between these two campaigns. The fraction of unknown DBP was thus higher in June than in September.

Meteorological data show that cumulative rainfall one month before the June campaign was 80 mm, and 100 mm before the September campaign. This increased precipitation contributed to changes in water quality, resulting in a slight rise in chloride concentration (from 7 to 11 mg/L), conductivity (from 162 to 263 $\mu\text{S/cm}$), absorbance (from 0.145 to 0.185), and TOC (from 4.4 to 5 mg/L), while NH_4^+ concentrations decreased (from 0.09 to 0.05 mg/L). These changes can possibly be attributed to runoff from heavier rainfall. Moreover, the increase in precipitation may have diluted organohalogen precursors, contributing to the observed decrease in AOX between the two campaigns. The increase in known DBP in June may be explained by the higher presence of short-chain DPB in the drier season than in the wet season.

A more complete analysis, including data from all four seasonal campaigns and inter-seasonal variations, will be presented during this presentation.

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O-109**INVESTIGATING THE OCCURRENCE OF CHEMICALS OF EMERGING CONCERN AND SAFETY OF SOIL-GROWN TOMATOES IRRIGATED WITH TREATED WASTEWATER**

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Reusing treated municipal wastewater for crop irrigation has gained attention as a sustainable solution to meet rising food production and the demand for fresh water. However, its use risks introducing chemicals of emerging concern (CECs) into the food chain. These are a broad class of compounds, including pharmaceuticals, personal care products, industrial chemicals, hormones, stimulants, and pesticides, that persist through conventional treatment processes and pose a risk to human health. This study explores the uptake and translocation of 27 selected CECs in soil-grown tomatoes (*Solanum lycopersicum* L. cv. Rally), chosen as a model crop. The field experiment was performed during the 2022 growing season and included four irrigation treatments: (i) potable water as a control (PW), (ii) treated wastewater (TWW), (iii) potable water spiked with a mixture of CECs (PW+CECs), and (iv) treated wastewater spiked with the same CEC mixture (TWW+CECs). Both irrigation matrices were spiked with the selected compounds at 1 mg/L (well above typical environmental levels) to facilitate detection and a better understanding of the uptake and translocation of CECs in tomato plants. Samples of irrigation water, soil, and tomato plant parts (fruits, leaves, stems, and roots) were extracted using optimised and validated methods (solid phase extraction, solid-liquid extraction and QuEChERS) for each sample type. The extracts were analysed using LC-MS/MS. Five CECs were quantified in potable water (up to 20 ng/L), while 24 were detected in treated wastewater (ranging from 200 to 7000 ng/L). When the spiked irrigation media were analysed, 17 CECs were significantly less stable in TWW. Analysis of the tomato fruits found that only carbamazepine (22 ng/g dw) was above the LOQ for tomatoes irrigated with TWW. In tomato fruits irrigated with PW+CECs, three compounds (acetamiprid, carbamazepine and dimethomorph) were above the LOQ (ranging from 30.32 to 1034 ng/g dw) and six compounds (acetamiprid, azithromycin, BPS, carbamazepine, clarithromycin and dimethomorph) in tomatoes irrigated with TWW+CECs (ranging from 15.59 to 734.1 ng/g dw). Analysis of plant parts revealed the highest number of compounds in roots (15), followed by leaves (10), fruits (6) and stems (5). Soil samples were analysed at three sampling times: before irrigation, after the experimental period, and two years post-experiment, where 16 compounds remained present in soil irrigated with spiked media after 30 months. Dietary exposure assessment for all compounds above LOQ, indicated that health risks only arose for tomatoes irrigated with spiked media for carbamazepine for infants. For tomatoes irrigated with wastewater, although carbamazepine was present in fruits, all values were three orders of magnitude below the acceptable daily intake, indicating its potential safety for wastewater reuse. However, metabolites and transformation products should be assessed in future studies.

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O-110

FRAGMENTATION OR DEPOLYMERIZATION? NEW APPROACH FOR IDENTIFYING PLASTIC BIODEGRADATION PRODUCTS

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While the breakdown of plastics into smaller particles through physical processes is increasingly documented, much less is known about bacterial-driven biodegradation. As interest grows in using bacteria to address plastic pollution, the detection of degradation products remains largely unexplored, the focus is on the discovery of new plastic-degrading bacteria and modifications to the original plastic (Lear et al. 2021). Preliminary studies have focused primarily on depolymerization where polymer chains are cleaved into smaller molecules (Liu et al. 2022), with little attention given to potential fragmentation into smaller particles (Andrady et al. 2022). However, from a toxicological point of view and to completely understand the degradation pathways, the full size and shape spectrum of degradation products and molecules must be characterized. The lack of analytical methods to identify and characterize plastic markers at different scales following bacterial degradation clearly highlights this gap in understanding (Gambarini et al. 2021). In this study, we experimentally initiated the biodegradation of polystyrene and polyvinyl chloride by worms microbiota (Zhang et al. 2022; 2022) to develop an analytical method coupling thermodesorption and pyrolysis with gas chromatography-mass spectrometry (Py-GC-MS) for the identification of specific biodegradation markers (Toapanta et al. 2021). A liquid-liquid extraction method, optimized in this method for both plastics, using dichloromethane (DCM) was applied to microbiota cultures incubated with plastics. The extracted compounds, obtained in varying proportions, were then analyzed, according to predefined parameters, in thermodesorption for depolymerization products and pyrolysis for fragmentation. Thanks to this analytical strategy, we have revealed the existence of depolymerization products derived from PS such as (2-phenylcyclopropyl)benzene, biphenyl (Savoldelli, Tomback, et Savoldelli 2017) and derived from PVC, some of which already known to be degradation products of other plastics have been detected, as well as additives as phthalic anhydride, 2-ethylhexan-1-ol or acenaphthene (Khandare et al. 2022). Furthermore, the methods have identified novel molecules associated with biodegradation such as (1E)-1-benzylideneindene, 2-ethylcyclopentan-1-one and benzene. Concerning fragmentation, PS has been found in different quantities in samples whereas PVC was not present.

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O-111**NON-TARGETED SCREENING OF EMERGING CONTAMINANTS IN GROUNDWATER FOR ADVANCING DRINKING WATER QUALITY MANAGEMENT****Roverso, Marco¹; Pettenuzzo, Silvia¹; Menghini, Michele²; Pedrazzani, Roberta²; Bogialli, Sara¹.***¹Department of Chemical Sciences, University of Padova, Padova, Italy; ²Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy.*

Evidence of the presence of emerging contaminants in various water compartments is continually increasing, raising concerns about potential risks to human health and the environment. The quality and safety of tap water is generally ensured by water suppliers by the routine monitoring of a limited number of parameters. In this light, the overall knowledge of the local water quality and safety is limited to well-known contaminants, totally excluding the evaluation of the potential presence of other unknown or unrecognized chemicals with potentially adverse effects [1,2]. The European Union has implemented the principle of Water Safety Plans (WSPs) in the recent revision of the Drinking Water Directive, as a flexible and site-specific tools for managing water quality throughout the drinking water supply chain. The availability of analytical monitoring protocols for the identification of emerging contaminants in the specific context of the site is of primary importance in this context. Modern chromatography techniques coupled with high-resolution mass spectrometry (HRMS) are the ideal tool to meet the needs of chemical risk characterization set by the WSP.

The present work aims to perform a non-targeted screening on groundwater used to produce drinking water, collected in different seasons from 20 selected aquifers in the Veneto region, in order to detect, identify and possibly quantify unknown contaminants not previously handled within the routine analysis.

In this light, both solid phase extraction (SPE) and on-line SPE techniques were evaluated and validated to perform the initial pre-concentration of samples. Methods were optimized to be as comprehensive as possible in terms of different chemical properties (mainly, molecular weight and polarity) and to ensure limit of detection in the range of ng/L for relevant emerging contaminants' classes. Data were obtained by HPLC-HRMS analysis using data-dependent acquisition. MS-DIAL [3] was chosen as software for the identification of the unknown chemical species and to assess their distribution in the selected aquifers. Finally, the identification and the rough quantification of the most significant contaminants were confirmed by an independent targeted method.

Results highlighted at least 20 identified contaminants, mainly pharmaceuticals, pesticides, and related metabolites, at concentrations always <10 ng/L.

In conclusion, the proposed workflow allowed a comprehensive insight in the contamination profile of selected groundwaters in the Veneto region. Integrating these findings into Water Safety Planning (WSP), as recommended by the World Health Organization (WHO), will support the development of early-warning tools and facilitate site-specific water quality management.

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O-112**INTEGRATED EXPERIMENTAL AND MODELING ASSESSMENT OF OZONE PRECURSORS AND AGED AIR MASSES IN THE CENTRAL ANDES MOUNTAINS****Campos, Valeria¹; Diaz-Robles, Luis²; Fadic, Ximena³; Barcaza, Gonzalo³; Gallardo, Benjamin³; Cereceda-Balic, Francisco³.**¹Universidad de Santiago de Chile, Santiago, Chile; ²Ingeniería y Gestión Ambiental PARTICULAS SpA, Santiago, Chile;³Centre for Environmental Technologies (CETAM), Universidad Técnica Federico Santa María, Valparaíso, Chile.

Tropospheric ozone (O_3) is a secondary pollutant formed through complex photochemical reactions involving nitrogen oxides (NO_x) and volatile organic compounds (VOCs). Ozone levels can reach unexpectedly high concentrations in mountainous regions, even without strong local precursor emissions. This study investigates the chemical drivers and atmospheric processes behind the elevated ozone levels observed in the Andes Mountains of central Chile, focusing on precursor predominance, air mass aging, low NO concentrations, and the potential influence of water vapor.

A high-altitude monitoring campaign was conducted in Portillo, located in the Andes Mountains of the Valparaíso Region, at the NUNATAK-1 monitoring station (3000 m.a.s.l.). These results were complemented by additional ground-level stations in Los Andes, a rural valley area also within Valparaíso, and Las Condes, an urban site Santiago, Chile's capital. The campaign included ozone, NO , and NO_2 measurements using UV photometry and chemiluminescence analyzers (Thermo Scientific™, 49iQ and 42iQ, respectively). In parallel, the Community Multiscale Air Quality (CMAQ) model was applied to simulate regional photochemistry and estimate the ratio of volatile organic compounds to nitrogen oxides (VOC/NO_x), as well as the ratio of nitrogen oxides to total reactive nitrogen (NO_x/NO_y), across the domain.

The results revealed persistently high ozone concentrations at the mountain site with minimal diurnal variability and a seasonal pattern that does not follow the typical summer-dominated ozone profile commonly observed in urban areas. This persistence of ozone throughout the day and year is particularly concerning given its potentially harmful effects on the unique high-elevation ecosystems of the Andes Mountains, where native vegetation and biodiversity may be especially sensitive to oxidative stress. This behavior was closely linked to two main factors: (1) extremely low concentrations of nitric oxide (NO), the main ozone sink, due to the absence of significant local anthropogenic emissions in Portillo, and (2) limited relative humidity, which restricts the formation of hydroxyl and hydrogen radicals ($OH\bullet$, $H\bullet$) from water vapor, a known pathway for ozone destruction. NO concentrations in Portillo were over 90% lower than in the urban site and more than 80% lower than in the valley site. Likewise, relative humidity at the mountain site was consistently lower, limiting the availability of water vapor necessary for $OH\bullet$ and $H\bullet$ radical formation and consequently reducing this ozone removal pathway.

The CMAQ model was applied to complement the experimental findings. Model performance was evaluated by comparing simulated concentrations with observations from the monitoring stations. Once validated, the model was used to estimate VOC/NO_x and NO_x/NO_y ratios across the study area. The model indicated a VOC-limited regime in urban areas and a NO_x -limited regime in Portillo, consistent with low NO and high ozone levels. The model also confirmed low NO_x/NO_y ratios at the mountain site, indicating that air masses arriving at altitude had undergone significant photochemical aging, likely during long-range transport.

These findings confirm that the high ozone levels observed in the Andes are not a result of local precursor emissions but rather a combination of aged air masses transporting ozone from sites with high anthropogenic activity, NO -depleted air masses and suppressed ozone destruction mechanisms. The results also highlight the importance of considering topographical and chemical contexts when assessing background ozone and supporting the implementation of region-specific air quality monitoring and control strategies in the Andes mountain region. This study represents one of the first comprehensive characterizations of high-altitude ozone dynamics in the Andes of South America, bridging the gap between surface-level chemistry and atmospheric transport in complex terrain.

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O-113

NOVEL APPROACH IN IDENTIFICATION A PANEL OF BIOMARKERS IN CARDIOVASCULAR DISEASES

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Cardiovascular diseases (CVDs) are a significant contributor to the worldwide health and economic burden. Hypertension and the problems that it causes are the primary contributors to the extraordinarily high mortality rate that is linked with CVDs. They are the main cause of death on a global scale. Using biomarkers, especially early-stage biomarkers of CVD, has the potential to save a great number of lives and assist us in overcoming the battle against CVDs, especially in the context of preventive medicine.

For the purpose of identifying and improving risk differentiation among individuals who have cardiovascular diseases, a multi-marker panel technique seems to be a valuable and promising path of investigation. The difficulty isn't restricted to finding the most effective panel of biomarkers; rather, it is expanding to include the implementation of their use in the clinic in a timely and cost-efficient way.

Within the context of inflammatory disorders, such as atherosclerosis, the presence of elevated levels of C-reactive protein (CRP), interleukin 6 (IL-6), and C-type natriuretic peptide (CNP) is directly associated with the development of future cardiovascular risks.

For the detection of the multi-marker panel, more precisely CRP, IL-6, and CNP, three 3D stochastic sensors were proposed and developed for the analysis of the biomarkers using a stochastic approach. The three sensors are based on carbon nanopowder that was decorated with Ag nanoparticles and further modified with three types of phthalocyanines. The solutions of CRP, IL-6 and CNP were made using the successive dilution approach and buffered with phosphate buffer solution (PBS) that had a pH of 7.40. The validation was conducted utilizing whole blood samples.

The novelty is represented by the fact that the sensors were able to detect a multi-marker panel in a short amount of time with great accuracy and sensitivity from biological samples. The linear concentration range, high sensitivities, and the unique signatures of the stochastic 3D sensors have facilitated simultaneous detection of the three biomarkers in whole blood samples.

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O-114**DIGITAL DIPSTICK: NEXT-GENERATION GREEN POINT-OF-CARE SOLUTION****Bouilly, Guillaume; Migliorelli, Davide; Disser, Jeremy; Dorrestijn, Marko; Petkus, Bradley; Chai-Gao, Hui; Paoletti, Samantha; Burr, Loïc.***CSEM SA, Neuchatel, Switzerland.*

Digital health monitoring offers a promising solution to improve the quality of life for individuals with chronic diseases while alleviating strain and costs on healthcare systems. Urine, being a readily available biofluid that can be collected non-invasively, serves as an ideal candidate for remote health monitoring. It provides valuable insights into various health conditions, including diabetes, kidney failure, and hypertension.

Leveraging CSEM's expertise in biosensor development and integration, we have developed an electrochemical sensor module capable of incorporating up to six sensors (pH, Na⁺, K⁺, glucose, creatinine, and albumin) within a microfluidic cartridge. This cartridge is designed to manage the diffusion of the calibration solution prior to measurement in urine. As illustrated in the figure, the digital dipstick offers a streamlined user experience, with the calibration solution conveniently pre-packaged in a blister, activated by the user. Following simultaneous calibration of all sensors, the user simply removes the protective tape and immerses the dipstick in urine.

Our primary goal was to demonstrate that the digital dipstick could simultaneously measure multiple analytes with a level of accuracy comparable to commercially available electrochemical sensors. The findings reveal that, following a brief calibration process, it is possible to achieve a stable signal in urine that accurately reflects the activities of the respective analytes. Furthermore, analyte concentrations measured using the dipstick closely align with those obtained from commercially available reference devices.

Moving forward, we will supply hospitals with a statistically relevant quantity of digital dipsticks for validation within clinically relevant environments, allowing benchmarking against commercial off-the-shelf instruments. By accomplishing these milestones, we aim to provide a dependable alternative to conventional urine tests, enabling convenient at-home use with data readily accessible to healthcare providers remotely.

Furthermore, we are focusing on sustainability by thoroughly researching and assessing the possibility of manufacturing the digital dipstick with environmentally friendly materials. This method strives to reduce the device's environmental impact over its entire lifecycle, from manufacturing to disposal, so fostering a greener and more sustainable future for healthcare technology.

O-115

NEXT-GEN DIAGNOSTICS: CRISPR-CHIP FOR MONOCLONAL ANTIBODIES QUANTIFICATION AT THE POINT-OF-CARE

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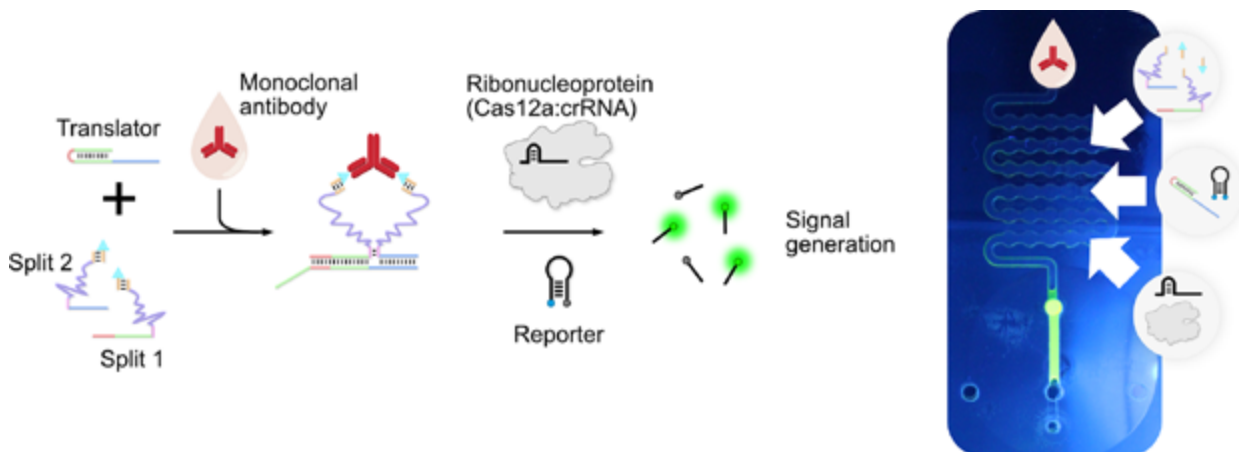
Monoclonal antibodies (mAbs) are used to treat numerous cancers, offering specific therapy that improves patient outcomes. Measurements of mAbs in the bloodstream during cancer treatment are essential for monitoring the therapeutic antibody dose, assuring treatment efficacy, reducing side effects, and tracking drug resistance.

Enzyme-Linked Immunosorbent Assay (ELISA) and High-Pressure Liquid Chromatography tandem Mass Spectrometry (HPLC-MS) are the gold standard for quantitative mAbs analysis in clinical studies. Both methods involve expensive, bulky, and complicated instrumentation, making them unsuitable for point-of-care (POC) use.

To address this need, we introduce a POC platform for quantitative mAb detection, which leverages a recently developed quantitative mAb detection method [1], based on the use of a DNA circuit for the recognition of the mouse anti-hemagglutinin (antiHA) from influenza, and the activation of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas). CRISPR systems' adaptability, low cost, high sensitivity and repeatability make our approach a great candidate for POC applications. Though promising, the conventional protocols run in test tubes, require four incubation steps and consecutive reagent additions. Thus, our study focuses on developing a simplified on-chip CRISPR-based assay without sacrificing on the performance.

For this purpose, a two-step protocol is developed, including sample dilution and injection, requiring only 10 μ L of volume, and providing results within a 60 minutes sample-to-answer timeframe. The assay is integrated on a cost-effective chip, less than 1 € for chemicals, and additionally it is verified that the dispensed reagents can be stored at -20°C for at least 1 month without degradation.

In conclusion, we demonstrate a proof-of-concept CRISPR-chip, which has the potential to impact modern diagnostics and the clinical market by introducing a new tool for protein quantification at the POC.



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O-116

ELECTROCHEMICAL DETERMINATION OF VITAMIN K₂ AND THE ROLE OF ITS HOMOLOGS IN COLORECTAL CANCER RESEARCH

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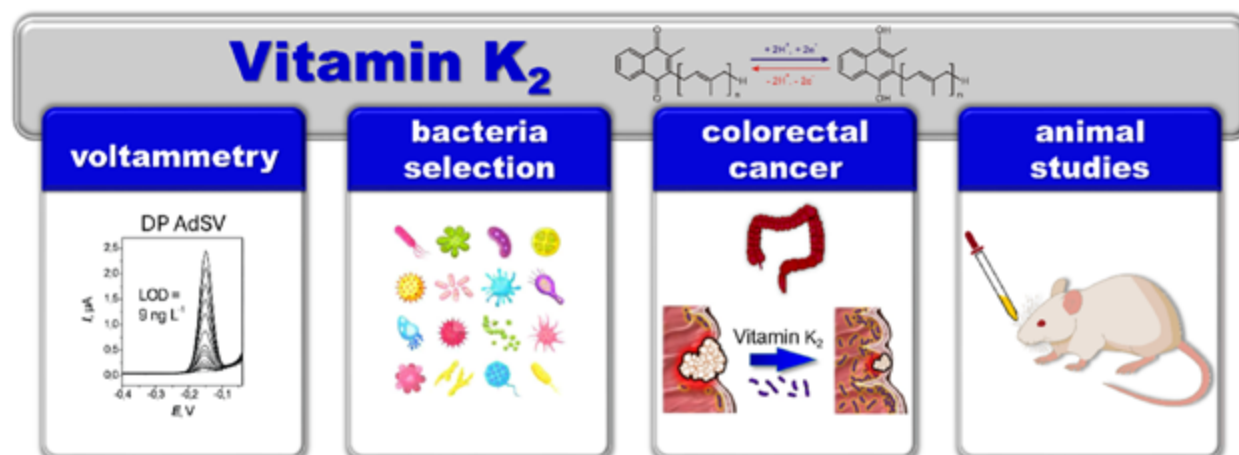
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Vitamin K₂ (VK₂) is a fat-soluble vitamin essential for calcium homeostasis, bone metabolism, and cardiovascular health [1-3]. Structurally, VK₂ belongs to the menaquinone group, distinguished by varying isoprenoid units (4 to 14) in its side chain, defining homologs with distinct biological activities and bioavailability [1,4]. VK₂ is primarily synthesized by bacterial metabolism and is present in fermented foods, dairy products, and certain meats [5-6]. Recently, an increasing number of studies have highlighted the beneficial effects of VK₂ in the context of various cancers, including colorectal cancer [1,2,7].

The determination of VK₂ has been traditionally performed using liquid chromatography (LC) and high-performance liquid chromatography (HPLC) coupled with different detection techniques such as fluorescence, ultraviolet (UV), and mass spectrometry (MS). While these methods offer high sensitivity and specificity, they often involve complex sample preparation and expensive instrumentation. To address these limitations, alternative analytical methods that are efficient and cost-effective are actively being explored [8].

This study presents a voltammetric approach for VK₂ determination. Several electrode types, including a Glassy Carbon Electrode (GCE) [9] and two renewable film electrodes [10], were tested, with the Controlled Growth Mercury Drop Electrode (CGMDE) ultimately selected as the most suitable. The electrochemical behavior of VK₂ was investigated using cyclic voltammetry (CV), while differential pulse adsorptive stripping voltammetry (DP AdSV) was employed for quantitative determination. Key parameters such as supporting electrolyte composition, instrumental parameters, and accumulation conditions were examined. The best results were obtained using a 70% methanol solution with 0.3M acetate buffer (pH 3.8) and 0.2M sodium perchlorate. The scanning range of -0.04 V to -0.40 V in both anodic and cathodic directions demonstrated a linear response for VK₂ concentrations between 0.2 and 8.0 mg/L ($R = 0.9987$) and 4.0 to 20.0 mg/L ($R = 0.9959$), with detection limits of 0.009 mg/L and 0.012 mg/L, respectively. The method was successfully applied to VK₂ quantification in pharmaceutical, microbiological, and food samples using the standard addition method.

The developed voltammetric method provided a foundation for a research grant titled The Role of Intestinal Bacteria Releasing Vitamin K₂-MK7 in the Process of Colon Carcinogenesis. Utilizing this method, 86 intestinal bacterial strains were analyzed, identifying Lactobacillus, Bifidobacterium, and Bacillus species as the most efficient VK₂ producers [11]. Further studies explored VK₂-MK4 homolog penetration into Caco-2 cells in an aqueous environment—without fats, pancreatic enzymes, or bile salts. Additionally, the effects of three VK₂ homologs (MK-4, MK-7, and MK-9) on apoptosis and necrosis in Caco-2 cells were examined, confirming their non-toxic nature. Moreover, long-chain homologs MK-7 and MK-9 exhibited unique anti-inflammatory properties by inhibiting the secretion of key pro-inflammatory cytokines, including IL-8 (in Caco-2 cells), as well as IL-6 and TNF- α (in RAW 264.7 macrophages) [12].



Expanding on these findings, an in vivo study was conducted using a mice model of chemically induced colorectal cancer. Forty-eight mice were divided into six groups and subjected to an 80-day experimental protocol. Following tumor induction, selected groups received various VK₂ homologs, including bacterial-derived VK₂. Fecal sample analysis and histopathological assessments of excised intestinal tissue postmortem were conducted to evaluate VK₂'s impact on tumor progression.

The results from both in vitro and in vivo experiments suggest that VK₂ may have anticancer properties by inhibiting tumor development and modulating inflammatory responses. Further research is required to elucidate the mechanisms underlying VK₂'s therapeutic potential and to explore its clinical applications in cancer prevention and treatment.

Acknowledgements

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O-117

FOLLOWING THE PERMEATION OF DICLOFENAC IN REAL TIME THROUGH A MODEL MEMBRANE OR SKIN

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Diclofenac is a nonsteroidal anti-inflammatory drug [1]. It is widely used to reduce pain and symptoms of osteoarthritis or rheumatoid arthritis. Diclofenac is also used in post-traumatic inflammation of tendons, ligaments, muscles, and joints (sprains, dislocations, and bruises). Diclofenac is popularly used in the form of a topic gel and its transdermal permeation must be studied.

Studies of drug permeation through skin, or some model membranes, frequently use a Franz Cells [1,2], where the membrane separates the donor and receptor chamber. The permeate is known by sampling and further analysing the fluid of the receptor chamber. This methodology does not allow continuous real time measurements, but forces to alter the concentration of the receptor chamber, as the removed sampled volume is generally replaced by clean fluid.

The use of a sensor in the receptor cell allows the continuous measurement of diclofenac without perturbing the chamber composition. A piezoelectric quartz crystal with an appropriate coating was used in the receptor chamber. Coating sensitivity to diclofenac was confirmed, but also its insensitivity to the gel excipients.

Permeation cells needed to be designed and fabricated in the University mechanical and glass workshops. All permeation studies were carried out at constant temperature of 32.0°C. A new cell was conceived for permeation studies through a membrane, from a diclofenac solution in the donor compartment to a receiver solution. A Teflon cell housing the coated crystal was connected to this receptor cell, so that the uncoated face of the crystal was kept dry, otherwise no series frequency could be measured. Special attention was devoted to the solutions stirring, so that good mixing was obtained without disturbing the frequency of the quartz crystal. For this, two cavities to house the magnetic bars were blowed at the bottom of the glass chambers. An ingenious solutions allowed to easily connect and disconnect the Teflon crystal cell to the glass chamber without leakage. This cell was used to study permeation through some model membranes, as peach, tomato and cellophane.

Another special cell was designed to test permeation of two commercial diclofenac gels. This time, permeation was studied through human skin stratum corneum, the main skin barrier. As human skin is hardly available for regular studies, fluxes and diffusion coefficients of diclofenac were also determined with cellophane, in order to evaluate this last membrane as a model. Results will be presented at the congress.

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M. Teresa S. R. Gomes is an associate professor at the University of Aveiro. She received a BS degree in Chemical Engineering from the University of Coimbra in 1983 and a PhD in Analytical Chemistry from the University of Aveiro in 1997. Her current interests are chemical sensors and analytical chemistry. Presently, she is vice-president of the forum Ibersensor.

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O-118

CRISPR-POWERED MONITORING OF DNA REPAIR ACTIVITIES USING SYNTHETIC DNA TRANSDUCERS

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DNA glycosylases are essential DNA repair enzymes that maintain genomic stability by identifying and excising damaged nucleotide bases, initiating the base excision repair (BER) pathway. BER protects the genome from mutations linked to diseases such as cancer.¹ However, traditional assays for measuring DNA glycosylase activity often suffer from multi-step procedures, reliance on auxiliary enzymes, or sensitivity limitations.

Here, we present a CRISPR-Cas12a-powered detection platform for the rapid and sensitive monitoring of both mono- and bi-functional DNA glycosylases. Our activity-based assay leverages CRISPR-Cas12a-mediated signal amplification, triggered by the molecular reconfiguration of a lesion-containing hairpin DNA probe (DNA Transducer, Figure 1). This DNA Transducer acts as both the DNA repair enzyme substrate and the Cas12a activator. Using a uracil-containing DNA Transducer, we enable single-step monitoring of uracil-DNA glycosylases (e.g., UDG, HSMUG1). UDG excises uracil bases incorporated into the DNA Transducer, leading to the generation of apurinic/apyrimidinic (AP) sites and subsequent destabilization of the hairpin structure. Repair-induced hairpin reconfiguration triggers Cas12a nuclease activity and fluorescence signal transduction. We demonstrate that the kinetics of hairpin reconfiguration are specifically induced by UDG activity and can be temporally regulated by uracil residues incorporated into the DNA transducer. Moreover, by incorporating 8-oxo guanine (8-oxoG) into the DNA Transducer, we assess the activity of 8-oxo guanine DNA glycosylases, including Fpg and hOGG1, via a release-upon-repair mechanism induced by the enzyme secondary lyase activity.

Beyond precise quantification of cellular DNA glycosylases in a single step (with recovery values of 98-122%), we also demonstrate its applicability for inhibitor screening, highlighting its potential as a versatile tool for clinical diagnosis and drug discovery.

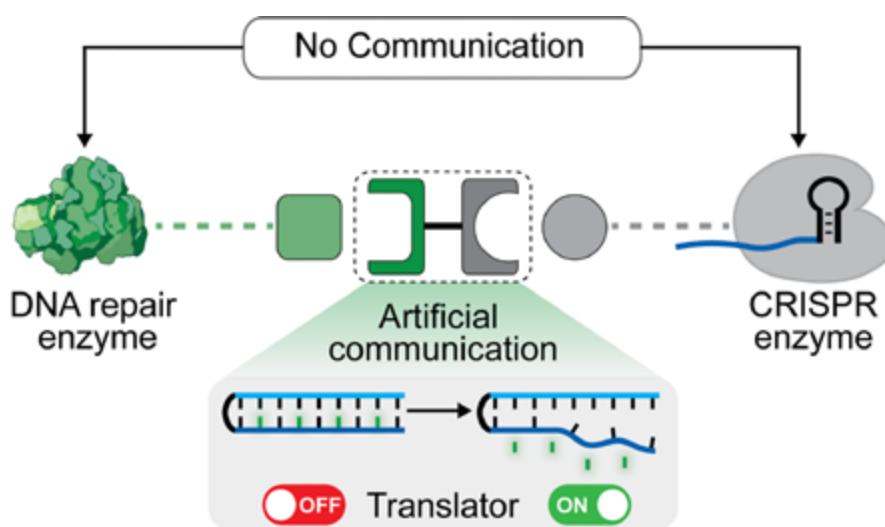


Figure 1. Schematic of the artificial communication pathway between DNA repair and CRISPR-Cas12a enzymes controlled by a DNA-based molecular transducer.

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O-119**COMPREHENSIVE CHARACTERIZATION OF SHEEP WOOL FROM DOMINANT CROATIAN BREEDS: A RENEWABLE RESOURCE FOR AGROECOSYSTEMS**

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The rising demand for meat, particularly lamb and mutton, has led to an increased focus on sheep farming, while the decreasing demand for wool, especially for low-quality EU wool compared to wool originated from major producers like China, India, and Australia, has left farmers grappling with wool's surplus. This imbalance creates both an economic and environmental problem. As a result, a substantial amount of sheared wool is being discarded or even burned, contributing to air pollution. One solution that has gained attention is the use of waste wool as a renewable, bio-based soil additive or hydroponic substrate in agroecosystems. Sheep wool contains essential nutrients such as carbon, nitrogen, and sulfur, and has a notable water retention capacity, which can improve soil fertility and microbial activity. The slow nitrogen release from wool may help reduce nitrate leaching into groundwater, which is a significant environmental concern linked to mineral fertilizers. However, factors like sheep's breed, diet, and climate conditions affect wool's properties, including biodegradability, nutrient content, and overall performance in agroecosystems. To address this challenge, this study focused on the characterization of wool from various dominant sheep breeds in Croatia, as well as feed hay and topsoil samples from 12 different locations across the country. This comprehensive analysis aimed to assess the wool's physicochemical, mineralogical, and radiological properties and to evaluate its potential for circular economic practices in agriculture. The research employed advanced analytical techniques including: Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) for elemental measurements, Fourier Transform Infrared Spectroscopy (FTIR) to study organic functional groups, Gas Chromatography-Mass Spectrometry (GC-MS/MS) for identifying persistent organic pollutants, Gamma-ray spectrometry for determining activity concentrations of gamma-ray emitting radionuclides, Scanning Electron Microscopy (SEM-EDX) and X-ray Diffraction (XRD) to provide high-resolution imaging and surface chemical analysis of the samples. The data from these analyses were further interpreted using Principal Component Analysis (PCA) and correlation analysis. Furthermore, the study incorporated a life-cycle assessment to explore the environmental impact of using wool-based products, focusing on sustainability and the broader implications of relying on wool in circular economies. This approach not only expands our understanding of wool's agricultural potential but also highlights the value of sustainable strategies in wool waste management, offering thus meaningful benefits for both farming systems and the environment.

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O-120

ENABLING SUSTAINABLE PROTEIN PRODUCTION: NMR AS A PROCESS MONITORING TOOL IN CULTURED MEAT (CM) PRODUCTION AND ENZYMATIC PROTEIN HYDROLYSIS OF BYPRODUCTS

Wubshet, Sileshi.

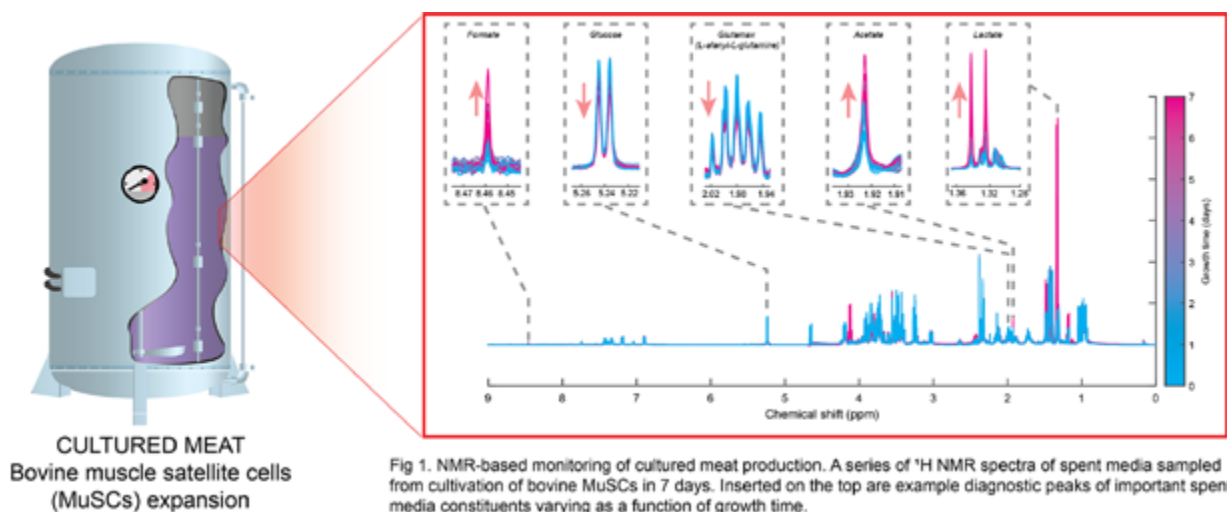
Nofima AS, Ås, Norway.

Biotechnological solutions present unparalleled opportunity to both ensure circular organic resource recovery as well as open novel ways of food protein production. Two of such solutions are: 1) enzymatic protein hydrolysis (EPH) [1] and 2) cultured meat (CM) productions [2]. EPH utilizes food-grade proteases to degrade a protein rich biomass (e.g., food processing byproduct) and enable extraction of water-soluble value-added ingredients in the form of peptides and amino acids. CM uses animal cells that are grown in a controlled bioreactor as an alternative to the traditional meat production. Both these processes have been recognized as central technologies for sustainable food protein production.

In both EPH and CM, process monitoring is an essential aspect in order to ensure controlled biomanufacturing with desired end product characteristics [3]. Nuclear Magnetic Resonance (NMR) spectroscopy provides a powerful platform to monitor such complex biotechnological processes. In CM production, NMR allows for both monitoring of nutrients (e.g. glucose and amino acids) and formation of critical metabolites (e.g., lactate). This allows for controlling depletion of vital nutrients and detecting build-up of toxic metabolites. Likewise, in EPH, NMR allows monitoring the release of small peptides and specific amino acids in the course of the process. In combination of advanced multivariate statistical methods, ^1H NMR fingerprint of hydrolysates from EPH can be used to predict important parameters such as degree of hydrolysis.

Here we are presenting two studies demonstrating NMR spectroscopy as a biotechnological process monitoring tool.

Study 1: CM production (Figure 1): Spent media samples from laboratory-scale cultured bovine cells have been analyzed using ^1H NMR spectroscopy (400 MHz). The experiment was designed to explore alternative growth factors to fetal bovine serum (FBS) in the cell culture medium, NMR was used to track the consumption of nutrients such as glucose, pyruvate, and free amino acids. Also, the formation of metabolites like lactate, acetate, and formate was monitored. Data analysis methods, including simple peak integration, and partial least squares regression were developed.



Study 2: Enzymatic protein hydrolysis: A total of 108 ^1H NMR spectra of hydrolysate samples was acquired from a laboratory scale EPH of poultry by-products. Processing parameters such as hydrolysis time, enzyme concentration and temperature were varied in a full factorial manner. The ^1H NMR fingerprints allowed to study the effect of the different processing parameters on the reaction and specifically on the release of free amino acids. Moreover, partial least squares regression based on the ^1H NMR fingerprints allowed prediction of important parameters such as average molecular weight of the hydrolysates.

In summary, NMR spectroscopy was demonstrated as a powerful tool for monitoring advanced biotechnological processes (i.e., cultured meat production and enzymatic protein hydrolysis of byproducts). In CM production, NMR allowed monitoring of the consumption of nutrients and the formation of metabolites. Similarly, during EPH, NMR was demonstrated as a viable technology for monitoring degree of protein hydrolysis as well as release of specific free amino acids. Such a method can have a pivotal role as integral part of process analytical technology (PAT) platform ensuring process monitoring, control and optimization.

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O-121

PRESSURIZED LIQUID EXTRACTION FOR THE SUSTAINABLE RECOVERY OF PROTEINS FROM LEMON PEELS. CHARACTERIZATION OF PROTEIN HYDROLYSATES BY UHPLC-MS/MS

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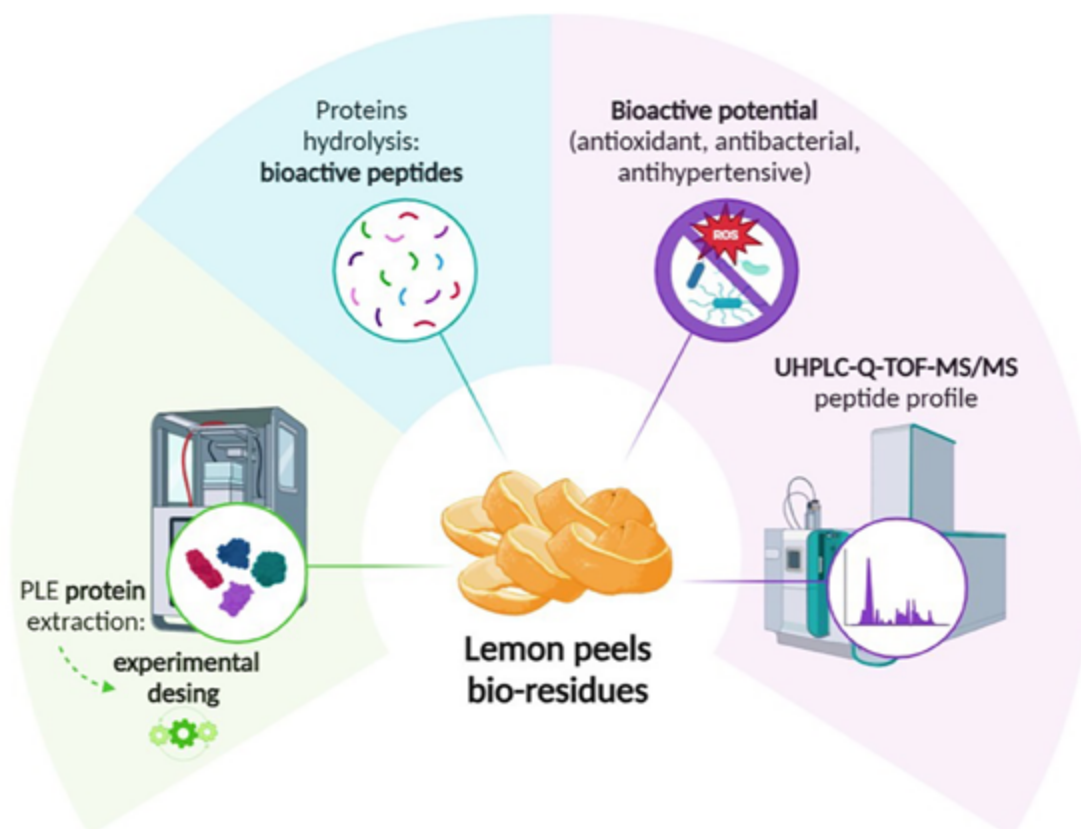
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Lemons, belonging to the *Rutaceae* family, rank among the world's most prominent crops, with global annual production consistently on the rise. This surge, however, has led to substantial waste, with up to 50-60% of the fruit's weight, primarily peels, being discarded. Consequently, sustainable waste management strategies have become increasingly vital, emerging as a priority for both the United Nations and the European Commission (Wedamulla et al., 2022).

The valorization of lemon peel biomass presents a renewable and cost-effective source of valuable compounds with industrial significance, including polyphenols, organic acids, terpenoids, and proteins (Wedamulla et al., 2022). Among these, proteins are particularly noteworthy due to the growing consumer preference for plant-based proteins (Xavier et al., 2025). Despite this trend, the exploration of citrus peels as a protein source remains relatively under-researched compared to other natural compounds, indicating a promising area for further investigation (Palma-Manrique et al., 2025; Sánchez-Elvira et al., 2024).

Proteins, while primarily recognized for their nutritional value, are also of interest due to their ability to release bioactive peptides upon consumption and hydrolysis by gastrointestinal enzymes. Hence, peptides can serve as bioactive ingredients across various industrial sectors (Palma-Manrique et al., 2025; Sánchez-Elvira et al., 2024).

In this context, a pressurized liquid extraction (PLE) method has been optimized using Box-Behnken experimental design and multi-response surface analysis. The factors examined included the ethanol-water ratio as the extraction solvent (0:100 - 80:20, v/v), static time (3-15 min), and temperature (100-180 °C). The responses considered were the total protein content and the formation of Maillard compounds. Additionally, the sustainability of the PLE method was assessed using the AGREEPrep tool (Wojnowski et al., 2022).



Upon establishing the optimal conditions, the protein extract was hydrolyzed with two enzymes (alcalase and thermolysin), and its antioxidant potential was characterized through various assays (reducing oxidizing compounds, scavenging ABTS radicals, and inhibiting the formation of hydroxyl radicals). Furthermore, its antimicrobial potential was evaluated against common food pathogens (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*), and its antihypertensive potential was studied via angiotensin-converting enzyme *in vitro* assay, monitoring inhibition by HPLC-DAD.

Finally, the peptide profile of both hydrolysates was determined using UHPLC-Q-TOF-MS/MS in both hydrophilic interaction and reverse phase modes. The results revealed that, in addition to peptides, various bioactive polyphenols and organic acids, such as coumaric acids, diosmetin 7-rutinoside, citric and malic acids, respectively, were identified in the extracts. Thus, the proposed method enables the recovery of a diverse array of high-value bioactive compounds, demonstrating antioxidant and antimicrobial potential, which could be utilized as bioactive ingredients in the agri-food and nutraceutical industries.

Acknowledgements:

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O-122**SUSTAINABLE VALORIZATION OF ORANGE PEEL WASTE: OPTIMIZED PRESSURIZED LIQUID EXTRACTION OF PHENOLIC COMPOUNDS AND HIGH-PURITY LIMONENE****Plaza, Merichel; Gallardo-Martínez, Rocío; Marina, María Luisa.***Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona Km. 33,600, 28871, Alcalá de Henares, Spain.*

✶Citrus fruit processing generates significant waste, primarily peels, which contain bioactive compounds such as phenolics and limonene. Valorizing these residues aligns with circular economy principles and promotes sustainability. However, optimizing extraction methods is crucial to maximizing the recovery of high-value compounds while maintaining environmental efficiency. Therefore, this work aimed to develop a biorefinery approach for the sustainable extraction of phenolic compounds and high-purity limonene from orange (*Citrus sinensis*) peel waste using pressurized liquid extraction (PLE). Consecutive extractions with water and ethyl acetate were optimized to achieve high-yield and high-purity extracts. PLE was applied using water and ethyl acetate as solvents to sequentially extract phenolic compounds and limonene. A Box-Behnken experimental design was used to optimize key parameters, including temperature and extraction time for both consecutive extractions. The optimal conditions were determined to be 100 °C for aqueous extraction and 125 °C for ethyl acetate extraction, with 9 min per extraction process. The aqueous extract involved three static cycles, while the ethyl acetate extract included one cycle with activated carbon inside the extraction cell to enhance limonene purity. Extract characterization was performed using high-performance liquid chromatography with a diode array detector (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). The aqueous extract was rich in flavanones, known for their antioxidant and health-promoting properties, while the formation of Maillard reaction products, such as hydroxymethylfurfural (HMF) and furfural, was avoided under optimal extraction conditions. The ethyl acetate extract yielded high-purity limonene, free from other volatiles or terpenes, making it suitable for food, cosmetic, and biopolymer applications. Statistical validation confirmed high reproducibility and efficiency of the optimized PLE process. This study demonstrates that PLE is an effective and green technology for valorizing citrus waste, offering a sustainable method to recover bioactive compounds. The proposed biorefinery approach contributes to a circular economy by efficiently utilizing orange peel waste, reducing environmental impact, and promoting the development of bio-based products.

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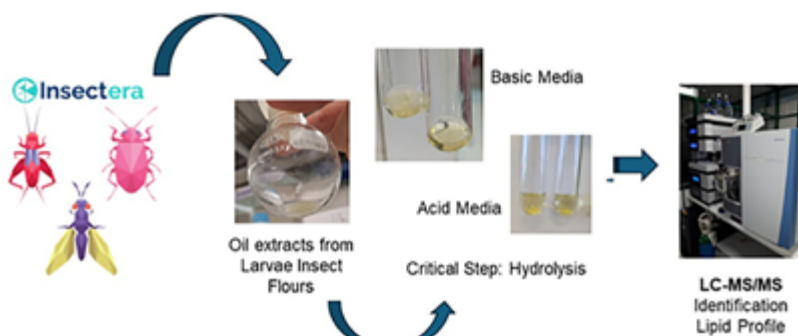
O-123

LIPID PROFILING OF INSECT FLOUR OILS FOR BIOMASS VALORIZATION AND ENHANCED NUTRITIONAL VALUE USING LC-MS/MS

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Monitoring the lipid profile is essential for ensuring the quality and authenticity of food products [1]. A detailed understanding of the lipid composition of oils and their metabolites significantly contributes to nutritional profiling. Within the framework of the INSECTERA – New Bioindustrial Sector Project, our research group aims to sustainably extract oils from insect larval flours and carry out a comprehensive characterization of their composition [2].



This study focuses on identifying metabolites in insect flour extracts to develop an additional and complementary lipid profile. This approach enhances food quality control and allows for a more thorough evaluation of lipid composition.

Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) is an emerging and powerful tool for lipid profiling. In this context, we have developed a simple and rapid LC-MS/MS methodology using electrospray ionization and a triple quadrupole system for the characterization of fatty acids (FAs). As a first step, we optimized an analytical procedure for the detection and analysis of free FAs, achieving direct analysis without the need for a derivatization step.

To accurately quantify total FAs, lipid hydrolysis is a crucial preparatory step. In this study, we developed and evaluated two distinct hydrolysis protocols - acidic and basic - tailored for effective lipid breakdown. Each protocol was systematically optimized by varying key parameters, including hydrolysis time, temperature, and solvent composition. Sample processing was performed in capped tube essays to maintain sample integrity and minimize contamination throughout the procedure. An aluminum thermoblock was employed to facilitate controlled thermal hydrolysis, ensuring efficient release of bound FAs under either acidic or basic conditions.

The resulting hydrolysates were analyzed via liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), enabling high-resolution quantification and structural characterization of individual fatty acids. Representative chromatograms and MS/MS spectra confirm the robustness of the workflow, supporting clear identification and differentiation of saturated, monounsaturated, and polyunsaturated FAs. This integrated approach; combining secure containment, optimized hydrolysis, and sensitive detection; offers a reliable platform for FA profiling in applications such as nutritional biochemistry, metabolomics, and biomarker discovery.

Preliminary findings suggest that basic hydrolysis yields superior results for oils extracted from insect flours derived from *Tenebrio molitor* (mealworm) larvae, *Acheta domesticus* (domestic cricket), and *Hermetia illucens* (black soldier fly). Ongoing efforts aim to further refine the analytical workflow to enhance the accuracy and reproducibility of total FA quantification post-hydrolysis.

Acknowledgements

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O-124

ADVANCES IN ANALYTICAL CHEMISTRY AND MASS SPECTROMETRY TO SUPPORT A SUSTAINABLE AND CIRCULAR ECONOMY

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Recent advances in analytical chemistry have significantly supported efforts to realize a sustainable circular economy and circular chemistry. By enabling rapid materials analysis and characterization of materials transformation throughout their life cycle, analytical methods are playing a key role in supporting waste reduction, resource recovery, pollution monitoring, and sustainable manufacturing.

In this presentation, recent developments in analytical chemistry and analytical instrumentation to support a sustainable and circular economy will be reviewed and selected contributions from our laboratories will be discussed.

For example, high-resolution mass spectrometry combined with chromatography is an indispensable tool for the identification and characterization of plastics. To support the transition from linear to circular chemistry, which prioritizes reuse, recycling, and resource efficiency, accurate and detailed chemical information about plastic materials is essential. Here, targeted and non-targeted screening approaches help to characterize, for example, the quality and safety of recycled plastics.

In addition, green analytical chemistry has emerged as a topic of interest, emphasizing the use of solvent-free and low-energy methods to minimize environmental impact during analysis. Here, ambient desorption/ionization high-resolution mass spectrometry (ADI-MS) is considered a very attractive tool because it does not require chromatography or large amounts of solvent, reduces sample preparation time, and produces virtually no chemical waste. Here, a solvent-free, plasma-based ADI-MS method is presented, which was carefully developed for the direct identification and fast screening of per- and polyfluoroalkyl substances (PFAS). PFAS are a large class of thousands of synthetic chemicals that are used worldwide. However, growing environmental and human health concerns in the last two decades have led to more stringent regulatory requirements and the development of quantitative analytical methods for PFAS detection. Today, standardized and powerful methods exist, e.g., for the determination of PFAS in water, sludge, compost, soil, and drinking water. While liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is often used, sample preparation, solvent consumption, and total analysis times can be challenging. Here, ADI-MS is considered interesting, because it requires only minimal sample preparation and little solvent consumption. In this work, a plasma-based pin-to-capillary flowing atmospheric-pressure afterglow source (FAPA) is used for the direct desorption/ionization of PFAS from thin-layer chromatography (TLC) plates. Selected PFAS samples were directly probed on functionalized TLC surfaces without a preceding planar chromatography step. In addition, direct analysis of PFAS mixtures and selected matrices was performed with little solvent consumption, no sample preparation and short analysis time.

O-125**SIMILARITY OF HUMAN SCENT AMONG BLOOD RELATIVES****Malá, Ulrika; Čechová, Jana; Kaminskyi, Oleksii; Škeříková, Veronika; Urban, Štěpán.***University of Chemistry and Technology in Prague, Prague, Czech Republic.*

Human scent samples offer a viable alternative to traditional crime scene evidence, as body scent emission cannot be prevented. Specially trained police canines are used for scent identification, but their reliability is often questioned in Europe due to possible misinterpretation. The method's subjectivity and lack of standardized procedures have raised concerns. To address this, an objective method based on analytical chemistry (olfactronic) is being developed, enabling statistical analysis and an international scent database. However, canine olfaction remains more sensitive than current laboratory devices.

Given the complexity of human scent samples, two-dimensional gas chromatography coupled with time-of-flight mass spectrometer appears to be the most suitable technique for analysis. In addition to individual identification, scent samples can provide valuable class identification, which may serve as useful evidence in court. Class identification categorizes samples into smaller groups based on shared characteristics such as gender, age or even metabolic changes or genetic kinship to other samples in forensic databases.

This study focuses on identifying similarities in the scent samples among blood relatives, particularly siblings and their parents. To leverage the advantages of both existing methods – olfactoric and olfactronic – both were used for scent analysis. Scent samples from the volunteers were collected on an inert, deeply cleaned sorbent (glass beads) and extracted into ethanol. The samples were then analyzed using GC×GC-MS/TOF or compared with each other by canines in a line-up procedure. The measured data were subjected to exploratory analysis, particularly Principal Component Analysis and Cluster Analysis.

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This study examines human scent as forensic evidence, emphasizing its reliability over traditional methods since scent emission cannot be prevented. While police canines aid scent identification, concerns over subjectivity have led to the development of an analytical chemistry-based method (olfactronic) for statistical analysis and database creation.

The study focused on scent similarities among blood relatives, comparing olfactoric (canine) and olfactronic (chemical) methods. Scent samples were analyzed with GC×GC-MS/TOF. Statistical techniques like Principal Component Analysis and Cluster Analysis were applied to interpret the results.

O-126**SIMULTANEOUS CHARACTERIZATION OF VOLATILE AND LOW-VOLATILE FRACTIONS OF BANNED CARBOFURAN FORMULATIONS FOR SOURCE TRACING USING A LIQUID-ELECTRON IONIZATION (LEI) INTERFACE****Grasselli, Genny¹; Arigò, Adriana¹; Famiglini, Giorgio¹; Skrob, Zdena²; Cajthaml, Tomáš²; Cappiello, Achille¹.**

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Carbofuran (CF) is a carbamate insecticide, nematicide, and acaricide widely used for its high effectiveness. Although it is no longer authorized in the European Union market, a case of intentional animal poisoning with CF occurred in the Czech Republic, but the origin of this pesticide remains unknown. In this study, 13 CF formulations from countries where the product is still available were analyzed using an innovative non-targeted analysis (NTA) approach. The characteristic compounds identified in these formulations were then searched in biological samples from poisoned animals to trace the origin of the substance used. Subsequently, a quantitative method was validated to determine the CF concentration in animal samples.

The analyses were performed using a liquid electron ionization (LEI) interface coupled with a high-resolution quadrupole time-of-flight mass spectrometer (Q-TOF). The LEI interface enables the combination of liquid chromatography (LC) with electron ionization (EI), extending the searchable EI spectral library to compounds not amenable to GC analysis [1]. Additionally, while conventional EI spectra often lack detectable molecular ions, the Q-TOF's low-energy EI capabilities allow the acquisition of highly informative spectra under standard EI conditions, as well as softer fragmentation at lower energies (15 and 9 eV), yielding molecular ions for most compounds.

A total of 13 CF formulations, both granular and liquid, as well as three real biological samples (one liver and two gastric contents), were provided by Charles University in Prague. Regarding the CF formulations, the extraction of compounds from granules was performed by immersing them in ultrapure water and sonicating for 10 minutes, followed by filtration with syringe filters. Liquid formulations were diluted in methanol (MeOH) and then filtered. The biological samples, including a fox liver and the gastric contents of an eagle and a red kite, were extracted using a modified Accelerated Solvent Extractor (ASE). Chromatographic separation was conducted using reversed-phase liquid chromatography (RPLC) on a Kinetex column (XB-C18 150x2.1, 1.7 μ m), with a gradient elution (5% B hold for 1 min; 5–100% B in 6 min; 100% B hold for 15 min) using ultrapure water + 0.1% formic acid (A) and ACN + 0.1% formic acid (B). Analyses were performed in Scan mode at 70 eV, at low energies (9 and 15 eV), and in MS/MS mode.

The NTA workflow developed in this study consists of several steps: (1) deconvolution of co-eluted compounds; (2) comparison of deconvoluted spectra with the NIST library; (3) molecular ion determination; (4) MS/MS analysis using the suspected molecular ion as the precursor ion; (5) molecular structure identification by comparing HR-MS/MS fragmentation with the ChemSpider database.

This approach enabled the identification of 7 compounds with high confidence levels, classified as level 1 or 2a on the Schymanski scale [2]. The library did not recognize 4 additional compounds, but their structures were proposed with high scores using the Molecular Structure Correlator (MSC) software, with satisfactory accurate masses, allowing their identification with confidence level 2b. For 3 compounds, molecular formulas were determined with high accuracy (<5 PPM), while for one compound, it was not possible to assign a molecular formula, likely due to its low concentration.

Regarding quantitative analysis, the method demonstrated excellent performance. As expected, negligible matrix effects (ME) were confirmed even without the use of an internal standard, verifying that the LC-LEI-QTOF-MS/MS system is a reliable alternative for the qualitative and quantitative analysis of volatile and low-volatility fractions in complex samples. The results showed that CF was detected and quantified in two of the three analyzed samples, however its concentration was well above LD50 values [3], confirming the suspicion of intentional poisoning.

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O-127**SMART AND GREEN FIRE DEBRIS CHARACTERIZATION USING HEADSPACE SORPTIVE EXTRACTION AND MACHINE LEARNING****Ferreiro González, Marta; Bikineyeva, Ramilya; P. Calle., José Luis; Palma, Miguel.***Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, Puerto Real, Spain.*

In arson cases, ignitable liquids such as gasoline, diesel, or kerosene are frequently used to initiate or accelerate fires. Consequently, detecting ignitable liquid residues (ILRs) at fire scenes is a critical step in forensic investigations to determine whether a fire was deliberately set. Currently, gas chromatography–mass spectrometry (GC-MS) is the most widely used analytical technique for fire debris analysis [1]. The American Society for Testing and Materials (ASTM) has established standardized protocols for ILR analysis and classification using GC-MS. According to ASTM E1416, IL classification into one of the eight ASTM categories relies on chromatographic pattern recognition, extracted ion profiling, and target compound analysis. However, accurately identifying ILRs in fire debris remains a major challenge for forensic analysts. The destructive nature of the fire, interfering pyrolysis compounds, fire suppression agents, and environmental factors such as weathering can alter the chemical composition of ILRs, making data interpretation complex. These challenges render the current methodology somewhat subjective, time-consuming, and highly dependent on the analyst's expertise. Moreover, the standard sample preparation method described in ASTM E1412 involves the use of activated charcoal adsorbents and toxic solvents such as CS₂, raising concerns about environmental impact. Given these limitations, there is an increasing demand for robust, rapid, and environmentally friendly analytical methods that enhance ILR detection and classification while minimizing analysis time and subjectivity.

This study aims to develop a faster, solvent-free, and more objective approach for ILR analysis and automatic classification using headspace sorptive extraction (HSSE) coupled with GC-MS and advanced machine learning (ML) algorithms. Fire debris samples were simulated by burning different substrates with and without ignitable liquids, including gasoline, diesel, kerosene, charcoal starter, lamp oil, and ethanol. HSSE optimization was performed using a Box-Behnken design and response surface methodology, yielding optimal conditions of 60 min extraction time, 80 °C extraction temperature, and a 50 mL vial volume. Following the thermal desorption of ILRs from sorptive stir bars, GC-MS analysis was conducted. To enhance objectivity in ILR classification, the total ion spectrum (TIS) was extracted from MS data instead of the traditional total ion chromatogram (TIC) [2]. Unsupervised ML algorithms, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), revealed three primary clusters: samples without ILs, gasoline-containing samples, and petroleum distillates. Additionally, supervised classification techniques—linear discriminant analysis (LDA), support vector machines (SVM), and random forest (RF)—were applied to three datasets: (A) ILR presence/absence detection, (B) ILR classification, and (C) a combined one-step classification approach. All supervised models achieved high classification rates ranging from 90% to 100%. The LDA model outperformed SVM and RF, achieving classification rates of 95.5%–100% across all datasets. Based on these results, a prototype web application was developed to deploy the trained models, enabling forensic analysts to use them for automatic ILR classification in fire debris data.

The results demonstrate that HSSE-GC-MS combined with ML provides a robust alternative to current ASTM methodologies. This approach offers a faster, fully automated, environmentally friendly, and highly accurate method for forensic fire debris analysis, reducing subjectivity in ILR classification and improving fire investigation outcomes.

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O-128

ESTIMATION OF BETA-BLOCKER CONSUMPTION IN THE URBAN POPULATION USING THE WASTEWATER-BASED EPIDEMIOLOGY APPROACH

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Wastewater-based epidemiology (WBE) is a relatively new approach that uses information obtained from the analysis of various metabolites or residues in wastewater with the aim of assessing the consumption or exposure to chemicals or pathogens in a population connected to a sewage system.

The aim of this work was to develop methods for the isolation and analysis of seven beta-blockers (atenolol, nadolol, pindolol, acebutolol, metoprolol, propranolol and betaxolol) in wastewater samples collected from the influent of the wastewater treatment plant in Cluj-Napoca, Romania, in order to estimate the consumption of these pharmaceuticals among the population in two time periods (February and October 2024) using WBE. The selected beta-blockers were extracted by solid phase extraction using a Strata C18-U cartridge and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The consumption was estimated using the daily mass load of pharmaceutical products reported per 1000 inhabitants (mg/day/1000 inh). Ammonia nitrogen was used to estimate the number of inhabitants connected to the sewage system.

The results obtained from the analysis of wastewater showed that the concentrations of the investigated beta-blockers expressed in ng/L varied as follows: atenolol (0.09-8.28), nadolol (0.02-0.3), propranolol (0.01-0.13), betaxolol (0.04-0.25) and metoprolol (25.51 – 124.59). Pindolol and acebutolol were detected in a single sample at concentrations of 0.04 and 0.25 ng/L, respectively.

Regarding the estimated consumption (mg/day/1000 inh) this parameter varied as follows: atenolol (0.03-3.74), nadolol (0.02-0.1), propranolol (0.04-0.74), betaxolol (0.05-0.38) and metoprolol (54.85 – 276.45). The median values for the sampling period are 1.22 for atenolol, 0.03 for nadolol, 0.335 for propranolol, 0.3 for betaxolol, and 110.37 mg/day/1000 inh for metoprolol. From the obtained results it can be observed that metoprolol is the most used beta-blocker in investigated population followed by atenolol and betaxolol. Other beta-blockers are consumed in small quantities or occasionally. As general conclusion, the WBE approach is an easy, rapid and non-invasive methodology for investigating the consumption behaviour of pharmaceutical products in a target population, providing valuable information in development of public health policies.

Acknowledgments: This study was performed in the framework of the PhD program from Babeş-Bolyai University, Cluj-Napoca, Romania, Environmental Science Doctoral School.

O-129**EVALUATION OF ECO-FRIENDLY SOLVENTS FOR HPLC****Michel, Frank¹; Peters, Benjamin²; Pieper, Anita²; Jung, Gisela²; Knoell, Peter².**

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High Performance Liquid Chromatography (HPLC) is known for its substantial solvent usage, resulting in considerable solvent waste. One effective strategy to mitigate solvent consumption in HPLC involves utilizing columns with reduced inner diameters or opting for more efficient column designs (such as those featuring smaller particle sizes or superficially porous particles) along with shorter column lengths. These methods align with principle #5 (miniaturization) of the 12 Principles of Green Analytical Chemistry [1].

However, a less frequently explored approach focuses on principles #10 (utilizing reagents from renewable sources) and #11 (substituting toxic reagents) of Green Analytical Chemistry. This can be achieved by transitioning to bio-based solvents or selecting solvents with lower toxicity levels (or ideally a combination of both) compared to conventional organic modifiers used in Reversed-Phase HPLC, such as acetonitrile and methanol.

This work will highlight recent studies investigating the use of ethanol, dimethyl carbonate and propylene carbonate as more sustainable solvents in HPLC. Given that ethanol (particularly its aqueous mixtures) exhibits significantly higher viscosity than acetonitrile and methanol, both monolithic and particle-packed HPLC columns have been utilized in this research. Monolithic columns offer advantages over particle-packed columns, including very low backpressure and enhanced resistance to matrix effects due to their high porosity. But also particle-packed columns can be used well with these more viscous solvents on today's HPLC and UHPLC instrumentation. It will be discussed how these greener solvents impact separation efficiency, selectivity, and backpressure. Finally, various examples will be presented showcasing the application of greener solvents in practical HPLC scenarios.

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O-130**IMPROVING EXHALED BREATH SAMPLING FOR ADVANCING VOLATILOME RESEARCH****Crescenzi, Carlo¹; Santarelli, Elisabetta¹; Caponigro, Vicky¹; Frattoni, Massimiliano²; Guerriero, Ettore².**

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Exhaled breath analysis has emerged as a powerful, non-invasive method for investigating human metabolism and holds significant promise for the development of diagnostic tools. However, despite its potential, obtaining accurate breath samples remains a challenge due to the complexity of the human respiratory system. Exhaled breath comprises a mixture of gases, water vapor, volatile organic compounds (VOCs), and aerosols. The diverse origins of these components—ranging from the alveolar region to the oral and nasal mucosa, and even gastrointestinal sources—pose substantial difficulties in acquiring samples that are truly representative of systemic metabolic processes.

In addition to high intra- and inter-individual variability, metabolite concentrations can fluctuate significantly throughout the day or over longer timeframes. Furthermore, the preconcentration of trace-level metabolites may be compromised by external contamination and inadequate storage conditions.

This presentation critically reviews and analyzes the key aspects of exhaled breath sampling, highlighting the technical and methodological challenges involved in achieving accurate and reproducible results. Factors influencing sample quality, including the anatomical and physiological characteristics of the respiratory system, are examined in detail.

Results from original experiments will be presented, suggesting strategies and solutions to improve sampling efficiency and accuracy, and contributing to the development of a standardized approach.

By exploring recent advances in breath sampling technologies and methodologies, this presentation aims to provide valuable insights into improving the reliability of exhaled breath analysis—ultimately enhancing our understanding of the human volatilome and its potential in non-invasive diagnostics.

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O-131

DEVELOPMENT OF ELECTROCHEMICAL SENSORS WITH 3D PRINTING TECHNOLOGY FOR THE ANALYSIS OF DRUGS OF ABUSE

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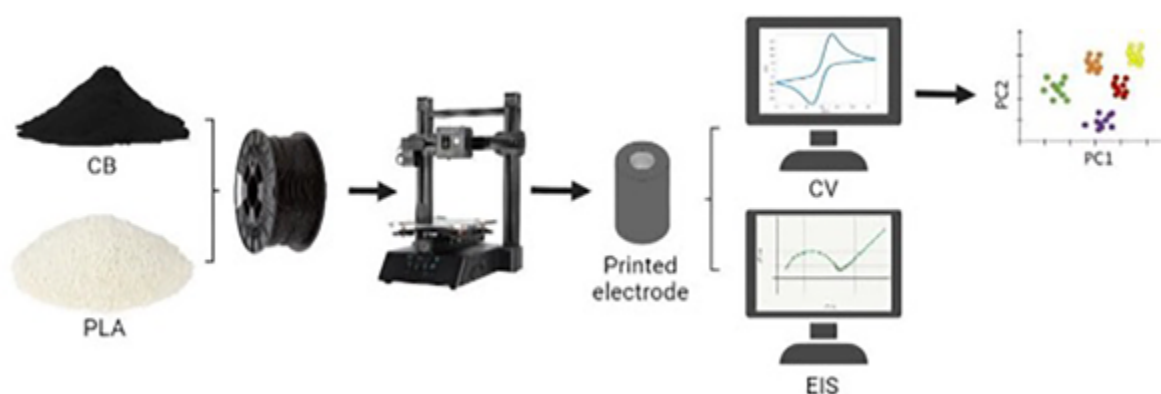
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Accurate and immediate analysis of illicit substances is crucial for handling social and public health challenges. The effective detection of these drugs can greatly enhance law enforcement efforts, public security and healthcare responses. However, current on-site drug testing methods often lack precision, while more advanced techniques tend to be expensive and require specialized equipment, making them less accessible and slower to implement [1]. In this scenario, electrochemical sensors represent a promising alternative for identifying illicit drugs and their precursors, given their characteristic electrochemical behavior. In particular, 3D printing technology, especially fused deposition modeling (FDM), stands out as an innovative and cost-effective approach to develop electrochemical sensors.

In recent years, commercially available filaments such as “Black Magic” or “Protopasta” have gained significant attention due to its excellent conductive properties. These filaments consist of polylactic acid (PLA), which serves as an insulating matrix, and some carbon-based material, which imparts the necessary electrical conductivity. Numerous studies, along with the experimental findings that will be presented herein, have demonstrated that sensors fabricated using these filaments have proven effective for the analysis of a wide range of molecular compounds.

However, dependency on external suppliers (e.g. “Black Magic” being no longer available) has proven problematic in the past. Such issues highlighted the necessity to develop custom-based alternative options with a focus on electrochemical sensing, which at the same time could offer several advantages by enabling precise modifications to its composition to tailor its electrochemical response.

For this purpose, a conductive filament composed of acrylonitrile butadiene styrene (ABS) as the base polymer, incorporating carbon black as the conductive agent was manufactured. Developed material was successfully extruded and 3D-printed sensors were prepared using this filament, which, following an activation process, exhibited a strong electrochemical response. A comparative analysis was conducted between the commercial “Protopasta” filament and our developed filament, both of which were utilized for the analysis of illicit drugs. Promising results have been obtained for the qualitative discrimination of different illicit drug substances such as cocaine, codeine, MDMA, methamphetamine or Ketamine. Finally, the developed sensors were also successfully applied to the quantitative analysis of cocaine in seized samples.



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O-132**3D PRINTING IN ANALYTICAL CHEMISTRY: REVOLUTIONIZING SAMPLE PREPARATION AND SENSORS**

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3D printing has gained significant interest in the field of Analytical Chemistry due to its ability to fabricate novel structures that are either challenging to achieve with conventional technologies or require prohibitively high manufacturing costs. Among the various 3D printing techniques available, the most widely used are Fused Deposition Modeling (FDM) and Stereolithography (SL), each offering distinct advantages and limitations. Despite their differences, these methods share a common principle: the layer-by-layer deposition of material to construct intricate structures, differing primarily in how these layers are generated and solidified.

In recent years, research on 3D printing applications in Analytical Chemistry has expanded exponentially, with sample preparation and sensor development emerging as key areas of innovation. This presentation explores how 3D printing is revolutionizing sample preparation by enabling the development of advanced solid-phase extraction systems and novel sensor-integrated devices.

Key examples from the CLECEM research group will illustrate the vast potential of 3D printing in this domain. These innovations include cutting-edge devices for rotating disk applications, sustainable systems utilizing eco-friendly materials, and multifunctional platforms that seamlessly integrate both extraction and sensing capabilities. Additionally, other promising alternatives will be discussed, highlighting the versatility and impact of 3D printing in modern analytical methodologies.

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O-133

FULLY 3D-PRINTED ELECTROCHEMICAL DEVICES: A GAME-CHANGER FOR (BIO) SENSING APPLICATIONS

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Electrochemical (bio)sensors have revolutionized the detection of target analytes across a wide range of applications, from clinical diagnostics and pharmaceutical research to environmental monitoring and agri-food safety^[1]. These devices provide high sensitivity and specificity, making them invaluable tools also for real-time analysis. While many fabrication techniques, such as screen-printing, inkjet-printing, wax printing, etc., have provided affordable and scalable solutions, the establishment of additive manufacturing, namely 3D printing, is transforming measurement science in unprecedented ways^[2]. 3D printing offers a unique ability to rapidly prototype and customize electrochemical devices, integrating complex structures that enhance both analytical performance and usability. This technological advancement has demonstrated to yield a big impact, in particular by facilitating the development of intricate well designs and improving fluid control, highlighting 3D-printed devices in user-friendly and low-cost application^[3,4].

This oral communication is aimed to present wide possibilities around the world of 3D printing for generating novel concepts of frugal diagnostics. In particular, its versatility will be demonstrated towards two successful cases of study.

The first results are related to the development of a fully 3D-printed electrochemical sensing device featuring a three-electrode system fabricated from conductive materials. This platform incorporated a microwell structure to optimize sample handling, and it has been applied to detect miRNA in serum samples. With a dynamic detection range from 0.001 to 400 nM and a lower limit of detection in the picomolar range, this sensor demonstrated superior performance compared to conventional screen-printed sensors. Its high specificity for the target miRNA underscores its potential for personalized diagnostics and prognostic healthcare applications^[5]. The second application goes beyond medical applications, providing 3D-printed solutions to be game-changers in agri-food monitoring^[6]. To showcase their versatility, we designed and optimized a novel trident-shaped, fully 3D-printed electrochemical system capable of direct fruit analysis—eliminating the need for complex sample preparation. This device enabled real-time, on-site measurements with remarkable portability and durability. As a proof of concept, we quantified ascorbic acid in oranges, achieving a 98% correlation with traditional LC-MS/MS methods. This level of accuracy confirms the reliability of 3D-printed sensors in ensuring food safety and quality.

As 3D printing continues to advance, its integration into sensor technology is driving innovation across multiple disciplines. This transformative approach is reshaping the future of electrochemical sensors, bringing us closer to a world where high-precision, cost-effective, and user-friendly analytical devices are accessible to all.

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O-134

3D-PRINTED MIL-100(Fe)-SO₃H COLUMN FOR THE PRECONCENTRATION AND EXTRACTION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs) FROM WATER

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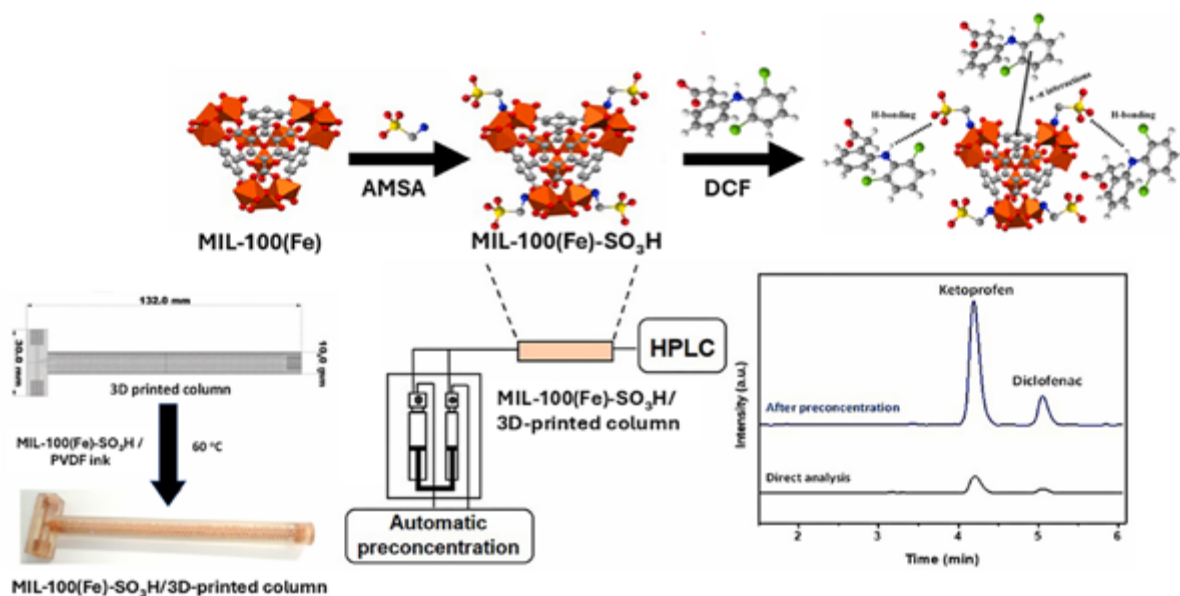
The increasing presence of pharmaceutical contaminants in aquatic environments represents a major environmental and public health concern. Among them, nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac (DCF) and ketoprofen (KET) are frequently detected in wastewater and surface waters [1][2]. Efficient removal and preconcentration of these pollutants are essential for environmental control and remediation.

Among the various available strategies, adsorption using porous materials stands out as one of the most cost-effective, simple, and efficient methods [3]. In particular, adsorption by metal-organic frameworks (MOFs) have attracted considerable attention due to their large surface area, tunable porosity, ease of synthesis, and their ability to interact with organic pollutants via multiple mechanisms, including electrostatic interactions, hydrogen bonding, acid–base interactions, and π – π stacking [4][5].

In this work, we developed a selective and reusable preconcentration and adsorption system by combining MOF-based adsorption with additive manufacturing. The iron-based MOF MIL-100(Fe) was functionalized via post-synthetic modification using aminomethane sulfonic acid (AMSA), introducing sulfonic acid (–SO₃H) groups at the unsaturated metal sites. This functionalization significantly enhances the MOF adsorption performance through hydrogen bonding with NSAID molecules.

The functionalized material (MIL-100(Fe)-SO₃H) was incorporated into a 3D-printed column designed for flow-through solid-phase extraction (SPE) of DCF and KET prior to HPLC analysis. The column, printed using stereolithography (SLA) at 0.500 mm resolution, consisted of 1016 layers forming interconnected cubic structures to optimize flow and mass transfer.

Batch adsorption studies demonstrated that MIL-100(Fe)-SO₃H exhibited significantly higher adsorption capacities for DCF compared to unmodified MIL-100(Fe), reaching over 80% removal for 100 ppm DCF in only 4 minutes. The material also demonstrated excellent reusability and regeneration capability, retaining over 93% performance after multiple adsorption–desorption cycles.



Under dynamic SPE conditions, the 3D-printed column achieved enrichment factors of 17 for DCF and 9 for KET from 1 ppm solutions, following elution with 2 mL of methanol. These results confirm its potential for trace-level detection and preconcentration of NSAIDs in aqueous matrices.

In conclusion, this work demonstrates the successful integration of MOF-based adsorbents with 3D printing technologies to develop a robust, efficient, and reusable system for the extraction and preconcentration of pharmaceutical pollutants in water. This innovative MIL-100(Fe)-SO₃H/3D-printed column represents a promising solution for efficient water purification and environmental monitoring.

Acknowledgement

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O-135**A LOW-COST 3D-PRINTED AUTOSAMPLER AND AUXILIARIES FOR LAB-IN-SYRINGE AUTOMATED LIQUID PHASE MICROEXTRACTION APPROACHES****Horstkotte, Burkhard; Sklenářová, Hana.***Charles University, Faculty of Pharmacy in Hradec Králové, Department of Analytical Chemistry, Hradec Králové, Czech Republic.*

3D printing continues to be a revolution in fabrication and prototyping. All related techniques produce the object in question by adding the prime material layer-by-layer by localized deposition or polymerization. 3D printing shows unprecedented advantages regarding fabrication speed, overall costs, shape flexibility, tuneability over classical substrative techniques.

For laboratory-based research, 3D printing is an enabling technique and offers a highly efficient way to generate either purpose-made or downloadable laboratory supports and auxiliaries with a wide range of uses, thereby facilitating the daily work in developing analytical methods and preparative procedures .

It enables fast replacement of broken instrumental parts, fabrication of sampling devices. Among the most interesting advantages is that needed devices can be designed and produced in the proximity to where needed, with affordable instrumentation and consumables, and by the final user her/himself. This allows straightforward design adaptation and minimizes “demand-to-obtain” time and effort [1].

While 3D printouts are not always suitable as active instrumental part, e.g., in contact with chemicals, it is an ideal approach to achieve improved or unique functionality of supports and auxiliaries, such as devices for sample preparation and tools for laboratory automation with examples to be shown.

We will present a 3D printable rotary 3-axis autosampler developed to be combined with the automation technique Lab-In-Syringe [2] that requires less than 150 Euro worth of material including the few not 3D printable elements (actuators, electronical control, screws, etc). A study of applicability for liquid phase microextraction will be presented. Herein, the void of a computer-controlled bidirectional syringe pump, connected to the autosampler, serves as for solution mixing, protein precipitation, and analyte extraction, and can be further coupled to optical detection or chromatographic separation of the extracted analytes. The features of the autosampler and combined system including challenges in design and fabrication will be highlighted as well as the optimization of the extraction system.

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O-136**3D-PRINTING IN ANALYTICAL CHEMISTRY: CHALLENGES AND OPPORTUNITIES****Cunha, Diana R.; Fernandes, Sara R.; Meireles, Andreia N.; Barreiros, Luisa; Segundo, Marcela A..***LAQV, REQUIMTE, Faculty of Pharmacy, Univ. Porto, Porto, Portugal.*

3D-printing transforms analytical chemistry by offering design flexibility for complex geometries, rapid prototyping, and cost-effective customization of devices like microfluidic chips and sensors. It enables miniaturization, integration of functionalities, and the use of different materials, potentially enhancing sensitivity and enabling novel analytical methods. Nevertheless, some challenges are still present, namely material limitations regarding chemical compatibility and purity, and resolution/accuracy constraints impacting elaborate designs. Additionally, post-processing for surface smoothness and support removal might be required, introducing also batch-to-batch variability.

These aspects will be discussed through different applications of 3D-printing, including developments in sample treatment and in the design of tailored devices to facilitate detection of analytes. For sample treatment, the development of an innovative device for the simultaneous sampling and extraction of four anticoagulant agents from biomatrices using filter paper as a sample collection material and sorbent will be showcased (1). The computer-aided design was performed using the Autodesk Tinkercad® free web app for 3D design, and the device was fabricated by 3D-printing using fused deposition modelling (TRILAB DeltiQ 2 printer), employing a black chlorinated polyethylene filament. To prototype the device, several parameters were evaluated, namely different configurations, infill percentages and materials, aiming at the inclusion in a flow system for automated sample treatment.

Furthermore, 3D-printing based on Liquid Crystal Display (LCD) technology allowed the production of solid-phase microextraction sorbents based on polymerized deep eutectic solvents using minimal volumes of printing material after printer customization (2). In this case, the printer utilized a parallel matrix of 15 LEDs, delivering a light intensity of 3500–4000 $\mu\text{W cm}^{-2}$, with a slicing layer thickness of 0.05 mm. Materials were tested for flexibility and elasticity, with best results found for a polymerization time of 12 s. Finally, the design of structures to house luminescence detectors in automated, miniaturized lab-on-valve platforms and the production of customized devices to accommodate smartphone-based colorimetry will be critically addressed, emphasizing the advantages and shortcomings of different approaches and configurations.

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O-137**IN-CAPILLARY ENZYMATIC DIGESTION FOR THE TARGETED BOTTOM-UP ANALYSIS OF PROTEIN BIOMARKERS BY CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY****Giménez, Estela¹; Pont, Laura²; Casas, Judith¹; Salim, Hiba¹; Benavente, Fernando¹.**

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Targeted bottom-up proteomics is of great interest for the straightforward, accurate, and sensitive measurement of specific protein biomarkers from surrogate peptide fragments. However, this approach typically requires a time-consuming off-line enzymatic digestion with trypsin, which may not be suitable for covering specific sequence regions containing important post-translational modifications (PTMs). Here, we present an in-line enzymatic digestion strategy for the targeted bottom-up analysis of α -synuclein (α -syn), which is a protein biomarker of Parkinson's disease (PD) [1]. The method involves in-capillary enzymatic digestion capillary electrophoresis-mass spectrometry (ICD-CE-MS) with endoproteinase GluC as an alternative to trypsin, which fails to map Ser-129, a phosphorylated site in PD [2]. The method was optimized and validated, focusing on the detection of potentially phosphorylated peptides using a recombinant α -syn standard. Under optimized conditions, the method was repeatable at $100 \mu\text{g}\cdot\text{mL}^{-1}$ of digested protein, yielding for the [124-131] α -syn peptide percent relative standard deviation (%RSD) values for peak area and migration time of 3.4 and 7.2, respectively ($n = 5$). The linearity between 25 and $200 \mu\text{g}\cdot\text{mL}^{-1}$ ($R^2 > 0.995$) was satisfactory, and the limit of detection was $15 \mu\text{g}\cdot\text{mL}^{-1}$, with a protein sequence coverage of 77.1%. The method was successfully applied to analyze endogenous α -syn in human blood [3], demonstrating the potential of ICD-CE-MS beyond trypsin for sensitive targeted bottom-up analysis of challenging protein biomarkers in biological fluids.

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AMBIENT DROPLET REACTOR: AN ANALYTICAL PLATFORM FOR SINGLE-MOLECULE MANIPULATIONS AND CHARACTERIZATIONS

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Droplet evaporation on superhydrophobic surfaces offers a powerful molecular enrichment mechanism by leveraging the movement of the three-phase contact line. This movement prevents molecular deposition on the interface, ensuring that molecules continuously migrate to the droplet's edge. As evaporation progresses, this leads to significant molecular concentration, making the process highly effective for applications requiring precision and sensitivity, such as analytical chemistry, biosensing, and environmental monitoring. Unlike conventional concentration methods, this approach minimizes contamination while enhancing the detection of target molecules. Beyond molecular enrichment, this method facilitates the separation of biological macromolecules, including DNA and proteins, from solution. Importantly, it preserves these macromolecules in their native conformations, which is crucial for studying their structure and function. By combining in-situ characterization techniques such as Raman spectroscopy, X-ray diffraction, and transmission electron microscopy (TEM), we gain detailed structural insights into these macromolecules. This enables a deeper understanding of complex biological systems and supports advancements in biochemical analysis, molecular biology, and nanotechnology. An additional breakthrough in our research reveals that evaporating droplets not only enrich molecules but also concentrate electric charge. Even when starting with an initial charge as small as a few tens of picocoulombs (pC), the charge progressively accumulates as the droplet shrinks. This continuous charge enrichment intensifies the electric field on the droplet's surface. When the charge reaches a critical threshold, known as the Rayleigh limit, the electrostatic repulsion overcomes surface tension, leading to spontaneous droplet fission—also known as Rayleigh Fission. This marks the first reported instance of spontaneous Rayleigh Fission driven purely by charge enrichment during evaporation. This phenomenon has significant implications for fields such as electrochemical reactions and bioreactors. The ability to manipulate charge concentration in droplets opens new possibilities for optimizing reactions at the microscale. By harnessing charge enrichment, researchers may develop innovative methodologies to control electrochemical processes, enhance reaction efficiency, and improve the performance of microfluidic systems. The potential applications extend to nanotechnology, where precise charge manipulation could play a role in advanced material synthesis and molecular assembly. The combination of molecular and charge enrichment through droplet evaporation presents a unique and versatile platform for scientific and technological advancements. It offers a novel approach to molecular concentration, separation, and charge manipulation, with applications spanning analytical chemistry, biosensing, nanotechnology, and electrochemistry. By further exploring this phenomenon, we can unlock new capabilities in biochemical analysis, develop next-generation diagnostic tools, and refine microscale reaction control techniques. This research paves the way for innovative applications, demonstrating how fundamental physical processes can be harnessed for practical and transformative advancements.

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FIT-FOR-PURPOSE DEVELOPMENT OF LATERAL FLOW IMMUNOASSAYS DETECTING INFECTIOUS DISEASES: UNRAVELLING THE IMPACT OF THE BIOANALYTICAL CHEMISTRY ON THE EPIDEMIC OUTBREAKS CONTROL

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More than sixty years after their introduction, lateral flow immunoassays (LFIA) have proven their essential role in managing infectious diseases. They are the most widely used diagnostic devices for large-scale screening, epitomizing point-of-care testing [1]. Authorities and reference laboratories typically develop in-house instrumental confirmation methods for health emergencies, while commercial POCTs, focussed on diagnostic sensitivity, specificity, and regulatory compliance, are used for screening. However, each infectious disease presents unique challenges, such as cross-reactivity with co-infections, interferences, variants and serotypes, unconventional samples or matrices. Therefore, LFIA development should include the communication between authorities and their expertise on specific clinical needs from the outset, the developers of the LFIAs can then exploit their expertise to make substantial improvements tailored to these needs, and companies that can support the device certification. Therefore, a collaboration was established between the Bioanalytical Chemistry group of the Department of Chemistry (University of Turin), the Infectious Diseases Unit of the Department of Veterinary Science (University of Turin), the Istituti Zooprofilattici Sperimentali della Lombardia e dell'Emilia-Romagna (IZSLER), dell'Umbria e delle Marche (IZSUM), della Sardegna (IZS-sardegna), and In3diagnostic srl. This multidisciplinary team has developed fit-for-purpose LFIAs for diagnosing animal and human infectious diseases with excellent diagnostic performance, successfully used in field applications, over the past four years [2–7]. Very important in this collaboration is the clear and distinct division of roles according to experience. The Department of Veterinary Science and IZSs provides the diagnostic need input, highlighting any needs related to the specific context, and generating and characterizing the monoclonal antibodies (mAbs) used in the devices. Nevertheless, the pivotal and crucial role of the LFIA developer is played by the bioanalytical chemist whose experience in developing LFIAs is exploited along the whole process, from taking the input of the IZS as the authority, conducting the cross-selection of bioreagents (mAbs), defining the optimal configuration, carrying thorough sensitivity studies and optimization.

Dwelling on the added value given by the bioanalytical chemist, when appropriately approached for specific diagnostic/application issues, this has led to results that surpassed the specific application. Examples are the “antigen saturation hook-effect” occurring when a single antibody is used both as the capturing reagent on the test line and as the detection reagent labelled with gold nanoparticles [2–4]; the use of hydrogen peroxide in samples from organs containing antibody binding factors [2]; the use of experimental design based strategies for the optimization of the sensitivity [2–5]; the choice of the role of cross-reactive mAbs to find an optimal compromise between an undesired cross-reactivity and the sensitivity; the positioning strategy of multiple test lines when the figure-of-merits are both diagnostic sensitivity and serotyping specificity [3,5]. This collaboration led to the development of two LFIAs for diagnosing and serotyping foot-and-mouth disease virus in cattle, achieving sensitivities between 75-94% and a specificity of 100% for the five most prevalent serotypes (A, Asia1, O, SAT1, and SAT2) on epithelial tissues [3,5,7]. For diagnosing Lumpy Skin Disease in cattle, a sensitivity comparable to the reference ELISA method (limit of detection $10^{3.4}$ TCID₅₀/mL) was obtained [4]. To address the recent African Swine Fever outbreak in Europe, an LFIA was developed with a sensitivity of 80-95% on organ and tissue samples from domestic, wild, and boar pigs [2]. Recently, the multidisciplinary team has been working on developing LFIAs to diagnose Brown Hare Syndrome and Rabbit Haemorrhagic Disease Virus infections in liver samples from hares and rabbits, and West Nile Virus infection in human serum samples. The impact of bioanalytical chemistry has been instrumental in discovering, describing and addressing peculiar clinical-analytical challenges and bringing performance to its fullest, rather than purely analytical, application potential.

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O-140**DIRECT INJECTION ESI-MS ANALYSIS OF PROTEINS WITH HIGH MATRIX CONTENT: UTILIZING TAYLOR–ARIS DISPERSION****Gaspar, Attila; Szabo, Ruben; Nagy, Cynthia.***University of Debrecen, Debrecen, Hungary.*

Recently we demonstrated the utility of the Taylor–Aris (TA) dispersion in avoiding serious interference issues commonly occurring in the electrospray ionization-mass spectrometric (ESI-MS) determination of therapeutic protein pharmaceuticals undergoing no pre-separation or sample purification. It was also pointed out that the TA dispersion conditions and its analytical utilization for proteomics can be easily accomplished in a commercial CE-MS instrument. In the proposed Taylor–Aris dispersion-assisted mass spectrometry (TADA-MS) analysis 0.5 μL sample is injected into a 65 cm long 50 μm i.d. capillary and pumped with 1 bar toward the MS. The procedure is efficient for the direct injection analysis of components having low diffusion coefficients (proteins) that are present in complex matrices of small organic and inorganic compounds. In the lecture beside the main features of the TADA-MS we demonstrate the exceptional utility of TADA-MS in native protein analysis as well: (i) a dramatic improvement in detection sensitivity was found due to its ability to strongly reduce matrix interferences, (ii) more "native-like" conditions can be used during analyses, (iii) the direct injection of non MS-compatible matrices is allowed into MS and (iv) a considerable simplification and economization of the workflow is ensured.

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Given the significance of these determinations, this study proposes the implementation of several electrochemical platforms for the simultaneous determination of biomarkers related to both the gastrointestinal (glial fibrillary acidic protein interleukins 12 and 23, fecal calprotectin, intestinal fatty acid binding protein) and oral (myeloperoxidase, matrix metalloproteinases 9 and 13, interleukin 6, receptor activator of NF- κ B ligand, protein arginine deiminase 4 and its corresponding antibody) microbiomes. These innovative platforms employ innovative nanomaterials, such as graphdiyne or hybrid nanocomposites (V_2O_5 /MWCNTs, rGO/CNC, rGO/MoS₂/AgNP, MWCNT/CNC or CeO₂NPs/MWCNT), to modify the electrode surface or as an electroactive label for signal amplification to improve the analytical properties of the developed immunosensors. The effectiveness of these platforms has been validated through the analysis of biomarkers in clinical samples and through comparison with reference methodologies.

The developed devices offer several significant advantages, including improved analytical characteristics, user-friendliness, reduced testing time and costs, and the ability to perform multiplexing. These features make these platforms suitable for clinical applications, paving the way for their widespread use in hospitals and outpatient care. This advancement contributes to validating biomarkers and supports the progress of personalized medicine. The main goal is to enhance diagnosis and improve the quality of life for patients affected by prevalent and life-threatening diseases.

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O-142**GAS-PHASE ELECTROPHORESIS (nES GEMMA INSTRUMENTATION) OF EXHALED BREATH CONDENSATE (EBC)****Weiss, Victor¹; Miskovic, Marija Zora²; Kuban, Petr³; Lado, Sara⁴; Steininger, Christoph⁴; Marchetti-Deschmann, Martina¹.**

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Human breath analysis is an attractive alternative strategy for non-invasive retrieval of representative, unbiased samples directly from the lung of a patient. In such a way, information on the health status of a person can be obtained. Significant effort has been put e.g. into volatile organic compounds (VOCs) that show characteristic patterns in lung cancer-related breath samples. Also, VOC analysis via GC-MS has been lately used for SARS-CoV-2 diagnostics.

Gas-phase electrophoresis on a nano electrospray gas-phase electrophoretic mobility molecular analyzer (nES GEMMA) also known as nES differential mobility analyzer (nES DMA) enables the separation and detection of (bio-) material in the size range from several few to several hundred nm diameter. [1] Analytes are electrosprayed from a volatile, liquid electrolyte solution, followed by drying of droplets and concomitant charge equilibration in a bipolar atmosphere induced e.g. by a ²¹⁰Po alpha-particle source, a corona discharge process, or similar. Subsequently, singly-charged analytes are separated according to their surface-dry particle size (electrophoretic mobility, EM, diameter) in a high laminar sheath flow of particle free air and a tunable electric field applied in an orthogonal direction. Particles are counted after separation yielding true particle-number concentration values in accordance with a recommendation of the EC for nanoparticle characterization. Finally, a spectrum of a corresponding sample relating particle size and respective number values is obtained. Based on our previous experience with the analysis of proteins, extracellular vesicles, viruses and virus-like particles we reasoned that such an instrument is also capable to contribute to the characterization of particles in exhaled air (PE_x), in particular in exhaled breath condensate (EBC).

Indeed, by applying EBC samples to our nES GEMMA instrument we were successful in obtaining corresponding spectra. For one donor, recorded spectra remained more or less constant over time (different time points of EBC collection). Low sample conductivity made as stable as possible nES conditions in terms of recorded current values a necessary prerequisite for data interpretation. Expanding our sampling strategy to more donors yielded spectra, some of which differed between individual donors and could be clustered via hierarchical cluster analysis. The overall biological and clinical relevance of recorded spectra will have to be targeted in a follow-up study. Likewise, this follow-up study will have to focus on clearly standardized collection, sample storage and measurement conditions.

Experiments presented in this study indicate that PEx detected during our experiments corresponds to unspecific aggregates. Particle collection together with further MALDI MS analysis will help in the future to deepen our knowledge on PEx.

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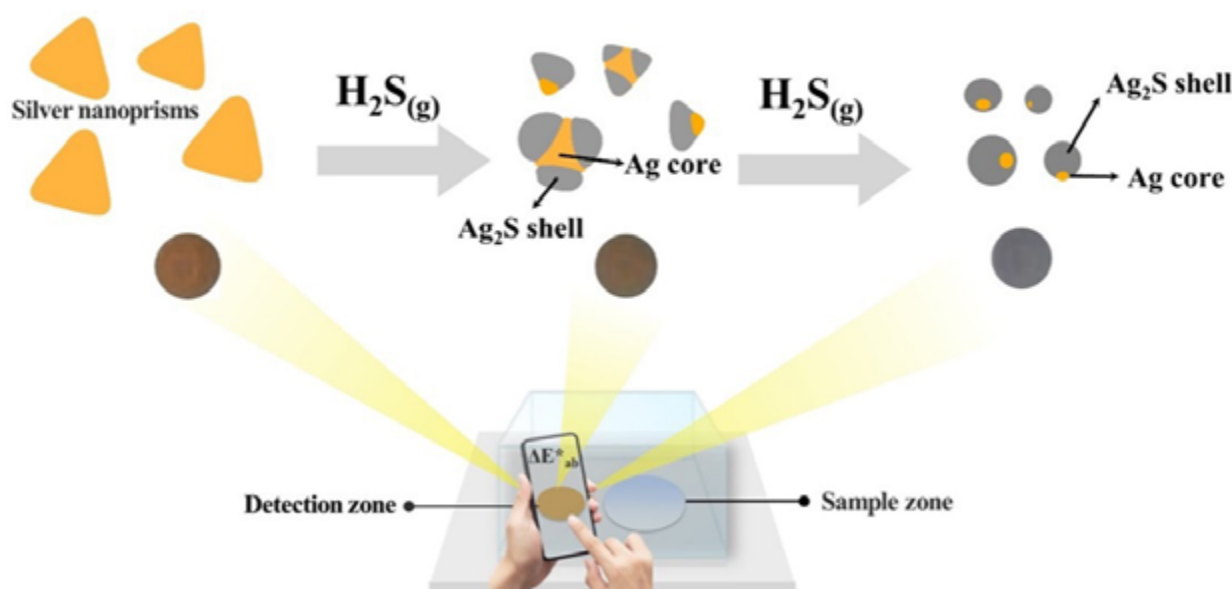
O-143

FACILE DETECTION OF HYDROGEN SULFIDE THROUGH DIGITAL IMAGE USING PHOTO-GENERATED CITRATE-CAPPED SILVER NANOPRISMS

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This study introduces an inaugural development of digital image colorimetric detection of hydrogen sulfide (H_2S) using citrate-capped silver nanoprisms (AgNPrs) synthesized by photo-mediated method. H_2S was in situ generated by acidification of aqueous solutions containing sulfide ions. Upon exposure to H_2S , the sensor undergoes a distinct color change from orange to gray, which can be attributed to sulfidation or the formation of Ag_2S precipitates. Key analytical parameters were optimized and found to be: buffer pH 9.0 of AgNPrs solution, a reaction time of 10 min, and a sensor volume of 60 μL . The method exhibited analytical performance with two linear ranges and limits of detection and quantification of 1.87 ppmv and 6.24 ppmv, respectively, aligning with World Health Organization safety standards. Accuracy and precision were validated through spike recovery analysis in natural water samples, intra-day and inter-day precision assessments, and comparison with a commercial lead acetate strip method. The proposed system demonstrates an environmentally friendly, reliable, portable, affordable, and reusable approach for onsite sulfide detection, highlighting its potential for practical and sustainable environmental monitoring.



Proposed mechanism of H_2S detection by using citrate-capped AgNPrs.

O-144**SURFACTANT QUANTIFICATION IN AGROCHEMICALS - OVERCOMING ANALYTICAL CHALLENGES****Falchetto-Bruckner, Jonathan; Czerwenka, Christoph.***Austrian Agency For Health And Food Safety - AGES, Vienna, Austria.*

In the course of official market control of plant protection products it is desirable to check conformity to the authorized formulation as completely as possible. Besides the active ingredients such agrochemicals often contain different co-formulants which are used to achieve certain physical or chemical properties in a formulation and should also be analysed. Surfactants are among the most frequently used co-formulants. These compounds are often technical mixtures of closely related chemical species, as opposed to, e.g. anti-freeze agents which are clearly defined molecular entities.

The quantification of surfactants is therefore particularly challenging. Analytical standards for the single molecular species which constitute a surfactant are usually not commercially available. Therefore, alternatives to the classical analytical approach need to be pursued.

The developed approach demonstrates how quantification can be achieved without neat analytical standards of the individual species. A combination of LC-HRMS, LC-UV and triple quadrupole LC-MS/MS allows for reliable determination of concentrations of individual molecular entities in technical standards. This trinity of detection techniques enables characterization of technical surfactant standards by LC-HRMS, determination of the concentration of each individual chemical entity by LC-UV and finally robust quantification in agrochemical formulations by LC-MS/MS.

Key aspects of the method will be demonstrated on the example of linear alkylbenzene sulfonates (LAS) and fatty alcohol ethoxylates. LAS illustrate a more easily realisable application of the developed approach, as this anionic surfactant possesses native UV-activity and a permanent charge. This circumstance makes the correlation of UV- and MS-signals easier. Fatty alcohol ethoxylates on the other hand represent a non-ionic surfactant possessing neither native UV-activity nor a permanent charge, thus making application of the approach more challenging. For both surfactant types strategies for a successful implementation into a quantitative method will be presented. The direct comparison of the strategies allows the strengths and weaknesses of the developed approach to be identified and potential pitfalls to be discussed. A method fit for validation and accreditation will be presented and its possible extension to further analytes will be outlined.

O-145**UNDERSTANDING PHTHALIC ACID ESTERS CONTAMINATION IN MANTA RAY AND STRANDED DOLPHIN TISSUES**

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Plastic pollution has become a critical global concern, with increasing attention on plastic additives such as phthalic acid esters (PAEs) due to their persistence, mobility, and endocrine-disrupting potential. Among marine organisms, cetaceans and elasmobranchs –such as dolphins and manta rays– are particularly vulnerable to these contaminants, owing to their long lifespans, high trophic levels, and coastal distribution. As sentinel species, their exposure reflects broader ecosystem health and pollutant burdens in marine environments [1,2]. However, despite growing awareness of the ecological risks posed by PAEs, data on their accumulation in marine megafauna remain scarce, especially in understudied tissues and species. This knowledge gap hinders comprehensive risk assessment and limits our understanding of how such contaminants may impact marine biodiversity and ultimately human health through trophic transfer.

In this study, the ammonium formate version of the QuEChERS method [3], with slight modifications depending on the nature of the sample, was applied for the first time to extract a group of up to twelve PAEs and one adipate from muscle, blubber, and brain tissues of stranded dolphins in the Canary Islands (Spain), as well as muscle biopsies collected from Chilean devil rays (*Mobula tarapacana*) from the Azores (Portugal). Stranded dolphins and devil rays tissues were analyzed using the validated methods, obtaining four of the studied plasticizers at quantifiable concentrations. In both species, the method demonstrated satisfactory performance, with mean relative recovery values within the range 69-115% and relative standard deviation values below 20%. Matrix-matched calibration curves showed determination coefficients above 0.99 in all cases, with limits of quantification ranging from 0.21 to 16.03 ng/g for cetacean matrices, and between 0.56 and 70.91 ng/g for devil rays. Matrix effects were generally soft (between -20 and 20%), except for six analytes in specific tissues where moderate effects (20-50%) were obtained.

The presence of PAEs in both cetacean tissues and devil ray biopsies highlights the widespread distribution of these emerging contaminants in oceanic ecosystems and raises important concerns about their potential physiological effects on marine megafauna, especially for filter-feeders such as the devil rays. These findings contribute valuable data to the limited body of knowledge on PAEs accumulation in lesser-studied species and tissues, emphasizing the need to expand analytical efforts across diverse biological matrices. As anthropogenic pressures on marine environments continue to rise, comprehensive monitoring of plastic additives is essential to inform conservation strategies, evaluate ecological risks, and protect both marine biodiversity and human health.

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O-146**USING FUNCTIONALIZED POLYMERIC MEMBRANES TO PREDICT METAL BIOAVAILABILITY IN AQUATIC ORGANISMS AND PLANTS****Anticó, Enriqueta; Molas, Alba; Fontàs, Clàudia.***Universitat de Girona, Girona, Spain.*

Micronutrients, among them Cu and Zn, are essential for the growth of living organisms and their deficiency or excess may cause severe symptoms in their development. The presence of these metals in surface waters and soils at high concentration is generally related to anthropogenic activities, for example mining activities or the use of copper-based fungicides in agriculture. Once in the environment, a fraction of the metal is bioavailable to the organism and can accumulate in the cell disrupting their normal functioning. For this reason, it is important to predict metal bioavailability using simple and reliable tools.

Functionalized membranes are polymer-based materials including specific extractants designed to selectively extract target analytes from aqueous environments. Upon adequate design of the membrane and when the membrane is interposed between the sample solution and a receiving phase, the analytes can be transported and recovered/preconcentrated in the receiving phase due to the chemical potential difference between the two aqueous phases. The choice of the right extractant is crucial for the membrane functionality and selectivity, and different extractant types can be selected for the transport of metal cations: commercial acidic reagents (di(2-ethylhexyl) phosphoric acid-D2EHPA), complexing agents (a mixture of 5-nonyl salicylaldoxime and 2-hydroxy-5-nonyl acetophenone-LIX984N), or ionic liquids (trihexyl(tetradecyl)phosphonium bis(2,4,4-trimethylpentyl)phosphinate-Cyphos 104). In previous studies we have demonstrated that functionalized membranes can be used to mimic Zn accumulation in fluvial biofilm affected by mine drainage, using the extractant Cyphos 104 [1]. We have also obtained information about metal internalization in potato roots (*Solanum tuberosum*) using a membrane composed of D2EHPA [2] or LIX984N. Zn and Cu accumulation in potato plants grown in hydroponic solution was compared with the transport of free Zn²⁺ and Cu²⁺ from the nutrient solution to the receiving phase.

The application of functionalized membranes to measure metal bioavailability in soils has not been explored yet. Traditional methods to measure metal bioavailability in soils include chemical extraction in single or sequential steps. Moreover, to investigate the dynamic release of heavy metals in soil, the diffusive gradients in thin films technique (DGT) has been developed as a passive sampling tool based on diffusive and binding phases. In our work, we explore the use of functionalized membranes to determine the labile copper from vineyard soil heavily contaminated with this element. The metal accumulated in the receiving solution has been compared with the metal obtained after chemical extraction and with the metal measured with the DGT technique [3]. Finally, the level of contamination in the leaves has also been determined. Altogether, the results obtained from the different studied cases allow as to envisage the functionalized membranes as a tool for environmental monitoring with special emphasis in the determination of the bioavailability of metals.

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O-147**RECENT DEVELOPMENTS IN SAMPLE PREPARATION FOR THE ANALYSIS OF VOC IN THE WOOD-BASED PANELS INDUSTRY**

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Wood-based panels (WBP) have become widely used in construction, furniture, and decorative applications and are nowadays considered a significant source of indoor air contamination due to the emission of volatile organic compounds (VOC). The emission of VOCs can result from the raw materials, chemical additives, or processes used during panel production [1]. WBP are made from wood particles or fibres glued together with an adhesive that is consolidated in a hot plate press, forming a solid panel. Different VOC can be released from these products, with the most important being formaldehyde, classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen.

Most methods for the determination of formaldehyde rely on expensive chamber systems or non-environmentally friendly extraction processes with toluene, usually accompanied by a derivatization reaction with acetylacetone and spectrophotometric analysis. For VOC sampling, most authors rely on solid-phase microextraction (SPME) coupled with GC-MS. In recent years, we have been focusing on developing simple and sustainable sample preparation strategies for the determination of VOC emitted from WBP.

In this work, the latest findings from the *Vo/Wood* project regarding the development of new methods for the analysis and characterization of the volatile profile of WBP are presented. Gas-diffusion microextraction (GDME), an innovative sample preparation technique for VOC in liquid and solid samples, was used to characterize the volatile carbonyl compound profile of particleboards and dry-process fibreboards (MDF) produced with different wood particles and formaldehyde-based adhesives. The method involved a derivatization reaction with 2,4-dinitrophenylhydrazine (DNPH) and subsequent HPLC-DAD-MS/MS analysis [2,3]. GDME was also used to determine the free formaldehyde content in amino and phenolic resins. Another approach that has been explored relies on the dynamic sorption of volatile analytes onto commercially available solid phase extraction (SPE) cartridges using a stream of N₂ followed by HPLC-DAD analysis. This method provided high recoveries and precision for the most relevant carbonyl compounds present in the samples, along with a high correlation with the formaldehyde emission determined according to EN ISO 12460-3.

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O-148

RAPID AND AUTOMATIZED EXTRACTION PROTOCOLS FOR THE MONITORING OF PESTICIDES IN *CANNABIS SATIVA L.* INFLORESCENCE BY MEANS OF CHROMATOGRAPHY TECHNIQUES COUPLED TO MASS SPECTROMETRY

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The goal of the present research is to develop of robust and automatized extraction protocol for the extraction and quantification of pesticide residues from *Cannabis Sativa L.* inflorescences. The cultivation of cannabis has significantly increased in proportion to market demand and with it the use of pesticides in order to increase the crop yield. Although there are numerous analytical methods for the extraction and characterization of bioactive compounds in cannabis [1], the determination of pesticides in this matrix is still a relatively new area that requires further study and evaluation. Bureau of Cannabis Control Text of Regulations, the California regulation concerning the limits admitted for pesticides in *Cannabis sativa L.*, describes the Residual Pesticides Testing criteria [2]. Pesticide residue admitted in cannabis plant materials in any case should not exceed action levels expressed as low part per billion (ppb) [lower limit of quantification (LLOQ)] [3].

In this research study particular emphasis has been placed on the development of miniaturized and automated extraction strategies for the extraction of pesticides from hemp inflorescences with the objective of reducing the analysis times and helping to prevent the exposition of operators to chemicals. The separation and quantification of target pesticides in hemp inflorescences was carried out by using chromatography techniques coupled to a triple quadrupole mass spectrometer (QqQ-MS). For this purpose, selective MRM transitions have been optimized for each pesticide by injecting pure analytical standards into the analytical system. One of the main issues in pesticides context concerns the large number of compounds belonging to a broad spectrum of chemical classes. As a result, particular care has been paid to the selection of pesticides present in the agricultural cannabis scenario in order to embrace a broader spectrum of compounds.

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O-149

DETERMINATION AND IMAGING OF VOLATILE FLAVOR COMPOUNDS BY GIRARD'S REAGENT T DERIVATIZATION TECHNIQUE

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Derivatization methods improve ionization efficiency and chemical properties such as molecular weight, polarity, and boiling point. Nonderivatized standard lactones evaporate over time due to their volatility. Thus, optimal lactone detection should be performed without delay. In this study, we aimed to detect Girard reagent T-labeled- γ -nonalactone (γ -NL), γ -decalactone (γ -DL), and γ -undecalactone (γ -UdL) stably regardless of the delay in analysis by matrix-assisted laser desorption ionization mass spectrometry. In addition, we analyzed the localization of lactones in beef meat (Japanese Black Wagyu) using the derivatization mass spectrometry imaging method and identified a border between lactones and fatty acids such as free oleic acid.

γ -NL, γ -DL and γ -UdL in methanol were mixed with GirT in 20% acetic acid. The mixture was allowed to react at room temperature for 10 min. For comparison, individual nonderivatized target solutions were prepared. The detected masses of GirT-standards by MALDI-MS, γ -NL, γ -DL and γ -UdL increased by 114.1 Da compared with the original masses. The detection efficiencies of GirT-lactones and nonderivatized lactones were calculated from the signal-to-noise (S/N) ratio. The results showed that both GirT- γ -NL and nonderivatized γ -NL were detected, and the S/N ratio was 2.5-fold higher for GirT- γ -NL than for the nonderivatized γ -NL. While GirT- γ -DL and GirT- γ -UdL were detected, nonderivatized γ -DL and γ -UdL were not detected. This result suggests that the ionization efficiencies of γ -DL and γ -UdL are quite low. Although the S/N ratios for GirT- γ -DL and GirT- γ -UdL were 8.2- and 2.9-fold lower than that of GirT- γ -NL, GirT derivatization enabled the detection of long-chain lactones.

Temporal stability analysis showed no change in the S/N ratio for GirT- γ -NL after 60 min, whereas the nonderivatized γ -NL exhibited a ~60% decrease, indicating volatilization. Similarly, GirT- γ -DL and GirT- γ -UdL remained stable over time. Nonderivatized γ -NL, γ -DL, and γ -UdL (boiling points: 470, 493, and 516 K, respectively) likely evaporated during measurement or were below detection limit, whereas GirT-lactones remained detectable. The detection efficiency over time was also examined. GirT- γ -NL showed no change in S/N ratio after 60 min compared with that at 0 min, whereas the S/N ratio for nonderivatized γ -NL decreased by about 60%. For GirT- γ -DL and GirT- γ -UdL, no change in S/N ratio was observed over time. Organic compounds with a boiling point (BP) below 523 K are defined as volatile organic compounds. Thus, nonderivatized γ -NL, γ -DL, and γ -UdL (BP: 470, 493, and 516 K) were not present or their concentration decreased due to volatilization during measurement. In contrast, the detection of GirT-lactones was stable over time.

GirT- γ -NL, GirT- γ -DL, GirT- γ -UdL, and OA were detected on beef sections and imaged at m/z 270.2, 284.2, 298.3 and 281.2, respectively. H&E staining and MSI images of GirT-lactones and OA in beef sections are shown in Figs. All lactones were imaged in fat regions [Figs. (b)–(d)]. On the other hand, free OA was mainly localized in muscle regions [Fig. (e)]. OA is a precursor molecule of lactones and is converted to lactones through aging and cooking; however, the evidence supporting this remains unclear. Most fatty acids in muscle are incorporated in triacylglycerols and not as free fatty acids, although some fatty acids are also present as phospholipids and free fatty acids for life-sustaining energy production. Lactones imaged at adipose region although OA marginally imaged at adipose region. The MSI data suggests that unsaturated fatty acids, such as OA stored in intramuscular fat as neutral fat, are immediately converted to the lactones that are detected in the adipose regions on the sections (1).

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O-150

INTEGRATING OMICS STRATEGIES TO DIFFERENTIATE PAPRIKA FROM KEY GEOGRAPHICAL PRODUCERS: UHPLC-Q-ORBITRAP-HRMS-BASED UNTARGETED METABOLOMICS AND LIPIDOMICS

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Paprika (*Capsicum annuum* L.) is a widely consumed spice with significant economic and culinary value, characterized by its rich red color and complex chemical composition. Its metabolomic and lipidomic profiles are strongly influenced by geographical origin due to environmental factors such as climate, soil characteristics, and cultivation practices. All these factors are englobed as "the terroir effect", an original concept of enology expanded to the food industry to refer to the influence of environmental factors, such as climate, soil, and cultivation practices, all of them specific to the production region, on the characteristics of agricultural products. While existing studies have investigated paprika from major production areas such as China and Zambia, other key producing regions remain largely unexplored. This study presents a novel approach integrating untargeted metabolomics and lipidomics using ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap-HRMS) to evaluate the terroir effect on paprika from two understudied yet significant producing regions: Peru (Ica and Arequipa) and Zimbabwe. A total of 60 paprika samples (30 from Peru and 30 from Zimbabwe) were analyzed using untargeted metabolomics and lipidomics workflows, revealing 27 key markers that differentiate samples from both regions. Chemometric analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), showed clear clustering patterns, demonstrating significant compositional variations driven by geographical origin. The application of UHPLC-Q-Orbitrap-HRMS combined with multivariate analysis enabled the development of robust classification models with high discriminatory power. The OPLS-DA models exhibited outstanding predictive performance, with Q^2 values exceeding 0.990 and 100% correct classification rates, confirming the reliability of the proposed methodology for paprika authentication. Among the identified markers, Peruvian paprika exhibited significantly higher concentrations of carotenoid-derived compounds such as capsanthin and *beta*-ionone, which contribute to its intense coloration and aromatic profile. Additionally, Peruvian samples were enriched in specific fatty acids, including stearidonic acid, vernolic acid, and 9-HODE, suggesting specific enhanced lipid derivatives potentially influenced by agro-climatic conditions. In contrast, Zimbabwean paprika was characterized by a higher abundance of capsaicinoids, including capsaicin and dihydrocapsaicin, which are responsible for the spice's pungency. The higher accumulation of these compounds suggests that environmental stress factors such as temperature and soil composition may enhance capsaicinoid biosynthesis. Additionally, L-proline and its Amadori compound derivative N-(1-deoxy-1-fructosyl)proline were up-regulated in Zimbabwean samples. Zimbabwean samples also exhibited significantly increased levels of phospholipids, including lysophosphatidylcholines (LPC 18:3, LPC 18:2/0:0, and LPC 18:1/0:0) and phosphatidylcholines (PC 36:5, PC 36:4, and PC 34:2). Compared to previous lipid-based paprika studies, the present lipidomics study further expands the existing literature (only focused on reporting well-known compounds such as lauric acid, oleic acid, and stearic acid) by revealing the presence of previously unreported lipids in paprika such as LPC and PC markers. These results highlight the potential of HRMS-based fingerprinting for traceability, food safety, and fraud prevention in the spice industry. In conclusion, this study provides the first comprehensive metabolomics and lipidomics characterization of paprika from Peru and Zimbabwe, demonstrating the impact of terroir on its chemical composition. The identification of 27 key metabolomic and lipid markers enhances the understanding of paprika's chemical complexity and supports the development of analytical strategies for origin authentication. Future research should extend this approach to other paprika-producing regions and further explore how environmental and agricultural factors influence the metabolomic and lipidomic signatures of this economically valuable spice. Acknowledgements: This research was funded by "PPITUAL, Junta de Andalucía-FEDER 2021-2027. Programa: 54.A". Grant reference: CPUENTE2023/21.

O-151**NEW PERSPECTIVES BEYOND TRADITIONAL APPROACHES IN WINE AUTHENTICATION BASED ON TARGETED AND UNTARGETED METABOLOMICS****Calvo, Júlia¹; Llopis, Gala¹; Deben, Anna¹; Morales, Laura¹; Ahumada, Diego¹; Sentellas, Sònia²; Saurina, Javier³.**

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The study of wine quality, traceability, and authenticity remains a hot topic with remarkable social and economic implications in the current food science landscape. In recent years, several papers have been published using different approaches, compound families, analytical techniques, and chemometric methods dealing with the characterization and authentication of wine attributes, including grape variety and quality, origin, agricultural practices, vinification, blending, aging, etc. However, noticeable oenological and analytical challenges remain still unresolved.

This work aims to develop new strategies for comprehensive wine characterization through targeted and untargeted metabolomics. We explore diverse analyte families, including phenolic compounds, organic acids, amino acids, and biogenic amines, using instrumental techniques and chemometrics. Among them, conventional high-performance methods (e.g., HPLC-UV and HPLC-MS/MS) or others that prioritize simplicity and speed (FIA-UV/Vis-FLD, FIA-MS/MS, or smartphone-based measurements) are considered. Regarding the assessed attributes, we focus on geographical issues (e.g., protected designations of origin), grape varieties, and aging. Anyway, other trending parameters deserve our attention, such as consumer ratings and wine prices; they are often more complex to model because they suffer from fashion or consumer subjectivity. Beyond the more traditional chemometric methods for data processing, we apply one-class models, as we believe this is the most realistic strategy for authenticating samples.

The calibration sets consist of several hundred of samples to ensure class representativeness. Truly external validation with new wines analyzed in independent batches avoids over-optimistic figures such as those generally given by cross-validation or random sample division into calibration and test subsets. The results obtained with instrumental fingerprints or compositional profiles of phenolics or amino compounds from HPLC-UV or HPLC-MS/MS are excellent, with sensitivity and specificity values exceeding 90%. Predictive efficacy decreases slightly with rapid FIA screening methods and smartphone-based imaging, but their high throughput makes them highly recommended for large wine sets.

O-152**USING ELECTRIC PULSES AS A PRE-TREATMENT FOR ENHANCING THE SONOTRODE EXTRACTION OF PHENOLIC COMPOUNDS WITH ANTIOXIDANT AND ANTIDIABETIC ACTIVITY FROM MANGO LEAVES****Razola-Díaz, María Del Carmen¹; Verardo, Vito¹; Gómez Caravaca, Ana María².**

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Mango leaves are a by-product rich in phenolic compounds whose extract could be used for food, nutraceutical, and cosmeceutical applications. The objective of this study was to improve the extraction of phenolic compounds using pulse electric fields (PEF) as a pre-treatment followed by extraction with ultrasound (US) technology via sonotrode. An experimental Box-Behnken design of 15 experiments and response surface methodology linked to a desirability quadratic function were used to optimize the PEF conditions. The independent factors evaluated were voltage (V), number of pulses and pulse width (μ s). The frequency was fixed to 50 Hz and the distance between the electrodes 10 cm. The response variables considered were the sum of phenolic compounds, the antioxidant activity measured by DPPH, ABTS and FRAP, and the anti-diabetic activity measured by alpha-amylase inhibiting activity. The optimal conditions established were 7492 V and 152 pulses of 123 μ s. All the extracts were analyzed by HPLC-ESI-TOF-MS and a total of 43 polar compounds were detected, 41 of them being phenolics. The combined use of PEF + US allowed the increment of the extraction of total phenolic compounds by 10% comparing to the only extraction using US. Mangiferin, one of the phenolic compounds present in mango leaves, has been attributed to several bioactivities. The use of PEF as pretreatment has made possible to increase up to 15% its content in the extracts. Moreover, alpha-amylase inhibitory activity increased 20% and the antioxidant activity 6-22% after PEF+US. The results confirmed that the use of PEF as a pre-treatment previous to US extraction under the established optimal conditions has proven to be effective in improving the extraction of phenolic compounds in mango leaves, probably due to the increase in the permeability of the cell membrane.

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O-153

BENZENE IN MECHANICALLY RECYCLED PET: ANALYTICAL ASPECTS AND NEW METHODOLOGIES TO REDUCE NON-INTENTIONALLY ADDED SUBSTANCES IN FOOD PACKAGING

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Polyethylene terephthalate (PET) is extensively used in food packaging, and more specifically for beverage bottles, due to its advantageous properties, such as low gas permeability, good mechanical strength, transparency, reflectivity, ease of processing, and affordability. Over the last decades, the recycling of PET has made significant progresses thanks to cleaning and sorting technologies. Mechanical recycling of PET is cost-effective and remains environmentally favourable compared to the production of new material. However, it often induces a partial decrease in PET quality, which may impair its use in food-grade packaging. Actually, to be approved in food packaging, mechanically recycled PET (rPET), as other polymers, must comply with the European food contact material (FCM) legislation. In particular, Intentionally and Non-Intentionally Added Substances (IAS and NAIS, respectively) are strictly controlled, as they may be transferred to food items and impact the health of consumers. Benzene, that is recognized as being carcinogenic, mutagenic and reprotoxic, is one of the most concerning NAIS contaminants in rPET. It mostly originates from heat-induced decomposition of rPET impurities such as polyvinylchloride (PVC), a secondary material coming from the sorting step of the mechanical recycling. Contamination of rPET by benzene, by the way of its potential migration into the contents of the package during conditioning and storage, represents a severe public health concern.

To evaluate the contamination level of rPET materials, an analytical method was developed for the direct determination of benzene in polymers, taking into consideration the following requirements and constraints: a sensitivity allowing the detection in the low $\mu\text{g/g}$ range, a selectivity reducing potential matrix interferences, as well as a quick sample preparation procedure. In addition, special attention was put on the robustness of the method to ensure its applicability on various sample types, from raw or treated rPET pellets to final packaging. As the golden standard in terms of sensitivity and selectivity for semi-volatile analysis, Gas Chromatography coupled to Tandem Mass Spectrometry (GC-MS/MS) was chosen as the quantification technique and was operated in Electron Impact (EI) ionisation and Multiple Reaction Monitoring (MRM) detection mode.

Due to its volatility and its inclusion in the deep structure of the polymer network, the extraction of Benzene from solid samples, which was the most critical step in the method development, was achieved by thermal desorption in gas-tight headspace vials coupled with preconcentration by Solid-Phase MicroExtraction (SPME). This 2-steps approach allowed adjusting the desorption temperature to measure selectively different fractions of Benzene (native or induced by thermal degradation of PVC). Plus, the selectivity of headspace SPME towards semi-volatile compounds contributed to the achieve a good sensitivity level. In these conditions, the method detection limit was evaluated at 5 ng/g, with a linear range up to 800 ng/g.

The method was applied on reference samples and commercial rPET bottles, then was used to control the efficiency of a new process aiming to reduce PVC and benzene contaminations in rPET. Our results demonstrate the possibility to reduce benzene levels while maintaining an acceptable level of rPET performance. These improvements are promising in the context of material quality, food safety and environmental impact for the use of rPET in packaging applications.

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O-154

PHARMACEUTICAL AND FORENSIC REAL-TIME ANALYSIS USING EXTRACTIVE-LIQUID SAMPLING ELECTRON IONIZATION-MASS SPECTROMETRY (E-LEI-MS)

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Extractive-Liquid Sampling Electron Ionization-Mass Spectrometry (E-LEI-MS) (Figure 1) is a recently developed real-time analytical approach suitable for the direct analysis of many compounds in different matrices [1]. The main features of the E-LEI-MS system are the absence of sample pre-treatment and the combination of ambient sampling with the high identification power of electron ionization (EI). The E-LEI-MS setup includes a solvent-release mechanism for extracting the analytes from the sample surface and a capillary system for transferring the solution to the EI source by the suction force of the MS high vacuum. The vaporizing process takes place in the vaporization microchannel using the high temperature of the transfer line, where the analytes reach the gas phase before entering the EI source.

The E-LEI-MS capability to provide real-time data was evaluated for identifying active pharmaceutical ingredients (APIs) and excipients in twenty industrial drugs, representing sixteen APIs belonging to different therapeutic classes in six different pharmaceutical forms. Furthermore, two supplement tablets were purchased as suspected counterfeits. Analyses of pharmaceuticals have been performed using the E-LEI-MS system coupled with an Agilent 7010 GC/QqQ-MS. HR data were obtained for the diclofenac pharmaceutical forms, tadalafil and supplementary tablets coupling the system with an Agilent 7250 GC/Q-ToF-MS. Extraction solvent, source, and Vaporization Microchannel (VMC) temperatures were optimized according to the chemical-physical properties of the analytes.

Nine out of sixteen APIs and four excipients were unambiguously identified using NIST and predicted spectra and one of the supplements resulted in containing sildenafil, known as Viagra.

The E-LEI-Q-ToF-MS system was also involved in the analysis of twenty benzodiazepines (BDZs) in solution. Among them, six BDZs were selected for their common consumption and used to fortify cocktails at concentrations of 20 mg/L and 100 mg/L. The fortified cocktails were analyzed on a glass surface as 20 mL dried spots to simulate the forensic scenario of BDZs being used as rape drugs, where glass represents a crime scene sample.

Nineteen BDZs analyzed as solutions were correctly identified by electronic libraries. BDZs contained in the residue of the fortified cocktails at a concentration of 100 mg/L were properly identified as well, whereas, to identify BDZ in the residue of fortified cocktails at a concentration of 20 mg/L, a lab-made HR library was created by collecting the accurate masses.

The results confirmed the E-LEI-MS capability in real-time targeted and untargeted analysis, providing results in less than five minutes. The pharmaceutical forms analysis and the accurate identification of BDZs demonstrated the potential of this analytical approach as a valuable screening technique in applications requiring the rapid acquisition of qualitative data as quality control tests, and detection of counterfeits and adulterations.

Acknowledgment

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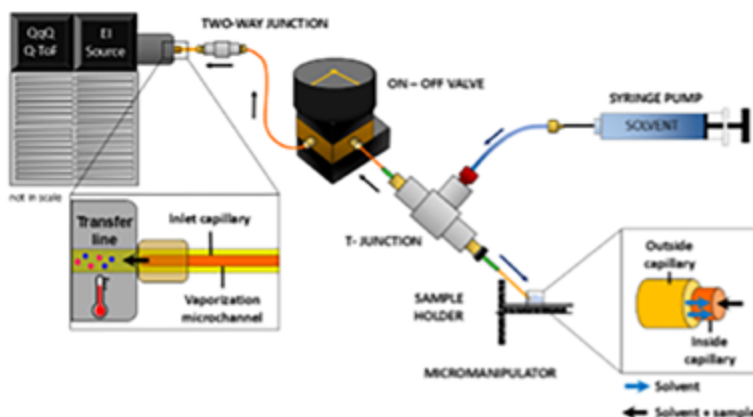


Figure 1. Scheme of the E-LEI-MS configuration.

O-155**RECENT UPDATE ON THE SENSOR EVENT-DRIVEN COLUMN CHROMATOGRAPHY SYSTEM****Lee, Jong Kwang; Park, Hwan Seo; Oh, Simon; Kim, Jiyong; Yoo, Jungbo; Park, Tae-Hong.***Korea Atomic Energy Research Institute, Daejeon, South Korea.*

In analytical chemistry, sample preparation is a crucial yet tedious, time-consuming, and error-prone process. Column chromatography-based chemical preconcentration, separation, and purification methods are widely used for the analysis of radiological, biological, and geological samples as well as the production of radioisotopes. Especially, systems for characterizing radioactive materials should be designed to effectively handle samples with considerable variations in radioactivity and matrix compositions. In these applications, carryover or cross-contamination is a major concern when designing automated systems. Eliminating this problem ideally involves using all fluidic components in contact with samples as disposable, but this is practically impossible for conventional pump-driven systems.

In our previous research, we developed a novel open column-based automated chromatographic sample preparation system (OpenPrep) that has been developed for precise column flow control and cross-contamination-free chemical separation¹. OpenPrep is operated based on a sensor event-driven flow control architecture. It consists of a liquid handling robot, resin column module, and fraction collector module, where all the modules are separately spaced without any connection through multi-way valves. The innovative valveless sample flow design, in combination with a compact robotic liquid handler and a motion stage-based fraction collector, reduces the sample flow path to a disposable component, enabling easy replacement. At each column, a peristaltic pump is used to generate a forced column flow whose flow rate can be automatically adjusted to the target value based on the online column flow calibration algorithm. A non-contact built-in sensor that generates a digital signal depending on the presence or absence of a liquid is installed outside the resin column. A local controller detects the sensor event that occurs when the upper surface of the supplied liquid leaves the sensing zone and then stops the flow. This ensures that a uniform amount of liquid is always maintained in the column, thereby preventing the bed from running dry. OpenPrep was developed to automate our in-house standard operating procedure (SOP)² for more efficient characterization of radioactive wastes, enabling the sequential separation of difficult-to-measure elements such as ⁹⁹Tc, ⁹⁰Sr, ⁵⁵Fe, ⁹⁴Nb, and ⁵⁹,⁶³Ni from a single sample solution. Through a proof-of-concept implementation using five different resin columns in sequence for the SOP, OpenPrep demonstrated key advantages: enhanced flexibility and reconfigurability, reduced risk of cross-contamination, improved operational efficiency, and cost-effective customization.

In this study, we enhanced the existing system by making it more compact and user-friendly. The sensor module was redesigned to be easily adjustable and replaceable, while also minimizing external exposure. By adopting a 50 mL centrifuge tube as the standard collection vessel—replacing the previous 200 mL vessel—the new system achieved an approximate 40% reduction in overall size. Verification tests are underway to implement the updated system in the radionuclide separation process.

Acknowledgments

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O-156**AN IR-TRANSPARENT CHIP FOR INFRARED SPECTROSCOPIC REACTION MONITORING IN DIGITAL MICROFLUIDICS****Fehse, Sebastian; Ungermann, Ben; Das, Anish; Belder, Detlev.***Leipzig University, Leipzig, Germany.*

Digital microfluidics (DMF) is a technique where micro- to nanoliter-sized chemical droplets can be electrically processed on an array of insulated and hydrophobic electrodes and research in this field has recently gained momentum [1,2]. DMF offers versatile sample handling of discrete droplets [3], offering the possibility to carry out chemical processes in an unrestricted and automated manner. To monitor these chemical processes, it is necessary to couple digital microfluidics with various analytical techniques. This has led to the hyphenation of DMF with mass spectroscopy [4] and surface-enhanced Raman spectroscopy [5]. However, coupling DMF with absorption-based spectroscopic (such as Infrared (IR)) techniques has not been reported to date. In order to extend the current analytical toolkit for DMF, we have now achieved the coupling of DMF with IR spectroscopy, as reported in this work.

To enable the first DMF-IR coupling, we developed an IR transparent DMF-chip. The novel DMF chip used calcium fluoride as the substrate, enabling real-time IR imaging not only in the reflection but also in the transmission, which enhances the measurement sensitivity. To combine conductive and insulating materials with suitable IR absorption properties, a thin film sputtering-based fabrication method was developed. In order to minimize evaporation during long-term measurements, a remodelled electrode array with evaporation control functionality was designed.

The performance of the developed chip was evaluated by screening various IR-compatible solvents and analytes in a proof-of-concept study. To demonstrate the potential use of the DMF-IR device, a long-term IR reaction monitoring was performed to follow the course of an imine-synthesis reaction. The chip provides an opportunity to study chemical processes in DMF using mid-IR spectroscopy as well as observation of droplets by *in-situ* IR imaging.

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O-157**DIRECT AMBIENT PLASMA MASS SPECTROMETRY ANALYSIS ENHANCED BY MOLECULARLY IMPRINTED PEI: SENSITIVE DETECTION OF HERBICIDES IN ENVIRONMENTAL WATERS****Nazim, Tomasz; Cegłowski, Michał.***Uniwersytet im. Adama Mickiewicza, Poznań, Poland.*

Molecular imprinting is recognized as an effective strategy to produce adsorbents with tailored selectivity and high affinity toward specific analytes. Polymers obtained by molecular imprinting (MIPs) are widely utilized as selective sorbents due to their capacity to specifically bind target compounds or structurally related analytes [1-3]. In the presented research, polyethyleneimine (PEI)-based molecularly imprinted polymers, functionalized to various extents, were developed and tested for efficient adsorption and quantification of phenoxy herbicides (specifically 2,4-D and 2,4,5-T) using ambient plasma mass spectrometry (FAPA-MS) [4-5].

PEI was chemically modified by introducing glycidol functionalities at varying functionalization degrees (from 0% to 30%), as well as through functionalization with alternative reagents, including furfuryl glycidyl ether (FGE), butyl glycidyl ether (BGE), and glycidyltrimethylammonium chloride (GTMAC). These chemical modifications changed polymer-analyte interactions by introducing diverse binding sites, which enhanced the overall adsorption efficiency and selectivity. The imprinting process involved cross-linking reactions utilizing epoxy-based cross-linkers such as 1,4-butanediol diglycidyl ether (BDE) and neopentyl glycol diglycidyl ether (NGDE) in the presence of template molecules.

The synthesized materials underwent comprehensive evaluations, including adsorption kinetics, equilibrium adsorption studies, thermodynamic characterization, and investigations into pH effects. Particular attention was given to the application of direct ambient plasma mass spectrometry (FAPA-MS) for rapid analysis and quantification. The obtained results clearly demonstrated the superior selectivity and adsorption capacity of modified PEI-based MIPs compared to their non-functionalized counterparts. Specifically, glycidol-modified MIPs showed significant improvements in sensitivity for 2,4-D detection, achieving detection limits substantially lower (approximately 200-fold) than conventional methods and approximately 20-fold better than non-imprinted reference polymers (NIPs) [4].

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O-158**AN ADJUSTABLE AND FLEXIBLE LABORATORY SAMPLE DIVIDER****Beleites, Claudia.***Chemometrix GmbH, Wölfersheim, Germany.*

Sample dividers split particulate bulk material into representative subsamples. In particular for e.g. agricultural research labs, the incoming materials can vary widely in particle shape and size. Also, several sub-samples may need to be prepared that can vary considerably in sample mass and volume.

As an example consider the calibration of a NIR-spectroscopic quantitation of essential oil in caraway during a breeding program [1]: the spectroscopic method typically measures a sample volume corresponding to about 1 g. Reference analyses may be performed either according to Pharmacopoeia [2], working on 10 g of caraway seeds, or with an in-house developed micro-extraction working on 200 mg of material. Both reference methods are destructive, and the non-destructive spectroscopic method is developed since the measured material then stays available for sowing. This however, rules out grinding the seeds in order to reduce top particle size as recommended by the Theory of Sampling (ToS) [3, 4]. Also, the amount of material per field sample is rather limited: on the one hand, by biological variation, also according to environmental influences such as weather conditions. On the other hand, from an experimental design perspective covering both the breeding and the analytical method, it is preferable to obtain more biological replicates (with fewer material each) than fewer replicates with ample amounts of material.

An additional difficulty that arises with any particulate matter, but can be particularly severe with plant materials is segregation: however well one may mix the bulk material, any agitation may immediately start segregation. This can be counteracted by forming a mixed sample from a sufficiently large number of increments. In turn, the increments should be small. Too small increments, however, risk a selection bias according to particle size. ToS thus recommends increment sizes of no less than 3 times the largest particle length.

Thus, we typically need as small as possible increments, and then check that a sufficient number of increments was collected into the sub-sample.

Commercially available sample dividers work most efficiently with spherical particles of the maximum diameter the divider is constructed for. For materials such as the caraway with elongated shape plus stem, the commercially available sample dividers are considerably less efficient. In addition, they can be adapted to particle size only in a very limited way (if at all). While some readily available construction principles such as Börner- or rotary type dividers can deal well with varying field sample volumes, others such as the widely used riffle splitters may not work well in practice with too small amounts of bulk material.

We present a sample divider that can easily be adjusted to a range of different particle sizes, works with elongated shapes or seeds with stem and allows small increment sizes without compromising the correctness of the splitting procedure. Our sample divider uses a mini-conveyor belt to form a material stream of small cross section from the bulk sample and then splits off given fractions of the bulk material. We typically operate it in restricted stratified random sampling mode [5]. Other operation modes are possible. E.g., equispaced sampling and preserving increments individually allows easier variographic analysis and in consequence to study effects of segregation of the bulk material.

Acknowledgements

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O-159

MULTIDIMENSIONAL PREPARATIVE GAS CHROMATOGRAPHY: A SUSTAINABLE APPROACH FOR ISOLATING TARGET ANALYTES FOR DOWNSTREAM BIOLOGICAL ASSAYS

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The increasing interest in terpene components arises from their diverse biological activities, rendering them valuable resources for the pharmaceutical, nutraceutical, and food industries. Within this class of compounds, β -caryophyllene (BCP) is of particular significance due to its antioxidant, anti-inflammatory, and anti-hyperglycemic effects, as well as its dietary bioavailability through the consumption of edible plants, including spices.

In this context, the isolation of chemical families through flash chromatography and solvent extraction is becoming a common practice to screen those chemical classes and/or components responsible for the corresponding biological activity [1]. In specific instances, aiming to reduce the number of candidates, a refined oil reconstitution process necessitates the use of multiple standard additions. Despite the several efforts, this procedure can be considerably intricate, particularly when considering the reconstitution of medium/high complex fractions. Consequently, the loss of trace components, as well as the addition of components in incorrect enantiomeric ratios, can significantly compromise biological test outcomes. The latter is a critical concern, as enantiomers exhibit distinct characteristic biological activities contingent upon their differing three-dimensional spatial disposition.

This research was designed to investigate the biological activity of essential oils derived from spices exhibiting consistent BCP content. An innovative analytical approach has been developed for the study of the biological activity of compounds present within the oil, specifically to delineate the influence of BCP.

In detail, a prototypical preparative system was implemented [2-4], employing a multiple heart-cut system and complementary chromatographic columns, culminating in the isolation of the desired fraction into a collection device. Initially, the activity of the essential oil, its sesquiterpene fraction, and standard BCP were evaluated. Subsequently, the sesquiterpene fraction was further isolated, explicitly excluding BCP, and subjected to assessment for potential synergistic interactions among specific terpene components.

Preliminary biological assays indicated that the sesquiterpene fraction exhibited a higher level of activity compared to BCP alone, suggesting potential synergistic effects.

The analysis of the isolated fractions facilitated the elucidation of the contribution of the various terpenes, independent of the presence of BCP, thereby demonstrating the efficacy of a combined approach utilizing gas chromatography (GC) and biological assays to establish the relationship between biological activity and specific sample constituents.

ACKNOWLEDGMENTS

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O-160**TOWARD UNRIVALED CHROMATOGRAPHIC RESOLVING POWER: DESIGN AND DEVELOPMENT OF COMPREHENSIVE SPATIAL THREE-DIMENSIONAL LIQUID-PHASE SEPARATION TECHNOLOGY****Eeltink, Sebastiaan.***Vrije Universiteit Brussel, Bruselas, Belgium.*

Spatial comprehensive three-dimensional chromatography (3D-LC) offers an innovative approach to achieve unprecedented resolving power in terms of peak capacity and sample throughput. This advanced technique separates components within a three-dimensional separation space, where orthogonal retention mechanisms are incorporated. The parallel development of the second- and third-dimension stages effectively overcomes the inherent limitation of conventional multi-dimensional approaches, where sampled fractions are analyzed sequentially. This contribution focuses on the design aspects of the microchip for spatial 3D-LC and the selection of orthogonal separation modes to enable the analysis of intact proteins. The design considerations for the flow distributor and channel layout are discussed, along with various approaches to confine the flow during the subsequent development stages. Additionally, the integration of stationary phases into the microchip is addressed, and interfacing to mass-spectrometry detection is discussed. According to Pareto-optimality, the integration of isoelectric focusing, size-exclusion chromatography, and reversed-phase chromatography in a spatial 3D-LC approach is predicted to achieve an exceptional peak capacity of over 30,000 within a 1-hour analysis, setting a new benchmark in chromatographic performance.



YOUNG ORALS



YO-01

OPTIMIZED FAST GAS CHROMATOGRAPHY COUPLED WITH PROTON-TRANSFER-REACTION TIME-OF-FLIGHT MASS SPECTROMETRY FOR THE SELECTIVE NEAR REAL-TIME ANALYSIS OF HERBIVORE- AND PATHOGEN-INDUCED PLANT VOLATILES

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The emission of volatile organic compounds (VOCs) by plants plays a key role in their defense against herbivores and pathogens. Analyzing qualitative and quantitative changes in emitted VOC profiles is essential for understanding plant-environment interactions and for detecting an infestation or infection. Proton transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS) is a highly sensitive analytical technique that can be used to monitor the emission of herbivore-induced plant volatiles (HIPVs) in response to various species [1]. Because isomers cannot be separated by PTR-TOF-MS, this technique is mainly used to obtain temporal patterns and to quantify compound classes of isomeric HIPVs. In some cases, however, knowledge of specific compounds within a group of multiple isomers may be crucial for the detection of infestation or infection. This is particularly relevant for the group of terpenoids, which are the most abundant group of secondary plant metabolites and comprise several thousand isomers. Specific terpenoids can serve as key airborne signals, attracting herbivore enemies or inducing defense mechanisms in neighboring plants [2].

In this study, an optimized fast gas chromatography (fastGC) system was developed for the analysis of isomeric terpenoids. The system is based on a modified version of the commercially available fastGC add-on integrated into a mobile PTR-TOF. The development and optimization of the system was performed on emissions from *Acer platanoides* trees infested by *Anoplophora glabripennis* (Motschulsky), commonly referred to as Asian longhorned beetle (ALB). The ALB is an invasive species originating from East Asia that causes enormous actual and potential damage in Europe and North America. The optimization of the fastGC-PTR-TOF focused on the analysis of sesquiterpenes, in particular α -longipinene, cyclosativene and α -copaene, which serve as strong indicators of ALB infestation [3]. These sesquiterpenes were separated in less than three minutes, with intra-day retention time RSD < 0.6% and peak widths < 1.4 s. Compared to the original system, the optimized fastGC demonstrates more than tripled sesquiterpene resolution, twice the sensitivity, and a reduction in total analysis time of about 10%.

In addition, the optimized fastGC-PTR-TOF was successfully applied to the analysis of microbial VOCs emitted by *Alternaria solani* and *Rhizoctonia solani*. These fungal pathogens are among the most important phytopathogens [4], whose early detection in potato stores is crucial in order to contain further spread and the associated yield-reducing damage.

In summary, the integration of a fastGC into a PTR-TOF overcomes the conventional limitations of real-time VOC analysis. It not only facilitates the identification of numerous VOCs with multiple product ions, but also enhances specificity when analyzing isomeric compounds. The developed and optimized fastGC-PTR-TOF allows for near real-time analysis of volatiles emitted in response to herbivores or pathogens, or by the pests themselves, making it a powerful tool for environmental monitoring, integrated pest management, and forest protection.

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YO-02

EXTRACTIONS AND ANALYSIS OF 8 CLASSES OF HALOGENATED DISINFECTION BY-PRODUCTS IN SOIL AND LETTUCE

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Growing pressure on water resources is prompting stakeholders to adopt more sustainable practices to preserve this resource. Among the solutions proposed, the reuse of treated wastewater is already widely employed worldwide, but its use remains highly disparate depending on local challenges. 1 billion m³ are already reused for agricultural purposes in Europe, the potential is estimated at six times this value and would lead to a reduction in water stress of around 10% [1]. The European Union has recently regulated the use of treated wastewater through Regulation 2020/741, followed in 2022 by guidelines to support its implementation. Depending on the quality, so-called “barrier” measures can be implemented to reduce the risks associated with the presence of microorganisms, in particular disinfection of the water after treatment in a wastewater treatment plant. However, it is now acknowledged that disinfecting water containing natural or anthropogenic organic matter leads to the in-situ formation of disinfection by-products, many species of which are characterized as toxic. Among the more than 700 halogenated species already identified, only a few are regulated in drinking water. In the case of treated wastewater, the organic matter load can remain quite high (BOD₅ >10 mg/L), leads to an increased production of disinfection by-products [2,3,4,5].

This research work aims to contribute to quantifying the impact of this by-product formation in the context of reuse of treated and post-disinfected wastewater for agricultural purposes. This presentation will focus on the analytical development required to quantify 8 families of halogenated disinfection by-products (commonly found in water), in two complex solid matrices: soil and lettuce. Indeed, although the literature on the extraction and analysis of these by-products in aqueous phase is substantial, it is still very poor concerning soils and plants, two matrices potentially impacted by the democratization of this practice [6].

This work led to the development of 4 solid-liquid extraction methods coupled with GC-ECD analysis for the quantification of 8 families of halogenated disinfection by-products in artificial soil and lettuce. These methods include an additional purification step using Envi-Carb or Florisil cartridges, which have not yet been reported in the literature for these families of compounds. This step aims to preserve the analytical devices from the various matrix components that can clog the system, such as chlorophyll and organic acids, and potentially reduce matrix effects. Various parameters such as pH, salt amount, and the use of ultrasound were tested to determine the optimal extraction conditions. Current results show excellent recovery of analytes after purification (71.5-121.0% for the Envi-Carb 250mg cartridge and 76.6-121.3% for the Florisil 1000mg cartridge), as well as reduced matrix effects for many compounds. Best extraction yields were observed for sequential extraction with MTBE, pH 4.5 buffered or acidified water, in the presence of sodium sulfate, under horizontal agitation, followed by centrifugation for 5 minutes. Purification is performed by percolating 3 mL of the organic extract in Pass-Through mode on an Envi-Carb cartridge (lettuce) or Florisil cartridge (soil). Extraction yields varied according to compound family and matrix, ranging from 18 to 123% and 33 to 144%, respectively, in lettuce and artificial soil. MDLs are of the order of a tenth to a ng/g.

This new cartridge purification approach enables large-scale analysis of complex matrices while preserving systems and maintaining extraction yields and MDLs comparable to those found in the literature [7]. In initial trials of irrigating lettuce with ultra-filtered and disinfected wastewater, these methods have already enabled the detection of trichloroacetic acid in soils and lettuces, a compound absent in controls irrigated with non-disinfected wastewater.

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YO-03**TOOLS FOR ASSESSING THE IMPACT OF PETROCHEMICAL INDUSTRIES ON THE POPULATION**

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The area of Tarragona hosts one of the largest chemical and petrochemical complexes of Europe, divided in two main zones: Tarragona's Northern and Southern Industrial Parks, which between them host more than 34 different companies. The Northern Park houses an oil refinery and two ethylene cracking plants, while the Southern Park houses mostly polymers production plants. The two parks cover an area of 1200 h, including the Port of Tarragona, one of the 5 most important ports in Spain and a leading port in Europe. They are located in the municipalities of Tarragona, la Canonja, Reus, Vila-seca, Constantí, el Morell, la Pobla de Mafumet and Perafort [1], and less than 1 km away from some of the main population centres.

This gives the area a singular situation regarding air quality, because air pollution does not originate in road traffic like in many major cities around the world, and therefore the contaminants that must be targeted are of a different nature. Whereas in major cities studies usually focus on particulate matter and gas compounds from exhaust fumes, like nitrogen oxides or ozone, in Tarragona area the focus is placed on all the volatile organic compounds (VOCs) produced and used by the petrochemical industry [1, 2].

Both active and passive sampling strategies are currently in use for the analysis of these VOCs in air and are considered suitable for their determination at low concentrations levels, since they include a preconcentration step which increases sensitivity when compared to whole-air sampling methods using bags or canisters. Out of the two, this group has a long trajectory using axial passive samplers [3] in transference projects with the companies in the petrochemical industry, with a method for determination of up to 75 of these compounds in urban areas. The 14-day averages obtained with these samplers allow for exposure studies and risk assessment. In the case of benzene, they also allow for calculation of the yearly average, which is limited in immission zones by current European law to 5 $\mu\text{g m}^{-3}$ [4].

The main limitation of this method is that the information is obtained as 14-day averages, so specific episodic peaks in the concentration of certain key compounds cannot be identified at the time they occur. This is the case of 1,3-butadiene and benzene, both classified as carcinogenic agents by the International Agency for Research on Cancer, and both produced in Tarragona's Northern Park [5]. For this reason, during the last four years the use of online GC-PID instrumentation [6] has been introduced in the towns of El Morell, less than 1 km away from the two 1,3-butadiene production plants, and Constantí, about 3 km away from the Northern Park.

This online method, with an in-situ analysis every 15 or 30 minutes depending on the compound, allows for the identification of concentration peaks in these two towns, where the historic data show that the concentration of these compounds can be more prominent. The availability of this new information enables the involved companies to relate the peaks to their industrial activities and to adopt measures towards the actions that generate them.

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YO-04**DETERMINATION OF HIGH PRODUCTION VOLUME CHEMICALS (HPVCs) IN PREPACKAGED FISH-BASED MEALS SOLD IN TARRAGONA****Borrell, Laura; Borrull, Francesc; Aguilar, Carme; Pocurull, Eva.***Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Sescelades Campus, Marcel·lí Domingo 1, 43007, Tarragona, Tarragona, Spain.*

Phthalate esters (PAEs), organophosphate esters (OPEs), benzenesulfonamides (BSAs), benzothiazoles (BTHs) and synthetic musk (MUSKs) are common chemicals used in industrial applications, all of them included in the list of High Production Volume Chemicals (HPVCs) created by the US Environmental Protection Agency (EPA) based on the Organization for Economic Cooperation and Development's (OECD) HPVC Screening Information Data Set (SIDS) Program, where chemicals with an annual production exceeding 500 tons per year are reported [1].

PAEs, OPEs and MUSKs are highly volatile, which facilitates their dispersion in air. Meanwhile, BSAs and BTHs have a more lipophilic character which enhances their dispersion in lipids, being easily found on the surface of bodies of water and soil sediments. Afterwards, these compounds released in the environment are absorbed by seafood species, causing negative health effects to humans, such as endocrine disruptive and reproductive issues, and even promoting the development of cancers when consumed [2].

Most studies to date are focused on raw samples cooked while controlling the temperature and fat percentage, assessing the impact of common cooking methods on the bioavailability of HPVCs [3,4]. In these works, several of these compounds were determined at levels of ng g⁻¹ wet weight (w.w.).

However, ready-to-cook meals are becoming increasingly popular nowadays due to their convenience. In precooked fish the influence of the packaging and of the cooking method is combined, making it an important field of study, due to the fact that ready to eat meals are commonly packed in plastic-based containers, which may release HPVC pollutants into their contents. Moreover, in general, the impact of packaging on food and its risks for human consumption is an important topic of study [2,5].

In this study compounds from these five families of HPVCs were investigated in ten different ready to cook meals. These purchased ready to eat meals included different cooking methods such as grilled, steamed, smoked, boiled, fried and in sauces. These 10 different supermarket fish-based precooked dishes included squid (*Loligo vulgaris*), salmon (*Salmo salar*), mussel (*Mytilus edulis*), shrimp (*Parapenaeus longirostris*) and sardine (*Sardina pilchardus*), all of them sold in Tarragona (Spain). For their analysis, we propose a simple treatment consisting of a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample extraction and a LipiFiltr clean-up, followed by a GC-MS/MS analysis, following a previous methodology developed in our research group [6]. To the best of our knowledge, this represents the first study addressing the relationships between packaging materials, HPVCs, and cooking methods, which were studied considering the overall fat content, a factor dependent on the different purchased fish species.

Acknowledgments

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YO-05**DISTINCT CIGUATOXIN PROFILES IN FISH INVOLVED IN POISONING EVENTS FROM THE INDIAN OCEAN: THE IMPORTANCE OF UPLC-MS/MS ANALYSIS**

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Ciguatoxins (CTXs) are highly toxic, lipophilic, cyclic polyethers primarily produced by marine microalgae of the genera Gambierdiscus and Fukuyoa. These toxins can bioaccumulate and undergo biotransformation in fish, posing a risk to human health. Currently, there is no universally accepted method for their detection in fish matrices. However, the European Food Safety Authority (EFSA) recommends initial screening using the mouse neuroblastoma cell assay (Neuro-2a), followed by analytical detection and quantification through LC-MS/MS. Metabolization through trophic networks adds complexity to the detection of these compounds, as it generates a high number of analogues. The complementarity of UPLC-MS/MS technique in multiple-reaction-monitoring (MRM) for the detection and quantification of specific CTX analogues with the UPLC-HRMS technique for the confirmation of CTX allows the characterization of CTX profiles.

This methodology has revealed distinct toxin profiles in fish involved in poisoning incidents. Analyses of fish samples from poisoning events in the Indian Ocean, specifically in La Réunion and the Seychelles, report three cases where the analyzed samples showed presence of CTXs but different toxin profiles. In one of the cases, ciguatoxins CTX3B (0.12 ng eq. CTX3C/g flesh), in one of the three Lutjanus bohar flesh samples, and 2,3-dihydroxy-CTX3C (0.062 ng eq. CTX3C/g flesh), in another, were detected. A second poisoning incident involving Variola louti revealed the presence of CTX1B (0.082 ng eq. CTX1B/g flesh) and its analogues CTX2 (0.043 ng eq. CTX1B/g flesh) and CTX3 (0.043 ng eq. CTX1B/g flesh). A third event revealed the presence of Indian Ocean ciguatoxins (I-CTXs) in a Thysitoides marleyi: I-CTX-1 (0.14 ng eq. CTX1B/g flesh), I-CTX-2 (1.01 ng eq. CTX1B/g flesh), and I-CTX-4 (0.34 ng eq. CTX1B/g flesh). The presence of CTX1B congeners and I-CTXs has been confirmed through LC-HRMS. For CTX3B and 2,3-dihydroxy-CTX3C such confirmation has not been possible due to the high limit of detection for CTX3C congeners.

These findings highlight the diversity of ciguatoxin analogues present in fish associated with ciguatera poisoning events in the Indian Ocean and emphasize the importance of advanced analytical techniques such as UPLC-MS/MS for accurate identification and quantification. The variability in toxin profiles among different species and incidents underscores the complexity of CTX biotransformation and the need for region-specific monitoring strategies. Continued efforts in toxin profiling are essential to improve risk assessment and management of ciguatera fish poisoning in affected regions.

YO-06

A NEW HIGH-PERFORMANCE CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (HPLC-MS/MS) METHOD FOR THE DETERMINATION OF HYDROXYLATED PAHS METABOLITES IN ANIMAL ORGANS

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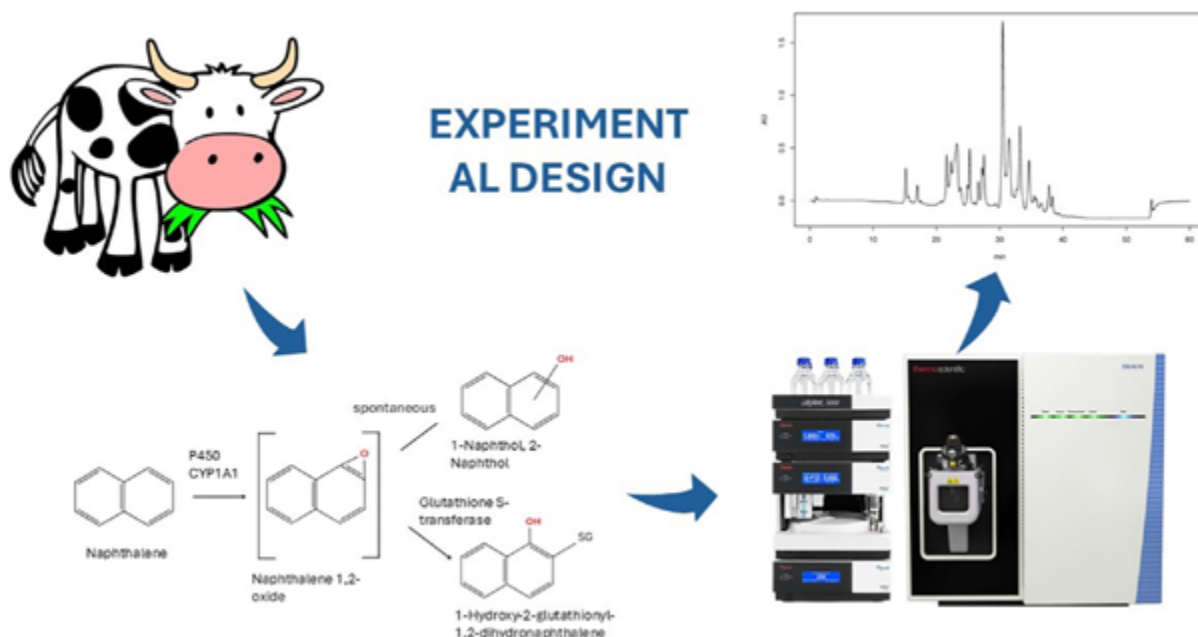
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Hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) are metabolites of polycyclic aromatic hydrocarbons that represent a critical class of biomarkers for assessing the exposure to persistent organic pollutants of high concern for human health due to carcinogenicity, neurotoxicity, and developmental impairments in pediatric populations^{1,2}. Exposure to PAHs mainly occurs through inhalation of contaminated fine particles, skin contact or oral uptake of soil particles.

Due to their lipophilic properties, PAHs diffuse across cellular plasma membranes, inducing upregulation of CYP1A1 transcription, the enzyme associated to metabolization of aromatic compounds to oxidated forms. Although its transcription is mainly regulated in the liver, which is the main organ carrying out the metabolism of many drugs and toxins³, it can be expressed in all tissues exposed to exogenous substances. In literature, the transcription of CYP1A1 was also investigated in the kidney, intestine⁴, and lungs⁵ as these organs are closely associated with exposure pathways involving inhalation and oral uptake. Oxygenated PAHs can also be generated directly within the environment through various processes. These compounds may subsequently enter the human body in their oxidized form. Specifically, atmospheric PAHs are oxidated in a structure-dependent manner: smaller PAHs (2-3 rings) undergo homogeneous gas-phase reactions with OH, NO₃, and O₃, while larger PAHs primarily engage in heterogeneous reactions⁶.

Traditional biomonitoring employs minimally invasive matrices such as serum, breast milk, and urine⁷. Conversely, research examining OH-PAH metabolites in animal organs is currently limited in the experimental design and restricted to a subset of these compounds¹. Advances in analytical methodologies are critical to elucidating the metabolic pathways, systemic distribution, and bioaccumulation potential of OH-PAHs.

The focus of this study is the development of a method for quantification of seven free OH-PAHs in animal organs. The analytical method was optimized by assessing operational conditions including different HPLC column, ionization sources, mobile phases composition, chromatographic gradient, ultrapure water quality, and extraction method. Evaluation focused on selectivity, resolution, and signal-to-noise ratio, balancing sensitivity with background noise impact. The protocol encompasses tissue homogenization, analyte extraction via vortex mixing in methanol with 0.5% formic acid, centrifugation, supernatant filtration, and dilution in ultrapure water. Quantitative analysis is conducted using high-performance liquid chromatography coupled to tandem mass spectrometry equipped with



an atmospheric pressure chemical ionization source (HPLC-APCI-MS/MS), The method was developed and validated for the analysis of the most metabolically relevant target tissue, i.e. bovine kidney, achieving detection limits in the range of 0.03 to 0.3 pg g⁻¹ depending on the compound. This study is, to the best of our knowledge, the first to validate the extraction of 9-hydroxyfluorene, 3-hydroxyfluoranthene, and 3-hydroxybenzo(a)pyrene in animal organs. Additionally, it pioneers the use of an APCI ionization source for PAH metabolite analysis, diverging from the conventionally employed ESI sources, with significant gains in background reduction.

Upon validation, the method has been applied to pilot testing other unfortified/unexposed animal tissues acquired from local butchers, including bovine (heart, liver, lung, trachea, kidney), swine (heart, liver), rabbit (heart, kidney, liver), and guinea fowl (heart, liver). The selection of tissues was based on existing literature on the distribution of CYP1A1 across the body and the anticipated patterns of PAH exposure. Despite none of the investigated OH-PAHs were detected at a significant level in the examined samples, the work provides a methodological background for both systematic implementation in the biomonitoring of exposed food matrices and human subjects; and the further extension of the analytical platform to other metabolites of PAHs. Namely, further research is ongoing to incorporate enzymatic hydrolysis in the workflow, so to determine also gluco-conjugated PAH metabolites. These findings have direct implications for ecological contamination studies in wildlife and human biomonitoring campaigns.

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YO-07

DEVELOPMENT OF MK-008: A SELECTIVE AND REVERSIBLE GUAIAZULENE-BASED CHEMOSENSOR FOR ON-SITE Cu^{2+} DETECTION: SPECTROPHOTOMETRIC, COLORIMETRIC, AND SMARTPHONE-BASED ANALYSIS

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Copper, the third most abundant essential trace element in the human body after iron and zinc, plays a critical role in biological processes such as iron absorption, red blood cell formation, and enzymatic activity¹⁻³. However, excessive Cu^{2+} exposure is associated with severe health risks, including Alzheimer's disease, Wilson's disease, and central nervous system damage⁴⁻⁶. Due to these potential hazards, regulatory agencies such as the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) have established strict maximum permissible limits for Cu^{2+} in drinking water at 2 ppm ($\sim 30 \mu\text{M}$) and 1.3 ppm ($\sim 20 \mu\text{M}$), respectively⁷. Conventional analytical methods like atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectroscopy (ICP-MS) remain limited by high costs, non-portability, required specialized personnel and operational complexity⁸⁻¹¹.

This study introduces the MK-008 chemosensor, a cost-effective, rapid, and portable solution for selective Cu^{2+} detection. The MK-008 probe was synthesized via a simple procedure and fully characterized using NMR, IR, MS, and single-crystal X-ray diffraction, confirming its structure. The sensor demonstrates exceptional selectivity for Cu^{2+} with no interference from competing ions. Use of EDTA as a chelating agent enables reversible and reusable functionality, regenerating the sensor through Cu^{2+} -EDTA complexation. In real-sample analyses of spiked drinking and tap water, the sensor achieved recovery rates of 84–104%, with a detection limit (LOD) of $9.45 \times 10^{-7} \text{ M}$ (55.4 ppb) and a linear dynamic range of $5.0 \times 10^{-7} \text{ M}$ to $3.7 \times 10^{-4} \text{ M}$ ($R^2 = 0.999$), well below regulatory thresholds. The sensor remained stable over 100 days and delivered near-instantaneous spectrophotometric and colorimetric results.

Complementing the chemosensor, the Android-based mobile application "MK-008 Cu^{2+} Detector" quantifies Cu^{2+} levels by analysing Red/Green intensity ratios in captured images, enabling on-site analysis with no specialized training. This integrated platform combines reusability, affordability, portability, and user-friendly operation, offering a transformative tool for environmental monitoring and public health protection.

Keywords: copper detection, chemosensor, smartphone-based sensing, colorimetry, environmental monitoring.

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YO-08**INNOVATIVE 3D MICROPAD FOR DUAL DETECTION OF PARACETAMOL AND ASPIRIN IN SALIVA AND URINE**

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The widespread use of analgesics such as paracetamol and non-steroidal anti-inflammatory drugs such as aspirin, over-the-counter drugs which can be acquired without medical prescription, can entail a health problem if they are administered incorrectly [1]. The development of analytical procedures for the rapid, sensitive and accurate determination of these kind of drugs in clinical samples is of utmost importance.

In the last years, *Point-of-Care Testing* (PoCT) systems have received much interest due to their ease of use, reliability, and cost-effectiveness for screening in suspected diseases [2]. In this context, paper-based analytical devices (PADs) have gained considerable attention as detection platforms due to their ease of fabrication, cost efficiency, and capacity for multi-element analysis, thereby reducing analysis time [3]. Until now, reported works based on PADs are focused on the individual analysis of paracetamol or aspirin. Furthermore, colorimetric micro-PAD systems have not been applied for the detection of paracetamol in clinical samples, such as saliva and urine. Application of colorimetric micro-PADs to clinical samples is highly interesting to assess multiplexed analysis allowing the simultaneous determination of different analytes saving cost and time.

The main objective of this study is to develop a three-dimensional (3D) micro-PAD as a chemosensor utilizing hydrophilic cellulose substrates for the simultaneous detection of paracetamol and aspirin in synthetic clinical samples [4]. Different parameters involved in the 3D micro-PAD system and the direct recognition of both analytes have been optimised. Under optimal conditions, detection limits of 0.004 mM and 0.013 mM were obtained for paracetamol and aspirin, respectively. Finally, the proposed methodology was applied to the determination of these drugs in synthetic urine and synthetic saliva. Recovery studies showed values ranged from 98% to 103%, with a relative standard deviation of 3–6% ($n = 6$) demonstrating the suitability of the developed approach. Image acquisition with a smartphone and its processing on the same electronic device allows the quantification of both analytes in a simple and fast way (analysis time less than 4 min). These findings underscore the potential of the developed 3D micro-PAD as a PoCT method for the total determination of paracetamol and aspirin in saliva and urine in cases of suspected deliberate self-intoxication.

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necessity of adding stabilizing agents to the ink formulation to avoid MNPs aggregation during printing and the fact that energy-intensive and time-consuming thermal treatments are required to remove all the auxiliary components from the ink (i.e. solvents, stabilizing agents) in order to obtain an adequate metal thin film purity and conductivity.

This contribution aims to present a one-step fabrication process for metal thin films by combining drop-on-demand inkjet-printing of metal precursor inks and simultaneous photochemical conversion of the metal precursors into pure metals and or respective oxides.

The process is known in the literature as Print-Light-Synthesis (PLS) and allows large-scale electrode production, no material waste, reduced fabrication time and costs. Metal precursors, which are present in the ink, are usually metal salts, which are reduced into metals by UV light while the heat which is locally generated on the substrate during UV irradiation of the ink allows all other ink components (i.e. solvents) to escape from the gas phase³.

We will demonstrate the use of PLS for the fabrication of disposable and reusable amperometric and potentiometric electroanalytical sensors. Gold (Au) and ruthenium oxide (RuO₂) thin film and NPs modified graphene electrodes have been fabricated by PLS and have been tested for several electroanalytical sensing applications.

Gold electrodes by PLS have been employed for the amperometric detection of bacterial markers and antioxidants exploiting high sensitivities respect to commercial screen-printed electrodes as well as electrocatalytical effects toward electroactive species.

Ruthenium oxide (RuO₂) electrodes by PLS have been used for the potentiometric pH sensing in cancer cell cultures showing a good response in the cell culture matrix and requiring a minimum amount of sample (< 1 mL)⁴. RuO₂ modified graphene electrodes fabricated by PLS, have been tested toward L-Cysteine sensing showing an electricatalytical effect toward L-Cysteine and allowing its detection also in the presence of interferent compounds.

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YO-10

MICROFLUIDIC BIOSENSOR FOR SIMULTANEOUS DETECTION OF GLUCOSE AND SUCROSE

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Glucose and sucrose are metabolically relevant key sugars as well as key analytes for food quality control and clinical diagnostic applications. Traditional sugar analysis is typically laborious and requires complex instrumentation and sample pretreatment. Excessive consumption of carbohydrate foods, particularly those with high refined sugar content, has been linked to metabolic diseases such as insulin resistance, obesity, and type 2 diabetes [1]. Thus, the quantification of individual sugars in food products is essential for health and regulatory control.

The microfluidic paper-based biosensor has been developed for the simultaneous and selective detection of glucose and sucrose. Prussian white is the chemosensitive element of this system, which is converted to Prussian blue upon reaction with hydrogen peroxide, generating an optical signal [2]. Due to the immobilized glucose oxidase (EC 1.1.3.4), the detection of glucose is enabled via the enzyme-catalyzed oxidation of this sugar to hydrogen peroxide. Furthermore, the presence of invertase (EC 3.2.1.26) permits sucrose hydrolysis to glucose, which is the initial step of a bienzymatic cascade detection method. Proper design of the microfluidic biosensor allows glucose interference, which is generated as a natural byproduct of the invertase-catalyzed reaction, to be removed. The developed biosensors exhibit good stability and satisfactory reproducibility of assays. Preliminary results show their ability to selectively detect both sugars in real beverage samples.

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YO-11**MULTIPLEXED ELECTROCHEMICAL IMMUNOSENSOR FOR THE DUAL DETECTION OF TETRODOTOXIN AND SAXITOXIN IN SEAFOOD SAMPLES****Reverté, Jaume; Diogène, Jorge; Campàs, Mònica.***IRTA, Marine and Continental Waters (AMiC), La Ràpita, Spain.*

Tetrodotoxin (TTX) and saxitoxin (STX) are potent neurotoxins found in several species of fish and shellfish that pose a significant food safety risk because of their potential to cause severe poisoning and, in some cases, even death. Conventional analytical methods have typically been developed to detect a single toxin family, which limits their effectiveness when both toxins may be present at the same time. With increasing reports of the co-occurrence of TTX and STX in seafood, there is an urgent need for advanced bioanalytical tools capable of simultaneous detection. In this work, we present a novel multiplexed electrochemical immunosensor designed for the dual detection of TTX and STX. The device incorporates a four-electrode array: two test electrodes for detecting the toxins in seafood samples, and two control electrodes acting as internal references aligned with current regulatory or safety advisory thresholds. This configuration enables the sensor to both identify which toxins are present and determine whether their levels exceed established safety limits. The result is a rapid, easy-to-use screening tool that supports consumer safety by facilitating early toxin detection. This dual-detection approach could form the basis of integrated monitoring systems capable of identifying multiple marine toxins in a single test, thereby improving seafood safety and protecting public health.

YO-12

HIGHLY SENSITIVE PRINTED pH SENSOR USING CB/PANI NANOCOMPOSITE FOR POC DIAGNOSIS OF ORTHOPEDIC INFECTIONS

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Orthopedic-related injuries, namely fractures, trauma, and bone replacement, are mostly treated by implanting medical hardware during current arthroplasty surgeries. Nonetheless, such a widespread surgical strategy significantly impacts on patient's health due to a relevant risk of post-surgery periprosthetic infections. The occurrence of bacterial infections leads to a critical implant failure along with long-term hospitalization, entailing costly and frequently unsuccessful antibiotic administrations.¹ The bacterial growth and metabolism on the orthopedic device surface trigger a pH drop within the local synovial fluid from its normal physiological range (pH = 7.4 - 7.7) to more acidic values (pH < 7), making it as a key diagnostic biomarker.^{2,3} Several hampering limitations, such as storage conditions and inadequate sensitivity, have been hindering the scalability of earlier developed potentiometric pH sensors as point-of-care diagnostic tools.^{4,5} Addressing this challenge, we introduced a potentiometric pH screen-printed electrode harnessing Carbon Black nanomaterial and pH-sensitive Polyaniline featured by an attractive chemical stability. This combination enables super-Nernstian (high-sensitive) point-of-care detection of slight pH variations, favoring the fast-up of restorative treatments by means of rapid orthopedic infection diagnosis. Drop-casting technique was used to deposit a dispersion of Carbon Black/Polyaniline nanocomposite onto the working electrode, ensuring a straightforward and reliable functionalization of the printed sensor. A thorough investigation of the optimal electrode modification was led by evaluating the size and the stability of various Carbon Black/Polyaniline composite mixtures using Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS), respectively. Furthermore, the morphological characterization of different compositions was carried out by Transmission Electron Microscopy (TEM). Following the evaluation of analytical performances, the CB 90%–10% PANI mixture was selected for electrode functionalization, as it exhibited an excellent dispersibility and an effective balance between linearity in the pH range 3–8 ($R^2 = 0.994$), valid reproducibility (RSD% = 0.9%, $n = 3$), and lack of memory effect, alongside noteworthy sensitivity (-74 ± 3 mV/pH unit). The designed sensor further demonstrated a remarkable selectivity, revealing negligible pH deviations in the presence of potential interfering agents in synovial fluid. Storage stability was also monitored over 28 days by weekly recording the potentiometric response of the developed sensor, held dry at room temperature. Specifically, a stable potential signal has been achieved since the second week with a performance consistency up to one month. Lastly, sensor validation was conducted in real samples of healthy and infected synovial fluid by comparing its response to the pH-meter reading and the outcome of microbiological analysis. The proposed device accurately measured pH variations across physiological and pathological conditions, detecting healthy synovial fluids in the pH range of 7.5–7.7 and infected samples with a pH value lower than 7. These results closely matched the pH-meter measurements and bacterial culture responses, thereby highlighting the sensor's diagnostic potential for point-of-care orthopedic infection monitoring.

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YO-13

TOWARDS ECO-FRIENDLY AND AI-POWERED BIOSENSING: A BIOCHAR-BASED PLATFORM FOR EMOTIONAL AND PHYSIOLOGICAL MONITORING

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Clothing and accessories are not only functional or aesthetic elements, but they also play a symbolic role in expressing emotions and identity. Building on this expressive potential, wearable systems that integrate biosensors into garments are emerging as powerful tools to monitor emotional and physiological states in real-time. This capability is becoming increasingly relevant in fields such as healthcare, safety, fashion technology, and performance optimization.[1]. Stress, enthusiasm, and exhaustion are examples of emotional stimuli that cause physiological reactions that change the makeup of biological fluids[2]. Among them, sweat and saliva are especially helpful for non-invasive analysis.

The proposed work is part of an ambitious Italian Project "Dress the future: novel combined wearable integrated systems (Stargate)" (PRIN-PNRR -P2022CZA3P), whose goal is the design of a Wearable System (WS), that embeds in a new wearable material a set of body-worn biosensors, reading and fusing different parameters such as temperature, pressure, and concentrations of some biomarkers in secretions; in particular this research work aims to develop environmentally friendly biochar screen-printed electrodes for monitoring cortisol and conductivity in emotional and physiological assessments. Perspiration rates can be altered by emotional states such as stress, fear, panic, or excitement, which can lead to changes in sweat ionic concentration. Single-frequency electrochemical impedance spectroscopy (EIS) is used to measure sweat resistance, an indicator of ionic concentration, providing rapid, low-energy measurements ideal for continuous monitoring[3], [4]. Experimental data confirm the sensor's ability to detect physiologically relevant variations in sweat resistance, forming a promising method for emotional assessment. For cortisol detection, a label-free immunosensor has been developed using SPEs modified with innovative carbon nanomaterials. These materials not only enhance electrochemical performance but also support covalent antibody immobilization, enabling precise detection at low concentrations. In this study, biochar is modified using a *Click Chemistry* reaction to introduce an aliphatic chain that terminates with a carboxylic group. This modification enhances the immobilization of antibodies by distancing them from the electrode surface, reducing steric hindrance, and allowing better penetration into the sample solution. As a result, antibody stability is improved, leading to a significant improvement in sensor performance.

A software platform that incorporates artificial intelligence and machine learning algorithms has been developed to complement the sensor hardware and analyze data. This solution automates data processing and interpretation, making the technology accessible and easy to use for non-specialized personnel. The Design of Experiment (DOE) approach is used to optimize each process, with the aim of minimizing the number of experiments, reducing reagent waste, and saving time, as demonstrated in literature studies[5]. This methodology enables multivariate analysis of such variables as antibody concentration and reaction time, thereby identifying optimal parameters that enhance sensor performance.

Integrating advanced electrochemical sensing, AI-driven data analysis, and sustainable materials, this project connects innovative technology with practical applications. The platform enables real-time monitoring of emotional and physiological states while promoting eco-friendly sensing. As part of a national research initiative, it contributes to developing a wearable multi-analyte sensor for tracking physical parameters (e.g., pH, temperature, ionic concentration) and biomarkers like cortisol, supporting comprehensive health and emotional assessment.

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YO-14

BIOCHAR TO PRODUCE PLASTIC ELECTRODES. ELECTROCHEMICAL APPROACH TO ENVIRONMENTAL MONITORING

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Marine pollution is largely driven by anthropogenic activities, with heavy metals like cadmium (Cd) and lead (Pb) posing serious threats due to their toxicity, persistence, and ability to bioaccumulate [1]. In Adriatic Sea sediments, reported concentrations reach 3 µg/g for Cd, 70 µg/g for Pb, and 150 µg/g for zinc (Zn) [2]. Inductively coupled plasma mass spectrometry (ICP-MS) is the gold standard method for detecting such contaminants, but its high cost and lack of portability limit field applications. Electrochemical methods, particularly electroanalytical sensors, offer cost-effective, selective, and portable alternatives. Among emerging materials, biochar, produced via biomass pyrolysis, stands out as a sustainable, low-cost option for detecting heavy metals like Cd, Pb, and Zn [3]. Its structural properties and conductivity make it highly suitable for environmental monitoring applications

In this study, plastic electrodes [4] were fabricated using biochar obtained from the pyrolysis of sunflower at 750 °C, followed by chemical activation with chitosan (CS) and NaOH. New electrodes, composed of 75% graphite (GP) and 25% activated biochar (BCS), designated as GP₇₅-BCS₂₅, were characterised by cyclic voltammetry in Fe(CN)₆^{3-/4-} (10 mM in 0.1 M KCl) (Fig. 1a). The results indicated that chemical activation significantly enhanced the electrochemical performance of the electrodes compared to those composed solely of GP, yielding electroactive surface areas of 0.0404 cm² for GP₇₅-BCS₂₅ and 0.011 cm² for GP, calculated from peak current (I_p) measurements at different scan rates (10–400 mV/s) (Fig. 1b).

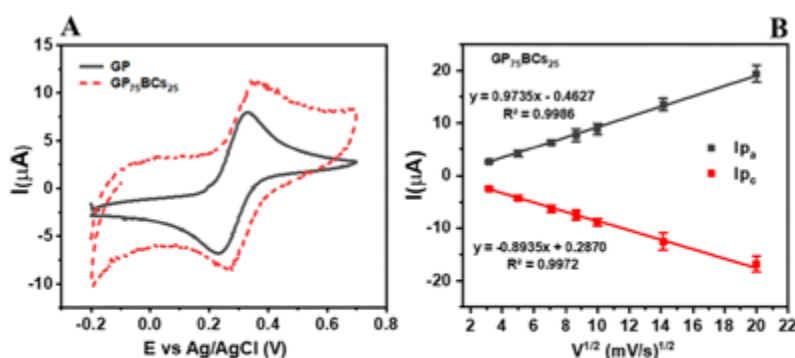


Figure 1. Cyclic voltammetry of Fe(CN)₆^{3-/4-} redox system (1 mM in 0.1M KCl at 50 mV/s) at: plastic electrodes manufactured using of GP and GP₇₅BCS₂₅ (A). Dependence of the current (I µA) vs concentration of Fe(CN)₆^{3-/4-} redox system using a potential window between -0.2 to 0.7 V for the plastic electrodes GP and GP₇₅BCS₂₅ (B).

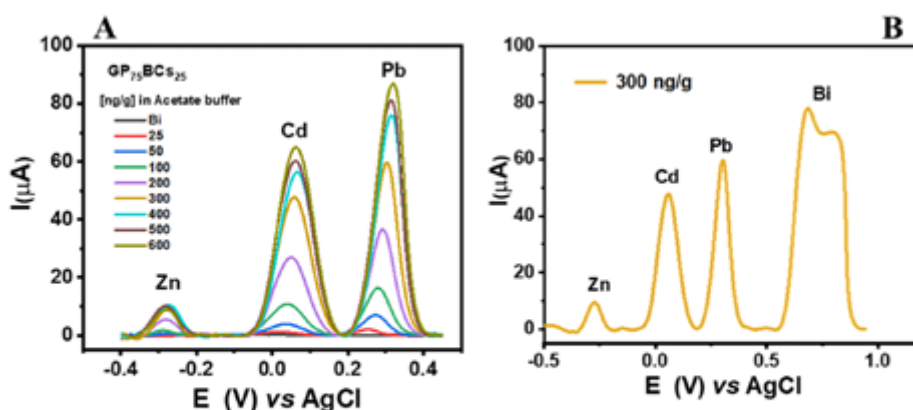


Figure 2. SWSAV curves of Zn, Cd and Pb solutions (25-600ng/g) with plastic electrodes GP₇₅BCS₂₅ in presence of Bi (500 ng/mL)(A-B) in acetate buffer (0.1M, pH 4.5).

The detection capability of the plastic electrodes was assessed in acetate buffer solution (Fig. 2) and in real marine samples (sea sand) (Fig. 3), without aggressive acid digestion, a procedure commonly used in heavy metal determination. The metal extraction protocol included the treatment of 1 g of sea sand with 5 mL of acetate buffer (0.1 M, pH 4.5), the solution was sonicated for 1 h in an ultrasonic bath and shaking overnight at room temperature. Finally, the solid was separated by centrifugation and subsequent filtration through a 0.22 mm filter. Electrochemical detection of Cd, Pb, and Zn was carried out using square wave anodic stripping voltammetry (SWSAV) in a range of 25–600 ng/g (Fig. 2A), with an optimal Bi^{3+} concentration of 500 ng/mL (Fig. 2B).

SWASV was performed using an accumulation potential of -1.2 V for 300 s, followed by a 60 s relaxation period. The scanning potential ranged from -1.1 to 0.6 V, with a pulse amplitude of 40 mV and a frequency of 10 Hz. Final metal concentrations were determined by linear regression of current responses using the standard addition method. Limits of detection (LOD), calculated with a signal-to-noise ratio of 3 ($n=3$), were 15.88 ng/g for Zn, 9.86 ng/g for Cd, and 6.66 ng/g for Pb, all below the legal limits for tap and marine waters. These findings support the viability of biochar as an effective electrode material, particularly when chemically activated after pyrolysis. In this study, treatment with CS and NaOH induced beneficial structural and morphological changes, enhancing ion exchange with heavy metals via functional groups such as OH^- , NH_2^- , and CO^- . Aligned with green chemistry principles, the results underscore the promise of activated biochar as a sustainable, low-cost electrochemical material for detecting environmental pollutants.

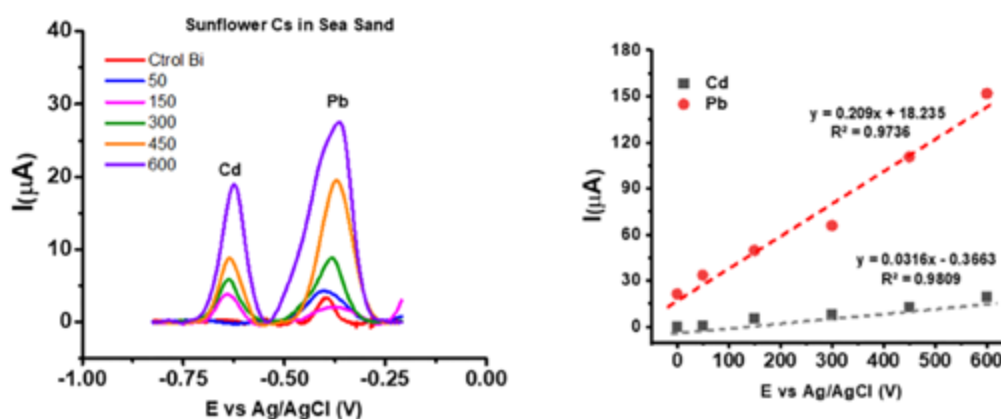


Figure 3. SWSAV curves of Cd and Pb (50–600 ng/g) in sea sand measured by mean of GP₇₅BCS₂₅ plastic electrodes in acetate buffer (0.1M, pH 4.5).

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YO-15

WEARABLE μ TPAD DEVICE FOR pH MONITORING IN CHRONIC WOUNDS

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As an intricate and delicate multi-phase process, skin wound healing pathway can often be corrupted by systemic or local factors, especially infections, that compromise the curative environment interfering with the repair progression. This can worsen simple acute wounds to chronic or even non-healing wounds. Thus, the need of less/non-invasive and user-friendly monitoring of the healing progresses could be of the outmost importance for management and treatment of the chronic wounds, improving patients' life quality. Within this framework, textile-based electrochemical sensors are gathering widespread interest, featuring high stretchability and adaptability, essential for the development of smart wearable analytical tools. Among textiles, common threads used for everyday apparels, namely cotton or polyester, can be easily functionalized by coating with conductive materials and integrated in electronic circuit, for the development of electrochemical sensors.

Threads are combined with paper, exploiting its unique features namely flexibility, foldability and capillarity as i) a support for threads embroidery and ii) a self-sampling system aiming to provide an integrated wearable device for pH detection as biomarker for infection assessment. In detail, when in contact with the wound area, paper samples biological fluids directing them to thread-based sensing area for the potentiometric detection of pH. The microfluidic thread/paper-based device (μ TPAD) includes a silver thread coated with graphite and silver/silver chloride (Ag/AgCl) ink for working (WE) and reference (RE) electrodes, respectively. Iridium oxide is chosen as a biocompatibility pH-sensitive material, since it allows to measure a wide range of pH with high reliability, stability over time, and ability to operate in complex matrices such as blood. [1,2] In detail, iridium oxide is electrodeposited via cyclic voltammetry onto the graphite-coated thread-based WE surface. The modified threads are then integrated into the paper-based sampling pad, designed to have circular hydrophilic sampling zones, delimited by hydrophobic wax.

As a preliminary study, the μ TPAD is tested using Open Circuit Potentiometry with a commercial potentiostat PalmSens4 (PalmSens BV, The Netherlands), by loading 15 μ L of standard pH solution (pH from 4 to 8) onto the hydrophilic area of the device, containing the thread-based electrodes. A slope of (0.054 ± 0.004) V/pH decade is obtained, with good inter- μ TPAD repeatability, showing an RSD% of 6%, calculated with three different μ TPADs at pH 6. The developed μ TPAD is then integrated with a self-developed Arduino module connected to a laptop. The calibration curve obtained with the Arduino module shows a sensitivity of (0.053 ± 0.003) V/pH decade, comparable with the one obtained with the commercial potentiostat, and an inter- μ TPAD repeatability with an RSD% of 7% evaluated with three different μ TPADs at pH 6.

Finally, a 3D printed flexible bracelet is designed as a scaffold for the wearable μ TPAD, combined to an Arduino module connected via Bluetooth to a smartphone enabling a readout throughout a downloadable application for conducting measurement in real samples.

This work was conducted in collaboration with European Horizon 2020 STRENTEx Project.

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YO-16**ARE ACTUALLY DEEP EUTECTIC SOLVENTS NEEDED IN LIQUID-LIQUID EXTRACTION?: DETERMINATION OF BISPHENOLS IN EDIBLE OIL SAMPLES****Zapater, Cristina; Vidal, Lorena; Aguirre, Miguel Ángel; Canals, Antonio.***Department of Analytical Chemistry, Nutrition and Food Science, and University Institute of Materials, University of Alicante, Alicante, Spain.*

Bisphenols (BPs) are organic compounds commonly used in plastic containers for food and beverages to provide strength and durability. However, there is growing concern regarding the migration of these compounds into food due to their adverse effects on human health [1]. These effects have been primarily associated with bisphenol A (BPA) due to its potential behavior as an endocrine disruptor. Nevertheless, various adverse effects on the nervous, immune, and reproductive systems have also been linked to BPA-analogue compounds [2,3].

The main goal of this research is to develop and compare three analytical methods based on dispersive liquid-liquid microextraction (DLLME) followed by liquid chromatography-diode array detector (LC-DAD) for the determination of four bisphenols (i.e., BPF, BPA, BPAF, and BPAP) in edible oil samples comparing the use of pure ethylene glycol (EG), a hydrophilic deep eutectic solvent (DES) composed of choline chloride and ethylene glycol (ChCl:EG, 1:2), and a hydrophilic magnetic deep eutectic solvent (MDES) composed of choline chloride, ethylene glycol, and iron (III) chloride hexahydrate (ChCl:EG:FeCl₃·6H₂O, 1:4:1) as extractant solvents. The use of DES and MDES aligns with current trends toward more sustainable practices in Analytical Chemistry, as they are less toxic and biodegradable compared to traditional solvents. In addition, MDES offers distinct advantages due to its magnetic properties, facilitating easy separation and recovery through the use of an external magnetic field, thereby removing the need for a centrifugation step. On the other hand, a comparison of DES and MDES with one of their pure components is particularly relevant, as it has not been previously investigated and would simplify the analytical process by not requiring a synthesis step.

The most promising results were obtained using pure ethylene glycol as an extractant, which achieved adequate linearity (i.e., $r \geq 0.990$), lower limits of detection (LODs) (i.e., $0.3 \mu\text{g kg}^{-1}$), and excellent repeatability (i.e., $\text{RSD} \leq 3.2\%$) for all analytes. Furthermore, ethylene glycol exhibited negligible matrix effects (i.e., 71–121 %) in the analysis of real samples, including olive, sunflower, rapeseed, soybean, and sesame oils. Consequently, pure ethylene glycol was identified as the most suitable extractant compared to eutectic mixtures and their magnetic variant. This finding underscores the necessity for further investigation of pure constituents of eutectic mixtures as extractant solvents.

Acknowledgement

The authors are grateful to the Spanish Ministry of Science and Innovation (PID2021- 126155OB-I00) and the Regional Government of Valencia (Spain) (CIPROM/2021/062) for the financial support. Also, authors would like to thank the Spanish Ministry of Science and Innovation for granting the Spanish Network of Excellence in Sample Preparation (RED2022-134079-T), and the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

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YO-17

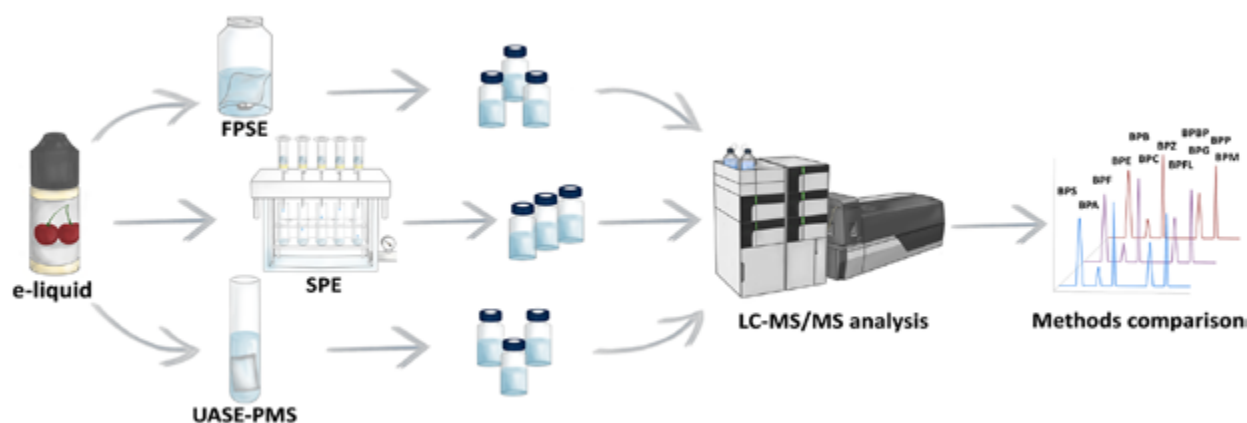
COMPARISON OF NOVEL EXTRACTION TECHNIQUES FOR THE DETERMINATION OF 12 BISPHENOLS IN E-CIGARETTE REFILL LIQUIDS

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In the presented research three distinct methods were developed, validated and compared to determine 12 bisphenols (BPs) in e-cigarette refill liquids. E-cigarettes have surged in popularity over the past few years. Consequently, monitoring both the liquids and the resulting aerosols is essential to assess the toxicity and health aspects associated with them. It is important to note that contaminants from plastic packaging can significantly impact the quality of these products. Previous research on BPs, which tend to migrate from packaging materials into the product, was primarily focused on their ingestion through food. However, the effects of exposure to these compounds from other sources, including inhalation, remain less understood. Refill liquids similar to food products, are stored in polymeric packaging, which may influence BPs content. Given the increasing popularity of e-cigarettes and the diversity of available liquids and devices, including disposable ones, this issue warrants attention. Therefore, developing new methods to control BPs content in e-cigarette liquids is crucial for determining the actual exposure to these compounds [1].

In this study, three extraction approaches were employed to isolate bisphenols from refill liquids. The first method utilizing fabric-phase sorptive extraction (FPSE), integrates the principles of microextraction and traditional solid-phase extraction (SPE) by applying a sorbent-coated fabric as the extraction medium. As proven before, FPSE has demonstrated remarkable effectiveness in isolating bisphenols across various matrices [2,3]. The second approach, ultrasound-assisted solvent extraction of porous membrane-packed samples (UASE-PMS), provides an alternative to SPE by enclosing the sample within a membrane bag. This technique was also successfully applied in determining bisphenols in food and human-related samples [4,5]. Lastly, the study included commercially available SPE cartridges with molecularly imprinted polymers (MIPs) as selective sorbents (AFFINIMIP® Bisphenol A). Designed for the targeted isolation of BPA and its analogs, these cartridges were applied for analyzing food and biological samples. Each extraction method was combined with ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) analysis. For the determination of 12 BPs (BPS, BPA, BPF, BPE, BPB, BPC, BPZ, BPFL, BPBP, BPP, BPG and BPM) each method was rigorously optimized and validated. For FPSE and UASE-PMS protocols, critical parameters—including FPSE membrane type, extraction solvents, UASE-PMS membrane bag size, and extraction duration—were carefully selected. Finally, results obtained using methods based on three extraction techniques were compared in terms of choice/adjustment of parameters, sample preparation and method development time, cost, labour efficiency and handling, availability and possible limitations. Sample matrix consisting of propylene glycol and vegetable glycerin in 8:2 ratio was fortified to assess recovery, precision, accuracy. Both FPSE and UASE-PMS achieved high recoveries and low variability. The UASE-PMS method exhibited recovery rates (average recovery in range 91-98%) and consistency across varying concentrations compared to the FPSE (average recovery for 5, 25 and 50 ng /mL equals 86, 78 and 67 % as follows) and proved to be a robust alternative for the method based on commercially provided SPE using MIPs.



The study conducted a comprehensive evaluation of three distinct techniques for isolating BPs from e-cigarette refill liquids. The UASE-PMS method was utilized to analyze eight real samples. BPA was quantified in all tested samples of refill liquids (in range from 4.48 ± 0.42 ng /mL to 14.73 ± 0.79 ng /mL). This research addresses a key shortfall in existing literature by providing a thorough analysis of BPs in e-cigarette refill liquids, which had not been previously investigated. The results highlight the critical need for assessing endocrine-disrupting chemicals in these human related samples, with potential implications for public health policies and regulatory standards.

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YO-18

DEVELOPMENT OF SELECTIVE APTAMER-BASED SORBENT FOR TRACE ANALYSIS OF CADMIUM

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Cadmium (Cd) is a toxic metal for humans, and its excess can lead to numerous diseases, mainly causing kidney and bone dysfunctions. This is the reason why the World Health Organization has limited cadmium concentration to 3 µg/L in drinking water. It is thus essential to quantify trace of cadmium in potentially complex matrices (water, serum, etc.). In this kind of samples, cadmium is mainly present as Cd²⁺ ions. Because of its high sensitivity and specificity, inductively coupled plasma mass spectrometry (ICP-MS) is a method of choice for its monitoring. However, this expensive instrument requires high dilution factor to limit matrix effects. We previously developed a specific sorbent based on immobilized aptamers, *i.e.* short DNA or RNA sequences having a high affinity for a target compound, for the selective extraction of Cadmium ions from serum. A DNA specific sequence for Cd²⁺ ions was covalently immobilized onto Sepharose, packed in a cartridge and used as an off-line SPE (solid-phase extraction) sorbent for the selective extraction of this target ion from serum ⁽¹⁾. The removal of matrix effect during the ICP-MS analysis of Cd²⁺ in serum sample was demonstrated.

The aim of this study is to develop a miniaturized monolithic support grafted with aptamers, in order to couple on-line this oligosorbent with ICP-MS as was done for an organic monolith modified with mercapto groups ⁽²⁾ while taking advantage here of the high specificity of aptamers. This selective microextraction approach will allow the reduction of percolated volumes of samples and solutions. Additionally, it can improve the sensitivity and the analysis time of the method. In the long term, if the enrichment factor is sufficient, it will be possible to combine it with less specific and less expensive tools than ICP-MS.

Hybrid monoliths including an amino-silane to facilitate the aptamer grafting were synthesized by Sol-gel reaction ⁽³⁾ *in situ* into a 533 µm internal diameter silica capillary. The permeability values associated with the synthesized monoliths were calculated by backpressures measurements. A permeability average value of $(2.90 \pm 0.81) \times 10^{-13}$ m² (n=6) was obtained, which is suitable for the on-line coupling. The aptamer sequence specific to Cd²⁺ was then grafted onto the synthesized 4 cm-long monolith. Grafting yields with an average value of 79 % (n=2) were obtained by ion-pair liquid chromatography coupled to a UV detector. These grafting yields allow to estimate a theoretical capacity of the supports equal, to 1,1 µg (mean value for n=2), assuming that one mole of grafted aptamers can trap one mole of cadmium. This low amount is compatible with ICP-MS analysis despite of the high dilution factor. Similarly, high grafting yields were obtained (77 % (n=2)) when a monolith was grafted with a sequence composed of the same oligonucleotides but in random order to get a control support. Once the supports were synthesized and functionalized, extraction procedures were performed to get SPE profiles. The control support is used to study the selectivity of these extraction procedure. The study of the specificity of the supports was also conducted by introducing other interfering di-charged ions into the percolation solution.

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YO-19**ANALYTICAL TOOLS FOR THE SCREENING OF PERSISTENT AND MOBILE CONTAMINANTS IN URINE**

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In recent years, concern about the adverse effects of Persistent Mobile and Toxic (PMT) and very Persistent and very Mobile (vPvM) substances on human health has significantly increased [1]. In response to this, the European Commission incorporated these substances into REACH CLP regulations in 2023 [2]. Due to their chemical properties, PMT/vPvM are ubiquitous in the water cycle and can e.g. contaminate drinking water sources reaching human beings [3]. Thus, developing analytical methods to detect PMT/vPvM substances in human biological matrices is crucial for effective human exposure monitoring.

This research aims to develop an analytical methodology based on liquid/supercritical fluid chromatography coupled to high-resolution mass spectrometry (LC/SFC-HRMS) for the determination of PMT/vPvM substances in urine. Different types of chromatographic techniques, including supercritical fluid chromatography (SFC), mixed-mode liquid chromatography (MMLC) and hydrophilic interaction liquid chromatography (HILIC) were tested as well as different sample preparation techniques approaches. Finally, solid-phase extraction (SPE) with 500 mg hydrophilic-lipophilic balanced (HLB) cartridges was selected as the sample preparation technique and SFC for chromatographic separation.

The instrumental method was validated in terms of linearity in the range of 0.1 µg/L to 1000 µg/L ($R^2 > 0.99$), repeatability ($RSD < 20\%$) and instrumental quantification limits (LOQs) in the range of 0.1-250 µg/L for a mixture of 32 model compounds. The SPE followed by SFC-HRMS method showed also good performance with recoveries higher than 60% in urine samples.

This method was then applied to 6 pooled samples of human urine corresponding to 115 individuals in separate groups of male, female, rural residence or urban residence. Individual samples were collected during 3 weeks in September 2020 from anonymized volunteers who work as public servants in the regional government administration in the city of Santiago de Compostela.

A total of 137 PMT/vPvM substances were identified using a HRMS spectral library of over 3,000 substances. The majority of the detected compounds were industrial chemicals (44%) followed by pharmaceuticals (35%), pesticides (10%), personal care products (5%), food additives (4%) and drugs (2%).

The findings highlight the widespread presence of these contaminants and emphasize the need for stricter regulations for minimizing human exposure. Thus, the developed methodology has been proven to be a reliable approach for detecting PMT/vPvM substances in urine samples, contributing to future environmental and public health risk assessments.

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YO-20

CHIRAL ANALYSIS OF MIRABEGRON IN PHARMACEUTICAL AND BIOLOGICAL MATRICES BY CYCLODEXTRIN-ELECTROKINETIC CHROMATOGRAPHY WITH DEEP EUTECTIC SOLVENTS AS ADDITIVES

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Keywords: *chiral electrokinetic chromatography, mirabegron, deep eutectic solvents, pharmaceutical formulation, urine.*

Chiral drugs can exist as one or more pairs of stereoisomers, with each stereoisomer presenting the capacity to exhibit different biological, pharmacokinetic, and pharmacodynamic activities. This phenomenon is attributed to the ability of drug targets to discriminate between stereoisomers [1]. Consequently, the pharmaceutical industry has advocated for the separation and analysis of these stereoisomers to ensure the safety, efficacy, and quality of pharmaceutical formulations. The ability to detect these stereoisomers in biological matrices such as urine is crucial for various applications, including the monitoring of drug metabolism and elimination, the assessment of pharmacokinetics, the identification of potential interactions, and the assurance of safe and effective treatment. In the field of chiral separations, Capillary Electromigration Techniques, mainly Electrokinetic Chromatography (EKC), has demonstrated to be a powerful technique due to its remarkable versatility [2]. In this research area, Deep Eutectic Solvents (DESS) have emerged as innovative additives suitable for tuning the selectivity in chiral analysis by EKC.

Mirabegron, a potent selective agonist for the human β_3 -adrenoceptor, constitutes a novel class of compounds developed for the treatment of overactive bladder (OAB) symptoms, including urinary frequency and urge incontinence. This drug presents a single chiral center in its structure, resulting in the existence of two enantiomers. The (*R*)-enantiomer is the pharmacologically active form, whereas (*S*)-mirabegron is considered a chiral impurity [3]. Consequently, the commercialization of this drug is restricted to its pure enantiomer.

In this context, the objective of this work was to develop an analytical methodology by EKC enabling the chiral separation of mirabegron. This methodology was based on the use of cyclodextrins (CDs) as chiral selectors and DESS as additives in the separation medium. The potential of various neutral and negatively charged CDs was assessed at two pH values (3.0 and 7.0). Among all the CDs tested, sulfobutylether- β -CD at a concentration of 1.25 % (w/v) was selected as the best option. Subsequently, two different DESS in combination with sulfobutylether- β -CD were tested at pH 3.0. The results obtained demonstrated that an increase in the concentration of the DES Choline Chloride-Citric acid enhanced the enantioselectivity of the method. Finally, to demonstrate the potential of the developed EKC method, it was applied (after evaluation of its analytical characteristics), to the determination of mirabegron in a pharmaceutical formulation and in urine samples.

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YO-21

DESIGN OF A STATIONARY PHASE FOR HYDRODYNAMIC CHROMATOGRAPHY AND ITS USES FOR THE SEPARATION AND DETECTION OF INORGANIC NANOPARTICLES

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Nanoparticles (NPs) have been introduced into virtually all industries due to their unique properties [1]. Due to their increasing use, it is not surprising that many of these engineered NPs end up in the environment and eventually enter the trophic chain. [2]

Several techniques could be used for the separation of NPs, such as Field Flow Fractionation (FFF), Size Exclusion Chromatography (SEC), capillary electrophoresis (CE), and Hydrodynamic Chromatography (HDC). [3]

In the current research, a stationary phase based on soda-lime glass beads with controlled porosity has been prepared and fully characterised. The prepared porous material was obtained by thermal treatment of soda-lime glass beads, which, under suitable sintering conditions, creates pores of suitable size between the particles, through which the material containing the NPs to be separated flows. The prototype was further used as a hydrodynamic column in high performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS) for the fractionation of silver nanoparticles (Ag NPs) in complex samples such as enzymatic hydrolysates from seafood (mussels). Under optimal manufacturing conditions, suitable fractionation between Ag NPs and ionic silver was attained with a high degree of repeatability. Analytical recoveries were found to be between 96 and 111% with relative standard deviation lower than 13%.

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YO-22**CHARACTERIZATION OF LIPID PROFILES IN BEE POLLEN AND HONEY BY MALDI-TOF MS**

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Lipids are a diverse group of biomolecules which are essential for various biological functions. They are categorized into multiple families, including phospholipids, sterols, and triglycerides, each with distinct structural and functional roles. The analysis of lipid composition can provide valuable insights into the botanical and geographical origin of different apicultural products such as honey and bee pollen, contributing to their authentication and characterization.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been previously used for lipid analysis in different biological and food matrices. However, its application to bee pollen and honey remains unexplored. Given the complexity and variability of these matrices, optimizing the conditions in both sample preparation and analysis is crucial for obtaining reliable lipid profiles. In this study, different extraction methods were tested to optimize lipid detection in pollen, light honey, and dark honey. In terms of the extraction of the analytes prior to analysis, various parameters were evaluated including solvent selection, solid-liquid and liquid-liquid extraction techniques, extraction time, and the use of assisting devices such as vortex, vibromatic, and ultrasound. For MALDI-TOF MS analysis, different matrices, sample-to-matrix ratios and ionizing agents (sodium, silver, and iodide) were explored to enhance lipid ionization.

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YO-23**COMPARISON OF NADES REMOVAL STRATEGIES FOR HYDROPHILIC AND HYDROPHOBIC EXTRACTS: OBTENTION OF PURIFIED EXTRACTS WITH ENHANCED BIOACTIVITY**

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Natural Deep Eutectic Solvents (NADES) have emerged as promising green alternatives for the extraction of bioactive compounds due to their high extraction efficiency, tunable properties, and low toxicity. However, their presence in final extracts can interfere with downstream applications, especially in the food, pharmaceutical, and cosmetic industries, where solvent-free or purified extracts are required. Despite their growing popularity, the removal or purification of NADES after extraction remains a significant challenge. This study addresses this critical gap by systematically evaluating and comparing multiple cleaning and purification strategies across different types of NADES.

Three NADES systems: two hydrophilic formulations and one hydrophobic NADES were evaluated. These solvents were used to extract flavonoids and carotenoids from *Citrus aurantium* leaves with previously tested neuroprotective activity. Extractions were performed using ultrasound-assisted extraction (UAE) and conventional solid-liquid extraction, and compared to ethanol and water controls.

Four different NADES removal strategies were tested: chemical precipitation, antisolvent extraction, physical destabilization, and solid-phase extraction (SPE). Each method was assessed for its ability to disrupt or separate the NADES matrix and preserve the target bioactive compounds. FT-IR was used to confirm the presence or absence of NADES components post-treatment. Results showed that the effectiveness of each cleaning strategy strongly depended on the type of NADES used.

This work, highlights the crucial importance of post-extraction purification when working with NADES, and demonstrates for the first time a comparative, multi-technique approach tailored to different NADES solvent types. The findings provide a foundational framework for future industrial applications of NADES-based extraction, ensuring both efficiency and extract quality.

YO-24

OPTIMIZING RADIOCHEMICAL SEPARATION FOR ACCURATE MEASUREMENT OF ^{93}Zr IN RADIOACTIVE WASTE USING TK400, ZR AND TBP RESINS

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^{93}Zr is among the difficult-to-measure (DTM) radionuclides important for assessing radiological safety of radioactive waste disposal due to its long half-life and potential radiological impact if leached into the environment [1]. It is a pure beta - decaying long-lived radionuclide produced in nuclear reactors either by neutron activation of stable Zr, or by nuclear fission within the nuclear fuel [2-4]. It is present in waste streams such as spent nuclear fuel, spent resins, stainless steel reactor components, control rods and reactor cladding [5-7]. Currently, the scaling factor method is used to calculate the activity of DTM radionuclides based on the measurement of easy-to-measure (ETM) radionuclides [8]. To define the DTM/ETM scaling factor, the accurate determination of DTM such as ^{93}Zr in waste samples is of paramount importance. For the separation of Zr, the literature has reported the use of ion exchange resins [2],[6],[9] and liquid-liquid extraction [5],[10-11], both of which involve complex handling and generate significant chemical waste. However, in the last decade, extraction chromatography has emerged as an effective and straightforward method for the separation of Zr, resulting in less waste and higher recovery rates compared to other separation methods [12-13]. For the quantification of Zr, Liquid Scintillation Counting (LSC) has been used, but it faces challenges due to the interferences caused by the presence of numerous beta emitting radionuclides [6]. Petrov et al. (2017) were the first to report the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to quantify ^{93}Zr in the presence of high concentrations its main isobaric interferences in ICP MS ^{93}Nb and ^{93}Mo , achieving a removal of these interferences up to five orders of magnitude. This advancement has made ICP-MS a widely used technique for ^{93}Zr quantification [7],[9-13].

This presentation will discuss preliminary results related to the development/optimization of a radiochemical separation method for Zr isotopes based on extraction chromatography using, a combination of different resins TK400/ZR, and TK400/TBP resins, all from Triskem International. TK400 resin has the specificity of retaining ^{93}Nb and ^{93}Mo , the main isobaric interference of ^{93}Zr in ICP-MS, in the presence of highly concentrated HCl [14]. ZR resin showed great selectivity for Zr in the presence of HNO_3 as the loading medium and oxalic acid as the eluting medium [12]. TBP resin is expected to show good selectivity for Zr in the presence of highly concentrated HCl [14]. The capacity of TK400 resin in retaining Nb, Mo and Fe interferences will be tested as well as the ZR resin potential reusability. The chemical recovery of the separation is monitored by ICP MS technique (Thermo Scientific Element II) using the ^{90}Zr and ^{91}Zr isotopes of Zr.

Keywords: Waste characterization, ^{93}Zr , extraction chromatography, TK400 resin, ZR resin, TBP resin, ICP MS

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YO-25**ADVANCING THE PREDICTIVE PERFORMANCE OF GENERALISED RETENTION MODELS: SERIALY COUPLED COLUMNS UNDER HPLC GRADIENT ELUTION****Peiró Vila, Pau; Torres Lapasió, José Ramón; García Álvarez-Coque, María Celia.***Department of Analytical Chemistry, Faculty of Chemistry, Universitat de València, Burjassot, Spain.*

Reversed-phase liquid chromatography (RPLC) remains the leading technique for separating non-volatile compounds, with C18 stationary phases being the most widely used. However, their limited versatility has driven the development of alternative stationary phases and strategies such as serial column coupling. By combining columns with different retention mechanisms, solutes undergo relative retention shifts as they migrate between columns, significantly impacting selectivity. This approach improves chromatographic resolution and reduces analysis time.

As with single-column approaches, method development with serial columns benefits from retention modelling. Generalised models (GEMs) have recently emerged as a holistic alternative to conventional individual solute models (ISMs). Unlike ISMs fitted separately for each solute, where all parameters are solute specific, GEMs integrate both solute-specific and common parameters, the latter capturing the combined effects of column and solvent interactions. In their current formulation, GEMs cannot predict changes in elution order within single-column systems. This study explores and confirms that, in serially coupled columns, where solutes traverse multiple stationary phases and encounter distinct solvent compositions, GEMs can anticipate significant selectivity shifts and even peak inversions.

To test this hypothesis, chromatographic fingerprints of *Melissa officinalis* (lemon balm) extracts were analysed using a single-node gradient design, employing three columns with distinct chemical properties (C18, phenyl, and cyano), both individually and in serial combinations. ISMs and GEMs were constructed for 16 peaks that could be consistently tracked. The predictive performance of GEMs was compared to that of ISMs. A systematic evaluation of selectivity changes in both approaches demonstrated that coupled-column systems enhance the predictive accuracy of GEMs, making them viable for modelling complex separations with improved selectivity control and resolution. These findings broaden the practical application of GEMs in gradient elution chromatography, offering new insights into retention modelling.

YO-26

PARAMETRIC MODELLING OF POLONIUM SOLID-LIQUID DISTRIBUTION COEFFICIENT IN SOILS

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Naturally occurring radioactive materials (NORM) are defined as materials containing naturally occurring radionuclides (NOR), such as uranium or polonium, among others. The accumulation of these materials due to mining and other industrial activities can pose a risk of environmental contamination through the potential leaching of NORM into soils and water sources, threatening ecosystems and human health (García-Tenorio et al., 2015). In fact, in the absence of accidental nuclear events, NORs are the main contributor to the radiation dose received by humans. It is therefore of great interest to study the interactions and mechanisms by which NOR are retained in soils or, conversely, become mobile and reach surface or groundwater. In this context, it is crucial to identify the soil properties that play a key role in soil-NOR interactions, and one of the most widely used parameters to describe soil-NOR interaction is the solid-liquid distribution coefficient (K_d), which is in fact used as an input parameter in radioecological risk assessment models (Sheppard, 2011). Therefore, it is essential to obtain datasets of K_d (NOR) values and to develop parametric models to predict K_d (NOR) values based on soil properties.

For polonium, the analytical determination of K_d (Po), usually performed by alpha spectrometry, remains a challenge due to its relatively short half-life and complex chemical behaviour. This study presents an analytical methodology to quantify K_d (Po) by liquid scintillation counting (LSC) in a collection of thirty extensively characterised uncontaminated natural soils with contrasting edaphic properties.

We initially optimised the measurement conditions to quantify Po in soil-derived solutions by LSC. After quantifying K_d (Po), the most relevant soil properties influencing Po sorption were identified using chemometric tools, such as principal component analysis (PCA) and partial least squares (PLS) regression. Subsequently, a comprehensive search for parametric models using the identified relevant soil properties was carried out. This search was performed using the *glmulti* package in R, which allows an exhaustive exploration of the parametric models that best explain the K_d (Po) parameter, ensuring a balance between robustness and model complexity, also considering the interaction terms between variables (Calcagno, 2010).

This study allows us to successfully predict K_d (Po), based on models ranging from simple parametric equations (i.e. based on specific surface area (SSA) and exchangeable sodium, which explain up to 70% of the variability of K_d (Po)) to more complex equations incorporating up to four variables such as SSA, dissolved organic carbon (DOC), amorphous iron and soil organic matter contents, including interaction terms, which explain about 90% of the variability of K_d (Po).

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YO-27

INTELLIGENT RECOMMENDATION SYSTEM FOR STRUCTURE-ENANTIORESOLUTION RELATIONSHIPS IN HPLC USING CELLULOSE-BASED CHIRAL STATIONARY PHASES

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Chirality is an essential property of molecular structures in biological systems. Due to the high level of stereospecificity in biological processes, enantiomers can have distinct effects on living organisms. Therefore, there is a critical need for the development of reliable analytical methods for analyzing chiral compounds. Currently, liquid chromatography using chiral stationary phases (CSPs) is the method of choice. However, determining the most suitable mobile phase (MP) for a specific CSP continues to be a process that relies on trial and error, which demands extensive experimental work, generates considerable waste, and incurs significant costs; thus, failing to meet the Sustainable Development Goals.

To provide a solution to this problem, artificial neural networks (ANNs) were trained to specifically recommend the best MP for each enantiomer from molecular descriptors and concrete chromatographic information. A novel parameter was proposed, efficient enantioseparation, $EES = f(R_s, k_2)$, where R_s is the enantioresolution and k_2 the retention factor of the most highly retained enantiomer (where k_2 values higher than 20 start penalizing EES relative to the R_s values). The CSP employed was Cell-1 (with the chiral selector tris(3,5-dimethylphenylcarbamate)). A large set of optimized ANNs were trained using a modified version of the Chaotic Neural Network Algorithm with Competitive Learning (CCLNNA) for the optimization of the internal architecture of the ANNs and to perform feature selection (FS) of the molecular descriptors. For the training set, 76 unrelated basic and neutral compounds belonging to 20 pharmacological and agrochemical families were employed. 62 molecular descriptors for each compound, and 9 values of EES obtained employing binary aqueous-organic MP based on acetonitrile (98 - 30 % (v/v)).

The CCLNNA algorithm employs a customized function to find the optimal values of the solution vector. The optimization function is a combination of rules for evaluating the quality of the MP-recommendation (like an analyst in the laboratory).

Excellent recommendation success has been achieved for all the compounds; thus, the proposed system has the potential to become an Intelligent recommendation system (IRS) for MPs for given CSPs.

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YO-28**DISCRIMINATION BETWEEN NATURAL HONEY AND “HONEY” PRODUCED BY SUGAR-SUPPLEMENTED BEES**

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In recent years, honey fraud has taken a special interest in our society, since fraud cases affect the consumer's trust and may cause health issues. In fact, a study performed by the UE called “From the Hives” concluded that 46% of imported honey in the UE was suspected of being adulterated with sugar-based syrups [1], resulting in products being non-compliant with the EU Honey Directive 2001/110/EC [2]. Considering the complexity of the composition of honey and the wide range of products, several fraudulent practices are known nowadays. For example, honeys produced by sugar-supplemented bees don't follow the given definition by the Codex Alimentarius of honey, which is the natural sweetener produced by honeybees from the nectar of plants or secretions from living parts of plants or insects. As a result, such products should be considered as fraudulent. For this reason, the development and improvement of analytical methodologies to address honey fraud is required.

The present contribution aims to characterize and discriminate natural honey from that produced by bees supplemented with sugar during honey flow season. For that purpose, several antioxidant indexes were determined (e.g. total phenolic content and ferric reducing antioxidant power) along with the measurement of physicochemical parameters, including pH, conductivity, sugar content (measured by refractometry and the DNS assay), and water content (measured by refractometry and gravimetry). Additional data consisted of HPLC-UV fingerprinting, elemental profiling (by ICP-MS and ICP-OES, monitoring a total of 22 elements), as well as the polyphenolic profiling by HPLC-MS/MS in multiple reaction monitoring mode (monitoring a total of 39 phenolic compounds). F and t statistical tests, PCA and PLS-DA were used to determine whether differences among natural and supplemented-based honeys were significant.

The two analyzed sample types were significantly discriminated with PLS-DA. Values of sensitivity and specificity were between 70 and 100%, observing classification error values between 0 and 20%. Among the parameters studied, the most discriminant descriptors were the water content, sugar content, some metal ions (such as Cu, Ca, and Sr), and some polyphenols (including hesperidin and *p*-coumaric and chlorogenic acids). In addition, HPLC-UV fingerprints demonstrated to be good chemical markers to discriminate honeys naturally produced and honeys produced by sugar-supplemented bees, observing classification errors of 10%. Thus, the studied properties demonstrated to be good chemical markers to distinguish honeys produced by sugar-supplemented bees and honeys naturally produced, providing straightforward methodologies to address honey frauds, especially those involving fraudulent practices in the hives by sugar-supplemented bees.

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YO-29**VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLS IN FRUIT JUICES****Pólit, María; Palma, Miguel.***University of Cadiz, Cadiz, Spain.*

Fruit juices hold a significant place in the daily diet across various age groups, social classes, and regions due to their nutritional and functional benefits. Most of their effects on human health are associated with their antioxidant content, particularly polyphenolic compounds. This study aimed to validate the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay for antioxidant activity and the Folin-Ciocalteu method for total polyphenol content in fruit juices. A variety of commercial fruit juices—including orange, pineapple, apple, and grape—were analyzed.

The validation of these conventional spectrophotometric methods included assessments of linearity, precision, accuracy, and robustness, in accordance with the guidelines of the International Conference on Harmonization (ICH). The analytical performance of both methods was confirmed across different matrices.

The results demonstrated high precision, and the accuracy tests yielded satisfactory recovery percentages, all within the acceptable range of 80–125%. Both spectrophotometric methods were successfully validated. Their analytical characteristics were established in various juice matrices, and robustness was confirmed under common experimental variations, including changes in sugar content, temperature, and analysis time. In addition, linear ranges were defined for the different juice types.

In conclusion, the methods for determining antioxidant activity and total polyphenol content in fruit juices were successfully validated and proven suitable for application across a range of commercial products.

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YO-30**USING THE AUTOMATED PEAK GROUP ANALYSIS TO DETERMINE PURE COMPONENT DECOMPOSITIONS FOR FT-IR AND RAMAN SPECTRA**

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The automated Peak Group Analysis (autoPGA) is a multivariate curve resolution technique that can be applied to spectral data sets of chemical reaction systems to determine pure component decompositions. Such decompositions into factor matrices containing the pure component spectra and the corresponding concentration profiles can be found according to the Lambert-Beer law.

The autoPGA uses the left/right singular vectors as linear bases for the reconstruction of the concentration profiles/pure component spectra. The linear coefficients are determined as a solution to an optimization problem based on properties aimed at chemically meaningful spectra, such as nonnegativity and smoothness. These additional constraints are applied within the objective function in the form of a sum of weighted functions, where the weighting factors can be chosen by the user according to preference and prior knowledge of the data set.

The existence of isolated peaks in FT-IR or Raman spectra is an essential prerequisite for autoPGA. The algorithm works as follows:

1. Automatic detection of all isolated peaks in the data set.
2. For each peak, autoPGA determines a linear combination of right singular vectors that provides a good approximation of the spectral data in a window containing that peak.
3. autoPGA claims that this linear combination can be interpreted as a pure component spectrum over the entire frequency interval.
4. Repeated detections of similar spectra are grouped into clusters.

The autoPGA algorithm is part of the free software package FACPACK^[3]. The graphical user interface and preset default parameters help with an easy start to using autoPGA.

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YO-31**FAST COCAINE PURITY DETERMINATION IN COMPLEX SEIZED SAMPLES USING NIR SPECTROSCOPY AND CHEMOMETRIC TOOLS**

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Cocaine trafficking and consumption represent a growing global challenge, with record seizures and a significant increase in availability. In the EU, cocaine detections have risen by 376% in the last decade. This trend reflects not only a greater supply but also an increasing sophistication in masking methods, complicating detection, identification, and quantification. Addressing this issue requires analytical methodologies capable of precise cocaine identification and quantification in different matrices, ensuring fast and reliable results. The reference methods for cocaine determination in seized samples rely on gas or liquid chromatography coupled with mass spectrometry, providing high sensitivity and selectivity. However, these techniques require high-cost instrumentation, sample pretreatment, and long analysis times, limiting their applicability for on-site analysis. [1]

In this context, near-infrared (NIR) spectroscopy emerges as a promising complementary alternative to conventional methods. It is fast, non-destructive, and portable, enabling direct cocaine detection in complex samples without extensive pretreatment. Despite its advantages, the presence of cutting substances can cause spectral overlap, necessitating advanced chemometric tools for improved quantification accuracy. [2]

This study evaluates the application of NIR spectroscopy for the rapid, direct, and non-destructive determination of cocaine purity in seized samples. A total of 155 samples were analyzed using liquid chromatography to determine cocaine concentration, along with the most relevant cutting agents. Subsequently, samples were analyzed via portable NIR spectroscopy to obtain their spectra.

The physical properties of the sample set represented the expected complex reality of forensic analysis, with a high degree of diversity in terms of particle size and sample amount. From the compositional point of view, analysis revealed a high degree of complexity of the dataset. Cocaine was represented in the whole range of concentrations, from 0.99 % to 88.05 %, namely levamisole and phenacetin.

The elemental composition of the samples was crossreferenced with the NIR spectra. To that end, adequate spectra pretreatment was applied through a 1st derivative (Savitzky-Golay smoothing, polynomial order 2, window width 3 nm) to account for additive and multiplicative effects. Subsequently, multivariate regression PLS models were created splitting the dataset into calibration and validation subsets (75:25 ratio). First, PLS1 models were tested to quantify cocaine as the main substance of concern, with Root Mean Square Errors (RMSE) of 6.40 %. PLS1 models were also built for two of the most concerning adulterants: levamisole and phenacetine. These models yielded RMSE of 2.93 and 3.38 %, respectively. Based on these promising results, a combined PLS2 model was also trained. In this case, the RMSE values for cocaine, levamisole and phenacetin were 6.55, 3.64 and 3.11 %, respectively. These values were slightly worse than the ones obtained through PLS1, except for phenacetin. However, this model implies a simpler approach that yields more information than the PLS1 models, as one can obtain information about three compounds with only one measurement.

This study shows a real forensic study case of NIR spectroscopy implementation and demonstrates the importance of well-optimized chemometric models for data extraction and interpretation. This tool can provide law enforcement agencies with a portable, affordable, and reliable screening method, minimizing the number of samples requiring chromatographic analysis to only those that are doubtful or of low concentration.

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YO-32**LOCALIZATION ANALYSIS OF TOXIC COMPONENTS IN HIGHER PLANTS BY IMAGING MASS SPECTROMETRY****Yamada, Soichiro¹; Taira, Shu².***¹the United Graduated School of Agricultural Science, Iwate University, Iwate, Japan; ²Graduate school of Agricultural Science, Fukushima University, Fukushima, Japan.*

Food poisoning caused by toxic components in higher plants can be particularly severe, making research into these toxic components a critical issue. Especially, for example, it is known that 22 cases occurred food poisoning across Japan in 2024. One of the cases involved two people who ate meadow saffron, which they had grown in their home gardens, mistaking it for *A. victorialis*, a type of wild vegetable, resulting in their deaths. Therefore, rapid and accurate analysis of such toxic components is crucial for public health and food safety. Simultaneous analysis using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has already been established for the qualitative and quantitative determination of toxic components in higher plants. To explore a new way of evaluating these toxic components from a different perspective, we aimed to observe their localization using imaging mass spectrometry (IMS)(1). This method provides new insights by presenting the results not only in numerical or graphical formats but also as spatial images, thereby adding visual information.

In this study, we focused on seven toxic components derived from three plants species that have caused multiple fatalities in Japan. Specifically, we examined aconitine, mesaconitine, and hyaconitine from aconite; lycorine, galantamine, and galanthaminone from narcissus; and colchicine from meadow saffron. The target plant parts were the leaves, stems, and roots of each plant. It is necessary to increase the spatial resolution of imaging to a level where differences in distribution at the level of microscopic plant tissues and cells can be detected. Thus, we compared matrix-assisted laser desorption/ionization (MALDI) with nanoparticle-assisted laser desorption/ionization (Nano-PALDI)(2) and selected the method that provided sufficient intensity due to its low noise levels in the low molecular weight region and high spatial resolution.

As a result, IMS was successfully applied to all three plants. We used MALDI for aconite, which contains relatively high molecular weight toxic components, and Nano-PALDI for narcissus and meadow saffron, which contain relatively low molecular weight toxic components. Hyaconitine was strongly detected in aconite, galantamine and galanthaminone in narcissus, and colchicine in meadow saffron. However, aconitine and mesaconitine were less strongly detected in aconite, and lycorine was less strongly detected in narcissus. These variations may be due to differences in ionization efficiency in the analytical method, or to variations in content depending on the site, species, or community from which the specimens were obtained. Further analysis of specimens from different species and communities is necessary to validate the applicability of this method. In all plants, we observed that toxic components were mainly localized in the subterranean parts. Additionally, we confirmed the detailed distribution of each toxic component: aconitine was primarily localized in the inner tubers of aconite, galantamine and galanthaminone in the outer bulbs of narcissus, and colchicine in the outer bulbs of meadow saffron. In conclusion, although many natural toxins are low molecular weight compounds, we were able to perform visualization analysis on all plants using Nano-PALDI IMS, which excels at measuring low molecular weight components, even those that are difficult to detect with MALDI IMS. Our findings suggest that the localization of toxic components tends to align with the structural characteristics of each plant.

As future work, identifying the spatial localization of compounds in plant tissues is expected to provide new insights not only into the causes of food poisoning but also into the synthesis of various endogenous substances and their functional roles.

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YO-33

A FAST-SCREENING CHOCOLATE AUTHENTICATION BASED ON SMARTPHONE DIGITAL IMAGING AND CHEMOMETRICS

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Cocoa beans are the primary raw material in chocolate production and are rich in lipids, proteins, carbohydrates, and bioactive compounds, including polyphenols and methylxanthines. These constituents play a fundamental role in defining sensory, physicochemical, and functional properties of chocolate, influencing its flavor, color, texture, and health benefits (1). The chemical composition and quality attributes of chocolate and other cocoa-derived products are influenced by various factors, including cocoa content, genetic variety, post-harvest processing, manufacturing techniques, cultivation conditions, and geographical origin. These variables not only affect the nutritional and organoleptic features but also influence its availability, market value, and consumer acceptance (2).

Traditional analytical methods for characterizing quality-related compounds, such as antioxidants and fatty acids in cocoa matrices, rely on chromatographic and spectroscopic techniques (3–6). These methods typically require sample pretreatment, clean-up, and derivatization steps, making them time-consuming and resource-intensive. Therefore, the trend is towards developing sustainable analytical methods, implementing emission reduction strategies, and using non-destructive techniques. Image analysis has emerged as a promising tool for obtaining information about chemical composition and quality and is increasingly used to discriminate and classify various samples and features (7,8). This study aims to assess the feasibility of employing digital imaging, using a standard smartphone as the tool for chemical screening analysis on chocolate samples. Additionally, it investigates the effectiveness of color space data as a discriminative measure to classify chocolate based on its origin, variety, or cocoa content through chemometric methods.

Ninety-one chocolate samples from various geographical origins, varieties, cocoa percentages, and cultivation methods were analyzed. Three different smartphone devices were used, and experimental parameters such as type, illumination, camera position, and sample holder were evaluated. Images were stored in PNG format to prevent information loss. Image processing, color correction, image segmentation, and chemometric analysis were conducted using Rstudio. The extracted color space data were analyzed using supervised and unsupervised methods, including principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA), among others.

The chemometric results revealed patterns associated with color space features and chemical properties, such as polyphenol and cocoa content. These findings suggest a promising application for an inexpensive and straightforward screening method to characterize chocolate samples.

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YO-34**EFFICIENT MULTI-CLASS DETERMINATION OF MYCOTOXINS AND PESTICIDE RESIDUES IN FISH FEED AND BEEKEEPING PRODUCTS USING UAE-QuEChERS-BASED EXTRACTION AND LC-MS**

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Mycotoxins and pesticides in food and animal feed pose health risks. Aquafeeds increasingly use plant-based ingredients, raising contamination concerns. Maximum levels for certain mycotoxins are regulated under Recommendation (EU) 576/2006 [1], and pesticide residues by Regulation (EC) 396/2005 [2] in fish feed. Bee pollen and honey offer nutritional benefits but are prone to contamination. Regulation (EC) 396/2005 [2] sets maximum residue limits of pesticides in honey, but no mycotoxin limits exist. Moreover, no legislation regulates those contaminants in bee pollen. Implementing rigorous quality control and promoting sustainable beekeeping practices are essential to ensure product safety and quality.

This work proposes a multi-analyte method for the determination of 28 compounds, including 10 pesticides and 20 mycotoxins, in fish feed, bee pollen, and honey. Sample treatment was optimized for each matrix using ultrasound-assisted extraction (UAE) combined with a QuEChERS approach using acetonitrile as the extractant. For fish feed, a dispersive solid-phase extraction (dSPE) cleanup using PSA and C₁₈ sorbents was necessary. For beekeeping products, extraction included n-hexane and an additional re-extraction step for bee pollen; dSPE cleanup was not required in these matrices.

Liquid chromatography (LC) separation was performed using a Hypersil GOLD aQ column (100 × 2.1 mm, 1.9 µm). Detection was performed using Q-Exactive Orbitrap-MS for fish feed and triple quadrupole (QqQ)-MS for beekeeping products. Methods validations were carried out according to the SANTE/11312/2021 guideline [3], with determination coefficients above 0.99 for matrix-matched calibration curves of all compounds in fish feed. Procedural calibration curves were evaluated for honey and pollen using weighted least squares (WLS) regression to correct heteroscedasticity, achieving good linearity. Limits of quantification (LOQs) ranged as follows: for fish feed, from 0.06 µg/kg (Pirimiphos-methyl) to 100 µg/kg (Ochratoxin α); for honey, from 0.11 µg/kg (ENNA1) to 31.3 µg/kg (3-AcDON); and for bee pollen, from 1.30 µg/kg (ENNA) to 289 µg/kg (DON). Trueness was between 70 and 120% for most analytes, and satisfactory precision (RSDs < 20%) was achieved in all cases.

The validated method was applied to 36 fish feed samples. Among pesticide residues, pirimiphos-methyl was the most frequently detected (75%). Among mycotoxins, enniatin B and B1 were the most prevalent (81%). Non-targeted analysis also revealed the presence of piperonyl butoxide in 81% of samples. Ochratoxin α was detected at the highest concentration (202 µg/kg). A total of 28 honey and six pollen samples were analyzed. In honey, acetamiprid and azoxystrobin were detected in 29% and 32% of samples, with maximum concentrations of 16.2 µg/kg and 1.57 µg/kg, respectively. No pesticide residues or mycotoxins were found in bee pollen. Importantly, none of the detected contaminant concentrations exceeded the maximum limits established by EU regulations.

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YO-35

EVALUATION OF THE DIETARY EXPOSURE TO PERCHLORATE VIA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Perchlorate is a ubiquitous inorganic anion that can contaminate food through both natural and anthropogenic sources, such as the use of fertilizers or the degradation of hypochlorite ion used for water disinfection. This contaminant is associated with the inhibition of thyroid iodine uptake and thyroid hormone production, which can lead to various health issues, including chronic hypothyroidism in children (European Food Safety Authority (EFSA), 2017) Given these risks, regulatory authorities have established maximum residue limits (MRLs) for perchlorate in various food groups to ensure consumer safety.

The European Food Safety Authority (EFSA) has conducted dietary exposure assessments on perchlorate, highlighting its presence in different food categories. The European Commission has responded by setting maximum legal limits for perchlorate in multiple food matrices, including teas and herbs used for infusions ($750 \mu\text{g kg}^{-1}$), leaf vegetables ($500 \mu\text{g kg}^{-1}$), *cucurbitaceous* and kale ($100 \mu\text{g kg}^{-1}$), fruits and vegetables ($50 \mu\text{g kg}^{-1}$), infant food ($20 \mu\text{g kg}^{-1}$), follow-on-formula and cereal-based products ($10 \mu\text{g kg}^{-1}$) (European Commission, 2023)

The aim of this research is to develop an accurate and sensitive methodology for the determination of perchlorate in food. The proposed method involves an extraction step using acetonitrile, followed by a clean-up step with dispersive solid-phase extraction (dSPE) utilizing C18 and graphitized carbon black (GCB) sorbents. The final extract is analyzed using liquid chromatography with a HILIC-OH5 column coupled to a tandem mass spectrometer (LC-MS/MS).

Compared to conventional methods, the developed methodology presents several advantages. Traditionally, perchlorate analysis has been performed using ion chromatography (IC) with conductivity detection or IC-MS. While these methods are effective, they often require complex sample preparation and are prone to matrix interferences. The proposed approach, based on QuEChERS extraction combined with dSPE clean-up, offers a faster, more cost-effective, and environmentally friendly alternative by reducing the use of hazardous solvents and minimizing sample handling.

The method validation was performed in accordance with official guidelines for analytical quality control and method validation. Linearity, limit of quantification, recovery, and precision were evaluated using different food matrixes, demonstrating robust analytical performance. The results indicate that the developed method provides satisfactory linearity ($R^2 \geq 0.99$), as well as excellent precision ($\text{RSD} \leq 18\%$) and accuracy (69 - 119%) for perchlorate determination in food. Several food samples from local markets were analyzed, all showing concentrations below the MRL set by European legislation. However, a significant percentage of the analyzed samples contained quantifiable levels of perchlorate, reinforcing the importance of continuous monitoring. Furthermore, the dietary exposure assessment suggests that certain population groups may be at greater risk of exceeding the TDI, emphasizing the need for regulatory surveillance and risk management strategies.

In addition to the development of an analytical method, this study includes a dietary exposure assessment of perchlorate based on food consumption data and contaminant levels in various food categories. The assessment was conducted considering the Tolerable Daily Intake (TDI) established by the European Union of $0.4 \mu\text{g kg}^{-1}$ (b.w) day^{-1} , using the hazard index (HI) as a risk indicator, providing a measure of potential health risks associated with perchlorate intake.

In conclusion, a precise, sensitive, and reliable analytical methodology has been developed for the determination of perchlorate in food. The method improves upon existing techniques by offering greater efficiency, reduced sample preparation time, and lower solvent consumption, making it a valuable tool for routine food safety monitoring and regulatory compliance. Additionally, the dietary exposure study highlights the importance of continuous assessment of perchlorate intake to ensure consumer protection and support evidence-based regulatory decisions.

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YO-36

LEVERAGING VOLATILE ORGANIC COMPOUND PROFILES FOR RAPID DIFFERENTIATION OF STINGLESS BEE AND HONEYBEE HONEYS USING HS-GC-IMS AND ULTRA-FAST GC-FID COUPLED WITH CHEMOMETRICS

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Stingless bee honey is gaining prominence for its distinctive flavour and health benefits. It differs compositionally from conventional honeybee (*Apis mellifera*) honey, particularly in its content of volatile organic compounds (VOCs). The increasing demand for high-quality and authentic honey products underscores the need for reliable analytical methods to authenticate and differentiate honey varieties—especially between stingless bee and conventional honeybee honeys.

This study investigates the potential of VOCs as chemical markers for distinguishing stingless bee honey from regular honeybee honey. Additionally, it evaluates the effectiveness of two rapid, non-destructive analytical techniques—Headspace Gas Chromatography–Ion Mobility Spectrometry (HS-GC-IMS) and Ultra-Fast Gas Chromatography with Flame Ionization Detection (Ultra-Fast GC-FID)—combined with chemometric analysis as efficient classification tools.

A total of 21 stingless bee honey and 24 honeybee honey samples were analysed using Headspace Solid-Phase Microextraction Gas Chromatography–Triple Quadrupole Mass Spectrometry (HS-SPME-GC-TQMS) to establish comprehensive VOC profiles. Distinct patterns were observed, with several VOCs—including alcohols, aldehydes, ketones, and esters—serving as potential discriminatory markers. Stingless bee honey consistently displayed unique VOCs, reinforcing its distinctive chemical identity.

Multivariate data analysis, including Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), applied to data from the HS-GC-IMS data, demonstrated significant discrimination between the two honey types. Notably, Discriminant Function Analysis (DFA) applied to Ultra-Fast GC-FID data confirmed the clear grouping of samples according to their entomological origin.

The integration of these analytical techniques with chemometric tools provides a reliable, rapid, and cost-effective approach for honey authentication. These findings highlight the potential of fast VOC-based analytical methods combined with chemometrics as powerful tools for honey classification, quality control, and fraud detection, offering promising applications in both regulatory and commercial contexts.

YO-37**DRY ASSAYS FOR THE DISCRIMINATION OF IRRADIATED FOOD SAMPLES USING A REACTION-BASED OPTICAL FINGERPRINTING STRATEGY**

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Fingerprinting methods have become a powerful tool for discriminating and classifying samples by their unique pattern without identifying particular components. These methods find application in diverse fields such as environmental monitoring, food quality control, pharmaceuticals, forensics, etc.

Food irradiation has gained popularity in many countries due to various advantages, such as suppressing pathogens, reducing spoilage, extending shelf life, stimulating and inhibiting sprouting. Since different purposes require different doses, irradiating with a wrong dose can lead to undesirable consequences. To avoid fraud, post-irradiation monitoring can be carried out by checking the absorbed doses declared by the manufacturers. Therefore, the development of reliable and straightforward methods for determining post-irradiation doses has crucial importance. Fingerprinting methods can provide a solution to this problem as they are sensitive to tiny differences in the chemical composition of samples.

Previously we proposed a reaction-based optical fingerprinting strategy that involves redox indicator reactions of carbocyanine dyes conducted in the presence of a sample [1, 2]. The technique is based on the photographic measurement of optical signals in several spectral ranges over a certain period of time and then analyzing the obtained data using machine learning methods. Periodical measurements increase the amount of information obtained from the sample, enabling their more precise characterization. Our current research focuses on developing dry test systems, which can be used in simple and express field testing. A carbocyanine dye solution, buffer, and oxidizer are added to the wells of a fluorimetric plate and dried in the oven at 70°C to obtain the dry assay form. To conduct the indicator reaction, a few microliters of 95% ethanol and an aqueous solution of sample are added to the wells, and the reaction is followed in a usual way. For the discrimination of samples, we use principal component analysis (PCA), linear discriminant analysis (LDA), softmax regression (SR), and *k*-nearest neighbors algorithm (kNN).

We have demonstrated the possibilities of the proposed approach through the discrimination of doses in potato tubers, turkey liver, and codfish irradiated with 1 MeV accelerated electrons. The samples were extracted by water for 24 h at room temperature, and the resulting mixtures were introduced into the indicator reactions. By combining the results for the most efficient reactions, it was possible to confidently discriminate between samples irradiated with doses from 0 to 1 kGy. Validation using LDA and SR showed a discrimination accuracy of approximately 90%.

The proposed dry assay strategy offers significant advantages over existing methods in terms of simplicity, short analysis time, inexpensive instruments, easy protocol, minimal sample preparation, and small amount of sample and reactants required for analysis.

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YO-38

SUSTAINABLE PRODUCTION OF GRAPHENE-BASED ELECTROCHEMICAL SENSORS USING INKJET PRINTING AND THEIR REVERSIBLE THERMAL TUNABILITY

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The societal demand for disposable, portable and miniaturized electroanalytical sensors has driven the development of printing-based manufacturing techniques for decades. The potential for low unit costs at industrial quantities has been the key catalyst behind some of the most successful innovations in point-of-care sensor technology, such as the electrochemical sensing of blood glucose.¹ Screen printing of graphite-based carbon pastes into thick film pattern of several μm thickness has been established themselves as the standard manufacturing technique for disposable electroanalytical sensors.² Carbon materials are regarded as sustainable and environmentally friendly, particularly in comparison to the mining and processing of precious sensing metals such as gold (Au) and platinum (Pt). In addition to their excellent intrinsic conductivity and biocompatibility, sensors based on graphitic materials are ideally suited for large-scale detection of clinically and biochemically relevant analytes. For many detection purposes, however, the sensitivities and selectivity achievable with graphite are insufficient, and other carbonaceous materials, such as graphene (G) have been introduced to improve sensing performance. The lack of precision and design flexibility resulting from the use of a mask makes screen printing of such graphite alternatives unsustainable and prodigal, particularly at an industrial scale. A large portion of the material is lost in the form of excessively thick electrodes and as ink residues left on the mask. Moreover, any modification to the pattern design requires a new master pattern of the screen.

Therefore, digital and mask-less deposition techniques, such as drop-on-demand inkjet printing (IJP), are increasingly replacing traditional mask-based methods (**Fig.1**). Highly reproducible picolitre droplets (e.g. of graphene-based inks) are jetted on-demand using piezo-electric driven print heads at kHz frequencies, giving generally better precision and accuracy than screen printing, with optimally zero material waste in a reasonably short time.³ Subsequent thermal processing, typically performed in ambient air, is necessary to remove ink solvents, decompose stabilizers and convert the as-printed ink pattern into solid, conductive and well-adhered graphene electrodes.

In this young oral talk, we will introduce sub-micrometer-thick graphene-based electroanalytical sensors, fabricated using the sustainability of large-scale inkjet printing. Defined post-print thermal treatments result highly reproducible electrodes and there voltammetric characterization demonstrates adsorption-driven enhanced sensitivities and selectivity for the detection of benchmark biomolecules (e.g., uric acid (UA)), outperforming state-of-the-art carbon materials like graphite and glassy carbon. We will show how the boosted sensitivities and selectivity of the IJP sensors can be repeatedly switched "on" and "off" on-demand by rapid and straight-forward reheating in ambient air and vacuum, respectively. Due to the rapid modulation between both sensing states, the electrode is proposed for potential use in antioxidant detection, allowing i) quantitative measurements of a global signal in the "off" state and ii) qualitative identification of the contributing compounds in the "on" state (**Fig.2**).⁴ Minimizing material waste for mass production of high performance sensors through inkjet printing, combined with repetitive modulation of the sensor performance, provides a disposable and flexible sensing device that opens up sustainable and interesting opportunities for future point-of-care sensing applications.

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YO-39**A MULTIPLEX METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF CONJUGATED SACCHARIDE IN GLYCOCONJUGATE VACCINES: MenACWY AS KEY STUDY****Santostefano, Giovanni¹; De Ricco, Riccardo²; Corrado, Alessio²; Di Bussolo, Valeria¹.**¹*Department of Pharmacy, University of Pisa, Pisa, Italy;* ²*GSK, Siena, Italy.*

Advancements in vaccine research have significantly enhanced human health by preventing many bacterial and viral infections. In this context glycoconjugate vaccines, which combine a polysaccharide (PS) or oligosaccharide (OS) antigen with a carrier protein, have been developed and commercialized [1]. Since only the conjugated portion is highly immunogenic, a key challenge in their production is the accurate monitoring of saccharide content throughout the entire process. Moreover, distinguishing between free and conjugated (or total) saccharides is essential to ensure regulatory compliance [2, 3]. Therefore, a robust analytical platform is essential to quantify PS/OS during various production stages, including the drug substance (DS) and the final drug product (DP). Current methods, such as HPAEC-PAD and colorimetric techniques require multiple sample manipulation steps (conjugate precipitation, ultrafiltration, and solid phase extraction), which are time-consuming and material-intensive [4]. This project aims to develop new, faster, and more accurate analytical platforms for saccharide quantification in multivalent glycoconjugate vaccines. To achieve this goal, various analytical tools were explored, incorporating both physico-chemical and immune-based assays, with MenACWY vaccines as case studies. The first attempt to analyse all four antigens in a one-shot analysis was performed using capillary electrophoresis (CE) techniques. Micellar electrokinetic chromatography with UV-Vis detection (MEKC-UV) showed good results in the oligosaccharides and their respective free saccharides separation at DS level. However, it showed significant limitations when all four antigens were analysed simultaneously. Moreover, the use of UV-Vis detection did not allow for accurate quantification of low levels of free saccharide, which lack suitable chromophores. To increase the sensitivity of our tools, we shifted our focus to immuno-based approaches, evaluating a multiplex bioassay. This method enables the selective and sensitive detection of all serotypes simultaneously using color-coded magnetic beads coupled with serotype-specific antibodies and a phycoerythrin conjugate species as reporter [5]. To this end, several configurations of the sandwich assay were designed and developed, aiming to create a sensitive method capable of detecting all four antigens simultaneously. Very promising results were obtained for the conjugate attribute, while the quantification of free and total saccharides together is still ongoing.

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The project is aimed to develop faster and more accurate analytical platforms applied on multivalent glycoconjugate vaccines. Current HPAEC-PAD methods for the various attributes of saccharide content are time-consuming and require extensive sample manipulation, so we selected other alternative tools. Utilizing Luminex technology, which leverages the multiplex approach through color-coded magnetic beads, we yielded great results for the detection of conjugated saccharides, demonstrating promising efficacy. Meanwhile, the development of a method for quantifying both free and total saccharides remains under investigation.

YO-40**EXPLORING STRESS GRANULES IN EXOSOMES AS NOVEL BIOMARKERS FOR EARLY ALZHEIMER'S DISEASE DETECTION****Shi, Jiayi; Rossi, Rosanna; Pividori, Maria Isabel; Marti, Mercè.***Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, Barcelona, Spain.*

Alzheimer's disease (AD) is the most prevalent form of dementia, accounting for 60–80% of cases. It poses a major socioeconomic burden, largely due to delayed diagnosis that limits opportunities for early intervention (1). Stress granules (SGs) are membraneless cytoplasmic structures that form in response to cellular stress, temporarily acting as protective compartments that sequester mRNA and translation-related proteins to help cells manage adverse conditions (2). Recent studies have shown that chronic stress in AD promotes the formation and persistence of SGs, with their abnormal accumulation correlating with disease severity.

Key components of SGs include RNA-binding proteins such as TIA-1 and G3BP1 involved in the formation of SGs. Normally, SGs disassemble spontaneously upon stress relief (2). However, prolonged SGs formation due to chronic stress disrupts RNA homeostasis, impairs translation regulation and interferes with cellular stress responses. Notably, SGs have been linked to the pathological aggregation of tau protein, a hallmark of AD (3). Exosomes are extracellular vesicles ranging from 30–100 nm in size, produced in the endosomal compartment under both physiological and pathological conditions by a wide variety of cells (1). A crucial aspect of exosomes is their detectability in multiple biological fluids, including blood, saliva, urine, cerebrospinal fluid, semen, and amniotic fluid, which makes them particularly interesting for minimally invasive procedures (4). In this study, SGs associated to exosomes as novel potential biomarkers for early AD detection are explored by analysing SG-associated proteins and RNAs using the SH-SY5Y neuroblastoma cell line as a model of AD.

RNAs are extracted under different stressed and non-stressed conditions and analysed via PCR for the presence of microtubule-associated protein tau (MAPT) mRNA, a key protein associated with AD pathology (1), along with TIA-1 and G3BP1 mRNAs, core SG components. A multiplex analysis of these three proteins is shown. Among the different combinations tested, the multiplex of tau, TIA-1, and G3BP1 yielded the most significant and relevant results for studying the relationship between SGs and AD. Preliminary findings confirmed the presence of tau, G3BP1, and TIA-1 in all experimental conditions under stress condition. These promising results establish a correlation between tau protein and SGs, supporting their potential use as biomarkers for AD, paving the way for the development of novel in vitro diagnostic tests (ITDs) for early-stage AD detection.

This study opens new avenues for early AD diagnosis by linking stress granules to tau pathology and validating their presence in extracellular vesicles. The potential to detect these biomarkers in exosomes could revolutionize diagnostic approaches, enabling non-invasive, cost-effective, and accessible testing in liquid biopsies.

Keywords: Alzheimer's disease, stress granules, exosomes, tau protein, liquid biopsy, non-invasive in vitro diagnostic.

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YO-41

AN EXTENSIVE TARGETED METABOLOMICS ASSAY FOR CLINICAL METABOLITE PROFILING IN SERUM AND PLASMA SAMPLES

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Background and objectives: Metabolomics is a powerful tool for studying the interaction between genetics and exposome factors in human health. However, due to the complexity of the human metabolome, novel methodologies are needed. With an expanded coverage of clinical metabolites and improved accuracy of metabolite quantification, this work shows the optimization and validation of a novel liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) based targeted metabolomics assay using derivatization and high-throughput metabolomics to analyze serum and plasma samples.

Materials and Methods: The methodology enables, for the first time, a simultaneous characterization of 721 metabolites from 20 different analyte groups. Sample preparation includes two derivatizations: phenyl isothiocyanate for amino acids, biogenic amines, and lipids, and 3-nitrophenylhydrazine for organic acids. Analysis was conducted on a reverse-phase UHPLC-6500 QTRAP system and direct flow injection MS (DFI-MS) in both positive and negative ionization modes to separate metabolites across four methods: Panel A (amino acids, biogenic amines), Panel B (organic acids), and DFI 1 and DFI 2 (lipids). Multiple reaction monitoring (MRM), in combination with isotopic standards and multi-point calibration curves, is used to detect and absolutely quantify the targeted metabolites. The assay has been adapted to a 96-well plate format to enable automated, high-throughput sample analysis. Data quantification is performed via an in-house web-based tool called LC-autofit, providing analysis and reporting within a few hours.

Results: This assay was satisfactorily validated in terms of linearity, recovery, matrix effects, and specificity, limits of quantification and detection, and intraday and interday precision. Limits of detection were determined to range from 1.4 nM to 10 mM, recovery rates were from 80% to 120%, and quantitative precision were within 20%. Using NIST SRM 1950 plasma standard, LC-MS/MS metabolite concentrations were found to be within 15% of NMR quantified levels. Additionally, it was successfully applied to a Mediterranean dietary intervention study. Further exploration of the data could provide more insights to metabolites linked to cognitive decline and brain health.

Conclusions: This methodology was designed to work using a 96-well format to support high-throughput automation. With as little as 40 µL of serum/plasma sample, simplified sample preparation and HPLC separation with shorter run times, the method permits a more affordable, accessible, sustainable, and automatable comprehensive quantitative metabolomics analysis that can be applied to large-scale clinical and nutritional studies.

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YO-42

DEVELOPMENT OF NEW SELECTIVE EXTRACTION AND HILIC SEPARATION METHODS FOR THE ANALYSIS OF hCG GLYCOFORMS AT THE INTACT LEVEL

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Human chorionic gonadotropin (hCG) is the key hormone of pregnancy. This protein is made up of two non-covalently linked subunits (hCG α and hCG β) and has multiple N- and O-glycosylation sites. Glycosylation is an enzymatic reaction in which an oligosaccharide chain is covalently bonded to a molecule, leading, in the case of a glycoprotein, to the formation of various glycoforms. For hCG, the glycosylation profile varies throughout pregnancy but also characterizes certain pregnancy-related pathologies (preeclampsia, choriocarcinoma, miscarriage)¹. Therefore, the objective is to characterize the glycoforms of hCG. To achieve this, it is necessary to develop a method for analyzing intact hCG glycoforms as well as an extraction method for processing complex biological samples.

Analyzing intact glycoforms using liquid chromatography coupled with mass spectrometry (LC-MS) preserves crucial information, particularly the structure and number of glycoforms. Over the past decade, hydrophilic interaction liquid chromatography (HILIC) has emerged as a highly promising separation method for the analysis of intact proteins². In our laboratory, a method based on a HILIC separation using TFA as ion-pairing agent in the mobile phase and a detection with a quadrupole time-of-flight tandem mass spectrometer (HILIC-QTOF) was developed³. It was observed that TFA induced a significant suppression effect during ionization. This is why, an alternative ion-pairing agent, difluoroacetic acid (DFA), was evaluated. Moreover, a higher mass resolution spectrometer, a quadrupole-Orbitrap (Exploris 120), was used, maintaining the nature of the stationary phase (Accucore amide, 150 x 2.1 mm, 2.6 μ m, 150 Å), the mobile phase flow rate, and the analysis temperature. Both urinary and recombinant hCG samples were analyzed. DFA enabled the detection of many more glycoforms, especially hCG β . Next, several parameters were tested, including DFA concentration, injection volume, and mobile phase gradient considering two main criteria: mass spectrometry signal intensity and glycoform separation resolution. The final method was then assessed for its analytical performance, including linearity, quantification limits, retention time repeatability, and peak area consistency.

However, to analyze biological samples which contain numerous other molecules, including proteins, often at much higher concentrations than hCG, a selective extraction step of all hCG glycoforms is required before separation and detection. Selective solid-phase extraction (SPE) was thus contemplated. One studied strategy was based on the synthesis of immunosorbents grafted with anti-hCG antibodies and the evaluation of their potential to extract the largest possible number of different hCG glycoforms. A second studied strategy was the synthesis and characterization of sorbents functionalized with aptamers. Oligonucleotide aptamers, synthetic DNA or RNA sequences composed of several dozen to a hundred nucleotide bases, are already recognized as valuable tools for the selective extraction and concentration of target compounds in complex samples. More recently, peptide aptamers, which are short sequences of about ten amino acids, have also been developed. Oligonucleotide and peptide aptamers targeting hCG have previously been described in the literature for sensor development^{4,5}. In this work, these sequences were evaluated for the first time in SPE. Thus, this study compared SPE procedures using immunosorbents and both kinds of aptamer-based sorbents for hCG glycoforms extraction.

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YO-43**A NOVEL INDIVIDUALIZED CELL TRAPPING DESIGN FOR NP DETERMINATION IN EXPOSED Caco-2 CELLS BY LA-ICP-MS**

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Nanotoxicological research at the single cell level has captivated the scientific's community attention in recent years. Since the boom of nanotechnology, many nanomaterial (NM)-based products have been introduced into the market. Nanomedicine and nanobiotechnology have gain considerable attention, specially for the use of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) since they hold potent antimicrobial and antiviral properties. The incorporation of NPs into medical devices, as well as in textiles, cosmetics and food items, has led to an increase in the direct contact to humans. At this point, NPs can penetrate the body via ingestion, inhalation, and dermal permeation and ultimately interact with cells. Understanding the NP-cell interaction is key for elucidating potential toxicological effects.

In the present study, the applicability of a microwell array for individual cell trapping and Ag and AuNP determination by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has been investigated. It is widely accepted now that a cell population exhibits great heterogeneity. For this reason, a method that can provide information on an individual cell basis is of great importance. A silicon master mold fabricated through photolithography was employed to prepare a polydimethylsiloxane (PDMS) replica-molding. Microwell characteristics are: 25 μm diameter, 100 μm depth, and 80 μm well-to-well distance. Parameters related to the fabrication of the PDMS mold, including PDMS-crosslinking mass, spin coater settings, and curation temperature and time, were optimized. In addition, the process of cell trapping within the microwells included the use of a custom-built CO_2 chamber to facilitate deposition. Human colon adenocarcinoma (Caco-2) cells were firstly exposed to Ag and AuNPs for 24 hours, then stained, and finally trapped into the microwell array. To evaluate the cell distribution through the microwells, the mold was examined under a microscope using a DAPI filter. Under optimum conditions, the percentage of microwells containing exclusively one individual cell was approximately 60 %, 23 % contained two cells, and 16 % contained more than two cells. Furthermore, LA-ICP-MS determination was conducted.

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YO-44**ANALYTICAL STRATEGIES TO EVALUATE CRITICAL QUALITY ATTRIBUTES (CQAs) OF MODIFIED mRNA IN BIOTHERAPEUTIC DEVELOPMENT****Losada-Baranera, Mar; Gotor, Gemma; Fornaguera, Cristina; Borrós, Salvador.***Institut Químic de Sarrià - Universitat Ramon Llull, Barcelona, Spain.*

Messenger RNA (mRNA) is essential for therapeutic applications, including vaccines and gene therapies, where the evaluation of critical quality attributes (CQAs) like sequence integrity, purity, and stability is crucial. This study focuses on analyzing mRNA CQAs using Ultra-High Performance Liquid Chromatography with UV detection (UHPLC-UV) and Mass Spectrometry (MS). UHPLC-UV is employed to evaluate mRNA content and integrity, while enzymatic digestion with specific endonucleases, followed by UHPLC-MS, enables precise detection of structural features such as 5' capping, nucleotide modifications, and poly(A) tail length [1]. These methods offer complementary insights into the modified mRNA structure, going beyond the assessment of translational efficiency. By combining UHPLC-UV and MS, this study provides a comprehensive framework for mRNA quality control, ensuring stability, purity, and efficacy in therapeutic applications [2].

Critical Quality Attributes (CQAs) for mRNA refer to the key properties that must be monitored and controlled to ensure the safety, efficacy, and consistency of the mRNA product, particularly in therapeutic or vaccine applications. These attributes impact the overall performance of the mRNA, including its stability, translation efficiency, and immunogenicity [3,4].

Different analytical workflows have been developed. The first one consists on the total digestion to nucleotides. In this approach mRNA is completely digested with nuclease P1, an endonuclease that cleaves single-stranded RNA into individual nucleotides, generating a nucleotide pool that is analyzed via UHPLC-QQQ-QTrap. This targeted method (MRM) provides a sensitive detection for different nucleotide molecules.

The second workflow Modified mRNA is partially digested with endonucleases that cleave specific nucleotides, such as RNase T1 (G-specific), RNase 4 (U/A and U/G-specific), or RNase A (pyrimidine-specific). The cleaved products are analyzed via IP-RP UHPLC-QTOF with Data-Dependent Acquisition (DDA), where the oligonucleotide's molecular weight is confirmed by MS scan, and the sequence is evaluated with the MS/MS fragmentation spectra.

The third workflow is for Intact mRNA. A UHPLC-UV method has been developed for analysing intact mRNA using Ion-Pair Reversed-Phase Chromatography (IP-RP) with a specific wide-pore column. The addition of TEA in the mobile phase facilitates the retention of intact modified mRNA, which is detected at 210 nm. Various mRNA standards were evaluated to determine the method's linear range (1.5 to 150 ng/μl) and sensitivity. This method is applicable for quantifying mRNA in applications such as assessing encapsulation efficiency in different types of nanoparticles, where the mRNA is isolated via chloroform extraction.

These complementary analytical workflows provide a robust and detailed framework for assessing the quality of modified mRNA, ensuring structural integrity, purity and stability. The combination of UHPLC-UV and MS-based approaches allows for a more precise characterization of mRNA CQAs, and contributes to the standardization of mRNA analysis methodologies, facilitating the development of mRNA-based biopharmaceutical.

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YO-45

3D HUMAN SKIN EQUIVALENT WOUND MODELS FOR STUDYING MEDICAL-GENERATED NANO-MICROPLASTICS PENETRATION, INTERNALIZATION AND CYTOTOXICITY

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Nano-microplastics (NMPs), ranging from nanometers to micrometers, are ubiquitous in the environment and pose significant health risks. Nanoplastics (NPs) are defined as particles smaller than 1 μm , while microplastics (MPs) range from 1 μm to 5 mm (1). In wound care, NMPs can be released from medical products such as bandages, surgical gloves, sutures, and dressings, which are commonly made of synthetic polymers (e.g., polyethylene, polypropylene, polyurethane, and polyvinyl chloride) (2). These materials degrade over time, shedding tiny plastic particles that may interfere with wound healing. The skin, consisting of the epidermis, dermis, and hypodermis, serves as a natural barrier, limiting the penetration of these particles and any toxic molecules they may carry (3). Due to their small size, NMPs have a high surface area and surface energy, facilitating deep biological penetration and potential

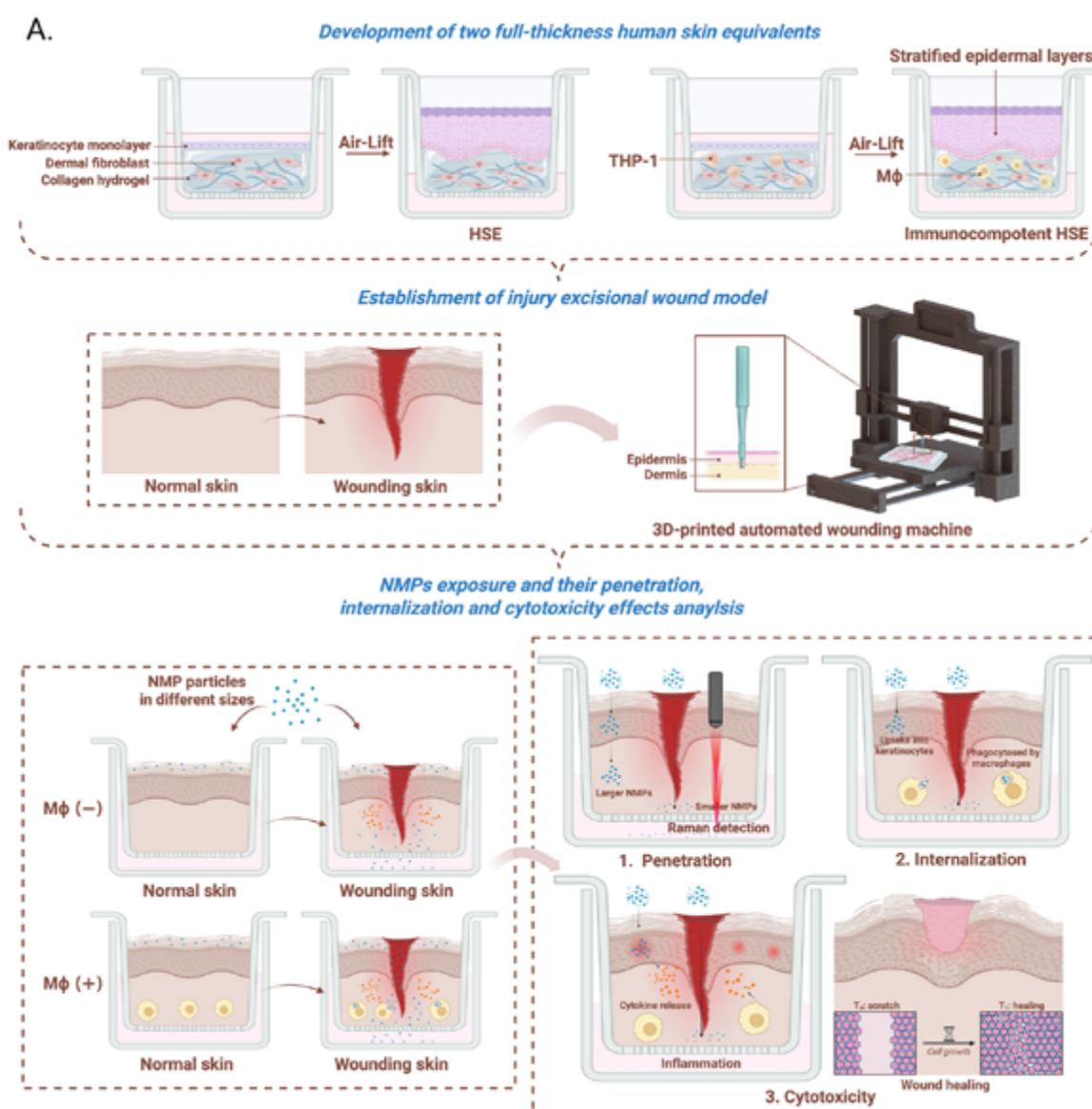


Figure 1. (A) Schematic illustration of a ftHSE as a 3D in vitro model for evaluating microplastic interactions with skin epithelium. M ϕ : macrophage. (B) After 14 days of air-lift culture, HSE morphology was characterized via spinning disk confocal microscopy, showing the stratum granulosum (filaggrin, magenta), stratum spinosum (cytokeratin 10, yellow), and basal layer (cytokeratin 14, red). Immunocompetent HSE characterization is not shown.

accumulation in vital organs (4). Particles smaller than 100 nm can pass through the stratum corneum and reach the dermis, while larger particles struggle to penetrate intact skin. However, wounds compromise this barrier, increasing permeability to NMPs (5). Once inside the skin, some NMPs are taken up by epidermal keratinocytes, while others reach the dermal layers via transepidermal transport, where they can be captured and phagocytized by circulating macrophages (6). This process is particularly active in the early stages of wound healing, as recruited monocytes differentiate into activated macrophages that engulf NMPs through phagocytosis (7). Retained NPs in the epidermis and dermis may exacerbate inflammation by triggering the release of proinflammatory cytokines, potentially deepening the wound and delaying the healing process (8). Understanding the mechanisms of NMP penetration, internalization, and cytotoxicity is crucial for mitigating their risks in medical settings, particularly in wound care.

Here, a 3D full-thickness human skin equivalent (ftHSE) composed of human keratinocytes and dermal fibroblasts serves as an alternative to animal testing for studying the harmful effects of medical-generated NMPs on excisional wounds (Figure 1A-B). Type I collagen acts as a scaffold, supporting keratinocyte differentiation into stratified epidermal layers, while fibroblasts form a dermis-like structure. Monocyte (THP-1)-derived macrophages, as innate immune components, are incorporated to create an immunocompetent HSE, accurately mimicking the *in vivo* skin microenvironment. A wound healing model is created using an in-house 3D-printed automated wounding device to remove a full-thickness tissue section from both the epidermis and dermis, forming a circular or elliptical excisional wound to simulate a real injury. NMPs of various sizes, ranging from nanometers to micrometers, are exposed to both normal and immunocompetent HSE in the wound healing model, with or without macrophages. The study aims to: (1) investigate their size- and dose-dependent penetration into dermal layers, with smaller particles passing through transwell membrane inserts, observed via Raman microscopy; (2) assess their size- and dose-dependent internalization by keratinocytes and macrophages; and (3) evaluate their cytotoxicity and potential to enhance the inflammation response in the injured tissue.

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YO-46**PRESERVING BIOLOGICAL INTEGRITY OF AIRBORNE MICROORGANISMS BY A CHARGED DROPLETS-ASSISTED BIOAEROSOL SAMPLER****Li, Yaxing¹; Kong, Ying¹; Tao, Yile¹; Kunert, Flavio¹; Tang, Jiukai²; Wang, Jing¹.**¹ETH, Zurich, Switzerland; ²Empa, Zurich, Switzerland.

Airborne microorganisms are composed of various components, including viruses, bacteria, fungi, spores, and pollen. These airborne particles, typically ranging from 0.001 to 100 μm in size, can travel long distances and remain suspended in the air over a long time. When exposed to human, pathogenic bioaerosols can cause severe respiratory diseases, allergic reactions, and other acute or chronic health effects, often in an invisible manner. Therefore, it is crucial to collect and quantify bioaerosols from ambient air for evaluating environmental risk and human exposure to pathogenic bioaerosols. Many existing sampling and sensor technologies have achieved high collection efficiency and sensitive detection. However, these methods often damage the biological integrity of airborne microorganisms to some extent, due to inertial impaction or unfavourable collection medium, leading to inaccurate assessments of environmental risk.

A promising sampling technology involves collecting bioaerosols through electrostatic force. Since most bioaerosols are either naturally charged or capable of being charged, they can interact with charged droplets via electrostatic force. Hence, we study and develop a sampling strategy that accurately reflects the level of live microorganisms in the air based on the interaction between charged droplets and bioaerosols, called charged droplets-assisted bioaerosol (CDB) sampler. Its basic ideal is shown in Fig. 1A. Firstly, the sampling liquid is atomized into charged droplets and dispersed in the form of an electrostatic spray. Secondly, the bioaerosols extracted from the environment are attracted and trapped by these charged droplets. Finally, all the droplets containing bioaerosols are deposited on the hydrophobic inner wall of the chamber and flow toward the collection reservoir for subsequent analysis. With this approach, the bioaerosols are collected and concentrated from air into liquid phase while experience low inertial impaction, therefore keep their biological integrity as much as possible. Moreover, the liquid medium provides microorganisms with essential nutrients and optimal osmotic pressure, which is compatible for long-term sampling and subsequent analysis technology.

The physical collection efficiencies for CDB and conventional electrostatic precipitators (ESP) were evaluated (Fig. 1B). Both sampling methods exhibited voltage-dependent enhancement: the physical collection efficiency increased sharply above 3 kV, followed by growth rate attenuation beyond 11 kV. The CDB sampler demonstrated superior overall collection performance compared to ESP, with consistently higher physical collection efficiencies across all tested voltage ranges. Furthermore, the CDB sampler demonstrated a 12% higher physical collection efficiency for negatively charged particles at 15 kV than ESP, surpassing its 7% improvement for neutralized particles. This marked difference suggests that the coulombic force between charged droplets and charged particles dominate the collection mechanism.

For biological assessment in Fig. 1C, *Escherichia coli* (*E. coli*) was employed as a model microorganism. The live and dead *E. coli* was quantified using a fluorescence-based assay according to the metabolism of microorganisms. The CDB sampler achieved a 5.1% biological collection efficiency, outperforming the SKC® BioSampler (0.69%), a widely used commercial bioaerosol sampler. Additionally, the viability of collected bacteria was 30% higher with the CDB sampler compared to the SKC® BioSampler. By simultaneously enhancing collection efficiency and viability during sampling, the CDB sampler achieves more accurate quantification of airborne microorganism, delivering reliable data for public health policy formulation.

YO-47**ANALYSIS OF PBDD/Fs IN AIR EMISSION SAMPLES: METHOD DEVELOPMENT, VALIDATION AND FIRST APPLICATIONS**

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Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) are persistent organic pollutants (POPs) with toxicological properties comparable to their chlorinated counterparts (PCDD/Fs). These compounds originate predominantly as unintended by-products of high-temperature combustion processes, such as waste incineration of organic matter in the presence of bromine, and can be released into the atmosphere, posing serious environmental and health risks [1]. Despite their increasing relevance, no standardized analytical procedures currently exist for quantifying PBDD/Fs in complex matrices. Some methodologies suggest adapting well-established PCDD/Fs procedures, but the different physicochemical properties of PBDD/Fs require a more tailored analytical approach to ensure their precise determination [2]. This study presents an analytical method for PBDD/Fs determination in atmospheric emission samples, emphasizing its development, validation, and first-practical applications.

The proposed methodology involves an optimized multi-step sample preparation strategy, consisting of Soxhlet extraction, a cleanup stage—including a multilayer silica gel column and a graphitized carbon solid-phase extraction (SPE)—and instrumental analysis by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). The method was validated, assessing precision, accuracy, linearity, detection limits, and recovery rates. The validation results proved its accuracy, robustness, and suitability for trace-level environmental monitoring of PBDD/Fs in air emission samples.

To evaluate the practical applicability of the validated methodology, atmospheric emission samples were collected monthly over one year from a waste incineration plant and subsequently analyzed. The results revealed the presence of PBDD/Fs, with brominated furans prevailing over dioxins. Notably, the most brominated congeners, along with tetra-brominated congeners, were detected at the highest concentrations. These findings were systematically compared with existing PBDD/Fs literature emission profiles, as well as with the concentration values and profiles reported for their chlorinated analogues.

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YO-48

ARE ANTARCTIC MARINE SPECIES CONTAMINATED? AN OPTIMISED QuEChERS EXTRACTION FOLLOWED BY LC-MS/MS ANALYSIS FOR EMERGING POLLUTANTS

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Emerging contaminants (ECs) definition encompasses a wide range of anthropogenic and naturally produced chemicals that, despite their ecological risks, remain largely unregulated [1,2]. These substances, including pesticides, pharmaceutical and personal care products (PPCPs), plasticisers, and hormones have been detected in various ecosystems worldwide [2]. Even Antarctica, once considered a pristine environment, has shown increasing signs of contamination due to long-range atmospheric transport (LTR) and local anthropogenic activities [2,3]. The accumulation of ECs in polar ecosystems is intensified by extreme environmental conditions, which inhibit degradation processes [3]. Consequently, ECs can bioaccumulate in polar organisms potentially leading to mutagenicity, genotoxicity, and reproductive damage in various species [4].

Among Antarctic marine species, bivalves are widely used as bioindicators of marine pollution due to their filter-feeding behaviour and widespread distribution. In particular, the Antarctic scallop *Adamussium colbecki* has been recognised as a valuable sentinel species due to its widespread presence in near-shore environments and its key role in the Southern Ocean ecosystem [5]. However, the high lipid and protein content of their biological matrix presents analytical challenges in contaminant detection [6]. Therefore, an effective sample pretreatment strategy is essential to ensure reliable quantification of ECs.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method, initially developed for pesticide analysis, offers a promising solution due to its efficiency, and adaptability to complex matrices [6]. This study optimised and validated a QuEChERS-based extraction protocol for ECs in *A. colbecki*, followed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. A multivariate experimental design was employed to enhance extraction efficiency, with recovery rates (R%) and matrix effects (ME%) serving as key performance indicators. Initially, a Plackett-Burman screening design was used to evaluate the influence of variables including solvent/sample ratio, sorbent amount (PSA, C18), extraction and clean-up times, and agitation mode. The most statistically significant factors (solvent/sample ratio, quantity of PSA, and clean-up time) were then further optimised using a Doehlert response surface design, resulting in an improved protocol that demonstrated satisfactory R% (46–123%) and ME% (62–103%) for 18 selected ECs.

The method was subsequently applied to other Antarctic species, including *Sphaerotylus antarcticus*, *Odontaster validus*, *Trematomus bernacchii*, and *Laternula elliptica*, with R% ranging from 42–143% and ME% from 62–108%, confirming its robustness across diverse biota. The method was finally applied to environmental samples collected during Antarctic expeditions from 2001 to 2022 and successfully identified trace levels of contaminants such as triclosan (TCS), perfluorooctanoic acid (PFOA), and octyl-dimethyl p-aminobenzoic acid (OD-PABA) in the samples. Furthermore, a suspect screening analysis (SSA) using high-resolution mass spectrometry (HR-MS) is currently being conducted to expand the scope of ECs identification in Antarctic biota's tissue.

This study represents the first validated application of QuEChERS for ECs analysis in Antarctic species, establishing a reliable and standardised approach for long-term environmental monitoring. The optimised method enhances the ability to extract and detect emerging contaminants, contributing to a more comprehensive understanding of EC distribution and its potential impacts on the Antarctic marine ecosystem. By facilitating a more thorough evaluation of ECs in Antarctic biota, this methodology contributes to the broader understanding of human-induced impacts on one of the world's most fragile and unique ecosystems.

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YO-49**RISK ASSESSMENT OF PHARMACEUTICAL RESIDUES IN CATALAN RIVERS****Olivella-Martí, Maria; Ortiz Almirall, Xavier; Gómez-Canela, Cristian.***Institut Químic de Sarrià - Universitat Ramon Llull, Barcelona, Spain.*

Water is essential for both human and wildlife survival, and maintaining its quality is critical. Monitoring groundwater and surface water is vital because these are the primary sources for domestic and industrial use. Recently, a group of chemicals known as Emerging Contaminants (EC) has posed a new challenge. Among these, pharmaceuticals are of particular concern since standard wastewater treatment processes often fail to remove them. Their persistence in water has been linked to harmful effects on aquatic organisms—such as interfering with fish endocrine systems—as well as potential risks to human health, including hormonal imbalances that could lead to reproductive and developmental issues. [1]

Increasing global pharmaceutical use is driven by an aging population, broader access to healthcare, and a rise in chronic diseases. Within Europe, Spain has shown notably high prescription rates compared to countries like France, Portugal, or the UK. Specifically, in Spain, the region of Catalonia stands out with significantly elevated rates; in 2019, it recorded 928 doses per 1000 inhabitants, surpassing the national average of 871 doses per 1000 inhabitants. Finally, Risk Quotients (RQs) were estimated using aquatic organisms as reference organisms to assess the environmental risk of the Catalan rivers. [2]

Given these concerns, it is crucial to assess the potential risk to the aquatic environment. For the first time, a study has evaluated 79 pharmaceuticals across the 14 main rivers of Catalonia, offering a broad perspective on the presence and distribution of these emerging contaminants. Water samples were collected from 52 sampling points distributed along the rivers Noguera Pallaresa, Noguera Ribagorçana, Tordera, Muga, Fluvià, Besòs, Llobregat, Segre, Ebre, Ter, Francolí, Gaià, Daró, and Foix, specifically at the source, a midstream point, and the mouth of each river.

To avoid pharmaceutical degradation, water samples were collected in amber glass bottles and promptly refrigerated. The extraction process began one day after the sampling campaigns. For the analysis, river water was filtered through 0.45 µm nylon filters to eliminate suspended solids and organic material. Then, 100 µL were directly injected without further sample treatment. Moreover, instrumental analysis was performed using an HPLC-MS/MS system. Chromatographic separation was conducted on an Exion LC system (SCIEX, MA) with a Kinetex Polar C18 column (2.6 µm, 150 × 2.1 mm). The mobile phase consisted of ultrapure water with 0.1% formic acid (Solvent A) and acetonitrile with 0.1% formic acid (Solvent B) in positive and negative electrospray ionisation mode (ESI+/ESI-). MS/MS detection was carried out with a Triple Quad™ 7500 MS/MS system (SCIEX, MA) in MRM mode, acquiring two transitions per analyte (quantifier and qualifier).

The findings of this research provide a comprehensive risk assessment of 79 pharmaceutical compounds across all major rivers in Catalonia. In total, 30 pharmaceuticals were detected in most of the analyzed samples, with concentrations on the order of µg·L⁻¹.

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YO-50

ASSESSING NEUROTOXIC AND CARDIOVASCULAR RISKS OF PHARMACEUTICAL POLLUTION IN SURFACE WATERS USING *DAPHNIA MAGNA* AND ZEBRAFISH MODELS

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The present study introduces a cost-effective and biobased testing framework with the objective of identifying and preventing the harmful effects of unintentional and hazardous mixtures of pharmaceuticals in aquatic ecosystems. This integrates advanced analytical procedures with toxicological and metabolomic evaluations in aquatic organisms to better understand the ecological and potential human health risks.

Using a robust large-volume solid-phase extraction (LV-SPE) method previously developed [1] neuroactive pharmaceuticals found in water samples collected across the main rivers of Catalonia (Besòs, Llobregat and Onyar) and wastewater treatment plants (WWTPs) were concentrated.

Water samples were enriched 5- and 20-fold and used to expose *Daphnia magna* (*D. Magna*) and zebrafish embryos (*Danio rerio*) to assess sublethal neurodevelopmental and neuro-disruptive effects. Seven behavioural responses in *D. magna*, were measured after 24-hour exposures, while zebrafish embryos were assessed over a 4-day period. These assays were complemented quantifying of 99 pharmaceutical residues and 31 neurotransmitters and related metabolites in both species using UHPLC-MS/MS.

Toxic effects were observed in 22.4% of *D. magna* samples and 41.6% of zebrafish embryos. Among the 32 chemical contaminants linked to the observed defects in both species, 4 neuroactive chemicals (topiramate, rasagiline, citalopram, and fluvoxamine) predominantly affected neurotransmitters and related metabolites in zebrafish, in line with their primary mode of action (MoA). Additionally, 7 chemicals (lidocaine, losartan, caffeine, tramadol, gabapentin, cloperastine, and chlorpheniramine) induced alterations through their secondary modes of action. Finally, toxicogenomic analysis and chemometric modeling (PLS) allowed the identification of significant cause-effect correlations and mechanistic pathways, particularly those related to amino acid and urea signaling. These findings confirm the utility of *D. magna* and zebrafish embryos as sensitive models in environmental neurotoxicity assessments.

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YO-51

ASSESSING THE TOXICOLOGICAL IMPACTS OF PHARMACEUTICAL RESIDUES IN WASTEWATERS: AN INTEGRATED CHEMICAL AND BIOLOGICAL STUDY USING *DAPHNIA MAGNA* MODEL

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The increasing scarcity of freshwater resources has led to the growing interest in wastewater reuse. However, the presence of residual contaminants in treated effluents remains a significant environmental concern. These compounds, often present at trace levels, may exert toxic effects even at low concentrations due to their biological activity, persistence, and potential to bioaccumulate [1]. In this context, the present study aimed to evaluate the toxicological impact of pharmaceutical residues found in multiple urban wastewater treatment plants (WWTPs) along the Costa Brava (Spain) by combining advanced chemical analysis with *in vivo* bioassays.

While chemical analysis often requires minimal or no sample enrichment, bioanalytical methods typically demand larger sample volumes and preconcentration steps to enable comprehensive testing across various biological endpoints [2]. Therefore, the first step of this study was to develop and validate an innovative large volume solid-phase extraction (LV-SPE) methodology for the efficient extraction and preconcentration of pharmaceutical residues from wastewater samples. For the optimization, quantitative analysis of target analytes was performed using a validated liquid chromatography coupled with a triple quadrupole mass spectrometer (LC-MS/MS, Xevo TQS, Acquity H-Class, Waters, Milford, USA) method [3,4], employing multiple reaction monitoring (MRM) in positive electrospray ionization mode (ESI+). The resulting LV-SPE technique demonstrated effective extraction and preconcentration of significant amounts of water, achieving recoveries above 30% for 87 out of 99 target compounds in spiked wastewater samples.

Then, the optimized procedure was applied to samples collected from 14 urban WWTPs located across the Catalan coast—Portbou, Colera, Llançà, Port de la Selva, Cadaqués, Roses, Empuriabrava, L'Escala, Torroella de Montgrí, Pals, Castell d'Aro, Tossa de Mar, Lloret and Blanes—targeting both secondary and tertiary effluents. The biological effects of these extracts were evaluated using the freshwater invertebrate *Daphnia magna* as a model organism. A series of behavioural assays were conducted, including startle, habituation, basal locomotor activity (BLA), visual motor response (VMR) and phototaxis. These tests allowed the detection of subtle sublethal effects that would not be observed through traditional acute toxicity endpoints. Significant behavioural alterations were observed in organisms exposed to secondary-treated samples, while tertiary-treated samples showed a lower degree of impact.

To complement the behavioural analysis, targeted metabolomic studies were carried out to quantify changes in key neurotransmitter levels, such as serotonin, dopamine, norepinephrine, and acetylcholine, as well as their metabolic precursors and degradation products. Neurochemical analysis was performed using an ExionLC System (SCIEX®, USA) coupled with a Triple Quad™ 7500 System (MS/MS) (SCIEX®, USA) [5], revealing alterations in some of the principal metabolic pathways, particularly in samples from plants with less efficient treatment stages. Overall, the results revealed clear and statistically significant differences between secondary and tertiary treatments. *Daphnia magna* exposed to secondary effluents exhibited pronounced behavioural disruptions and altered neurotransmitter profiles, particularly in the serotonergic and catecholaminergic pathways. In contrast, tertiary-treated samples showed a lower toxicological impact, confirming the added value of advanced treatment processes. Even though, residual compounds remained detectable, indicating that complete removal is not always achieved.

Thus, this integrated chemical-biological approach provides novel insights into the ecotoxicological risks associated with wastewater reuse. Our results underline the necessity of implementing more robust treatment and monitoring strategies to reduce the presence of neuroactive contaminants in treated effluents.

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YO-52

SECRETS IN THE SNOW: NONTARGETED ANALYSIS OF CECs IN MOUNTAIN USING UHPLC-HRMS

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Abstract:

This work is part of the IN2AQUAS project, financed within the EU Horizon program, which aims to secure water quality from remote regions to the tropical belt. This study specifically focuses on detecting contaminants of emerging concern (CECs) in mountain snow, applying a nontargeted approach using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS). Given the growing concerns regarding environmental pollution and its widespread effects, monitoring CECs in high-altitude snow is crucial for understanding the extent of anthropogenic contamination in secluded environments.¹

To achieve this, three continuous snow sampling campaigns were carried out in the Monte Bianco area (Valle d'Aosta, Italy), allowing an assessment of both the presence and temporal variation of CECs in snow. The collected snow samples underwent solid-phase extraction (SPE) to concentrate for enhanced detection.²

Three types of Waters™ Oasis® cartridges were used for SPE to capture a wide range of contaminants, enhancing the analysis of CECs, including polar and non-polar compounds. The applied cartridges were:

1. Hydrophilic-Lipophilic Balance (HLB)
2. Weak-Anion Exchange (WAX)
3. Mixed Anion Exchange (MAX)

The samples were analyzed using UHPLC coupled with HRMS (Orbitrap Exploris 120, Thermo Scientific), a crucial technique for high-accuracy detection of CECs in environmental studies.³

Data processing and identification were performed using Compound Discoverer 3.3 (Thermo Scientific), which enhances analysis through advanced spectral matching and suspect screening.⁴

The findings reveal the presence of a broad range of CECs, including pharmaceutical compounds, personal care products, pesticides, phthalates, parabens, industrial chemicals, and other compounds.

These results emphasize the far-reaching influence of anthropogenic pollution, even in remote, high-altitude environments.⁵ The detection of CECs in mountain snow highlights the necessity of implementing comprehensive monitoring strategies to assess pollution impacts in remote ecosystems and better understand the mechanisms of their mitigation.

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YO-53

ASSESSING THE EFFICIENCY OF BIOCHAR FOR THE REMOVAL OF LANTHANIDES IN CONTAMINATED WATERS

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The increase in lanthanides (Ln) mining and the difficulties associated with its recycling have led to an increase in environmental levels of Ln. Due to their large impact, especially in water bodies, strategies based on the use of sorbents for remediation actions are increasingly being considered. In this context, an alternative sorbent material of growing interest is biochar, a sustainable and readily available material obtained from the pyrolysis of plant biomass. Identifying in the laboratory whether a sorbent material is effective for sorption is a mandatory step for the implementation of its use, in this case in the removal of Ln from wastewater. In this work, we test different laboratory methods to evaluate Ln sorption in a set of biochar materials. On the one hand, the classical and widely used batch sorption method allows to easily obtain the solid-liquid distribution coefficient (K_d) at different concentration levels, which indicates the affinity of the sorbate for the sorbent material as key information for the evaluation of the suitability to use biochar in remediation activities. On the other hand, the less commonly used continuous-flow sorption method has been examined to extract meaningful information about the sorption process between a contaminant and a sorbent material, in this case Ln and biochar, respectively. The experimental set-up, depicted in Figure 1, consisted of a cartridge filled with approximately 3 to 4 g of biochar, with porous polyethylene frits at the top and bottom of the biochar column. The cartridge was connected to a pump on one side and to a fraction collector on the other. The Ln-enriched water matrices were dispensed by a peristaltic pump at a fixed speed, providing a solution flow rate of around 0.25 mL min^{-1} . The output solution was automatically collected at specified time intervals. The breakthrough curves were obtained by plotting the ratio of the metal concentration in each fraction to the initial metal concentration (C_t/C_0) at each elapsed time (t). The breakthrough curve shows a first zone ($C_t/C_0 = 0$) where complete sorption of the contaminant occurs, followed by the beginning of the contaminant elution, and ending with a plateau indicating the complete saturation of the biochar. The breakthrough curves are then fitted to obtain the maximum sorption capacity of the sorbent material and to analyse the dynamics of the sorption process. This setup better simulates the filtration of contaminated water through a semipermeable biochar filter for remediation purposes.

In this study, both batch and continuous-flow sorption experiments permitted to satisfactorily evaluate Ln sorption capacities of tested biochars. While batch tests were useful for identifying the materials with the highest affinity for Ln, the continuous-flow sorption experiments enabled the determination of maximum sorption capacities and the identification of cationic exchange as the main mechanism involved in Sm sorption. In this context, the results confirmed that biochar are promising sorbent materials, offering a sustainable and cost-effective alternative for removing Ln from contaminated waters at environmentally relevant concentrations.

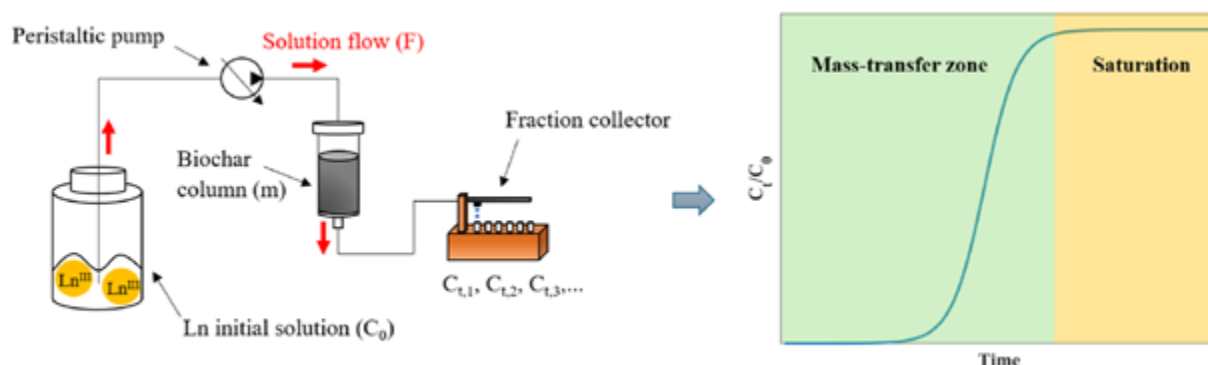


Figure 1. Experimental set-up and theoretical breakthrough curve for the continuous-flow sorption experiments.

YO-54**ASSESSING THE INFLUENCE OF DIFFERENT FORMALDEHYDE-BASED RESINS ON VOC EMISSION FROM WOOD-BASED PANELS USING A SIMPLIFIED GAS-DIFFUSION MICROEXTRACTION APPROACH**

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Wood-based panels (WBP), such as particleboard (PB) and dry-process fibreboard (MDF), are engineered wood products manufactured by compressing or binding wood fibres, particles, or veneers with an adhesive under heat and pressure. Although widely used by the construction and furniture industries for their affordability and versatility, these products have been associated with the deterioration of indoor air quality, primarily due to the emission of volatile organic compounds (VOCs). The extent and composition of VOC emissions are influenced by several factors, including the natural composition of the wood, chemical additives and adhesives used, and the manufacturing processes involved [1]. Among these emissions, the most important is formaldehyde, classified as a carcinogen by the International Agency for Research on Cancer (IARC), posing potential health risks with prolonged exposure. However, growing concerns about indoor air quality have led to increased efforts to monitor the emissions of other VOCs beyond formaldehyde.

Gas-diffusion microextraction (GDME) [2] is a simple, efficient and adaptable sample preparation technique that enables the simultaneous extraction, derivatization, and concentration of volatile compounds. Volatile compounds released from the sample will diffuse through a gas-permeable membrane and are absorbed into a liquid acceptor solution. This solution, which is relatively free of interfering compounds, can be directly analysed using the most common analytical techniques. In a recent work, a GDME-HPLC-DAD methodology was optimised for the selective sampling of volatile carbonyl compounds emitted from PB and MDF panels, through a derivatization reaction with 2,4-dinitrophenylhydrazine (DNPH) [3-4].

In this study, GDME-HPLC-DAD was used to analyse volatile carbonyl compounds emitted from PB panels produced with different UF and MUF resins. The resins were all formaldehyde-based, differing in formaldehyde-urea ratio and melamine content. Results indicated that formaldehyde emission was greatly affected by the resin composition, while the type of wood particles mainly affected the emission of other volatile carbonyl compounds. These differences were further elucidated through Principal Component Analysis (PCA).

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YO-55**DEVELOPMENT OF A NEW COATING BASED ON METAL-ORGANIC FRAMEWORK MATERIAL FOR STIR-BAR SORPTIVE EXTRACTION OF ACIDIC PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES**

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Pharmaceuticals are widely consumed by millions of people to improve their health and well-being. Due to this consumption and excretion, residues of these compounds have been found in influent and effluent wastewater, and eventually in surface water. To monitor and quantify these pharmaceuticals at very low concentration levels ($\mu\text{g/L}$ and ng/L), sample treatment step followed by an advanced analytical technique, such as liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS), are essential [1]. Among all the different sorptive extraction techniques available, stir bar sorptive extraction (SBSE) is an option.

As a sorptive extraction technique, the coating of the bar is crucial in the extraction process. None of the commercially available materials (polydimethylsiloxane (PDMS) and ethylene glycol-silicone (EG-silicone)) have demonstrated to retain effectively the most polar compounds. To this end, novel in-house coating materials for SBSE have been developed [2,3]. Recently, metal-organic framework (MOF) materials have been a subject of focus [4] due to their easy preparation, high stability and high surface area properties. Another characteristic of these materials is their potential to be further functionalized with ion-exchange moieties capable of selectively retain polar ionic compounds [5], which may allow the incorporation of an exhaustive clean-up step. However, MOFs in SBSE have been hardly applied.

This study presents the preparation and evaluation of MIL-101 (Al) and UiO-66 (Zr) as hybrid monolith coatings with MOF modification for SBSE. These coatings are functionalized with amine moieties, which facilitate weak anion exchange (WAX) interactions for the selective SBSE of a set of acidic pharmaceuticals from environmental water samples. The synthesis procedure involved the external surface activation of a common Teflon magnet followed by vinylization and covalent attachment of the amino-modified MOFs (NH_2 -MIL-101 (Al) or NH_2 -UiO-66(Zr)). A preliminary comparative study demonstrated that UiO-66 (Zr) showed better performance and reproducibility, leading to its selection for further application. After optimization, the final conditions presented good recoveries (from 48 to 54%) for the analytes of interest, considering the non-exhaustivity of the technique. Method validation was performed using an LC-MS/MS in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), matrix effect, repeatability and reproducibility. Finally, the applicability of the method was confirmed with the analysis of environmental water samples, such as Ebre river and effluent wastewater.

Acknowledgements

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YO-56**ADVANCED SCINTILLATING IMPRINTED POLYMERS FOR RADIOCHEMICAL IRON DETECTION**

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Several radionuclides are generated in nuclear power plants as byproducts of reactor operations. During the dismantling of nuclear facilities, authorities must ensure that all generated waste is free of radionuclides, necessitating extensive analysis, including the assessment of Difficult-to-Measure (DTM) radionuclides. According to ISO 24390:2023, a DTM radionuclide is defined as a “radionuclide whose radioactivity is difficult to measure directly from the outside of the waste packages by non-destructive assay means.”¹ DTM radionuclides are characterized by their low-energy emissions, the wide range of matrices in which they occur, and interferences that hinder their precise isolation for measurement. The accurate determination of these radionuclides is essential, particularly in the decommissioning of nuclear facilities and nuclear waste management. However, conventional methods continue to face challenges in their quantification across diverse matrices.

Key DTM radionuclides produced in nuclear reactors arise from neutron interactions with reactor materials, with some generated through neutron activation of reactor steel. Although primarily originating from steel activation, these radionuclides can migrate into cooling water or other reactor components. In particular, this study focuses on the determination of ⁵⁵Fe, a radionuclide of significant concern during decommissioning due to its high production levels resulting from the extensive use of iron in nuclear power plant structural components. ⁵⁵Fe poses health risks to workers handling, classifying, and characterizing radioactive waste and may spread into environmental media if not properly managed. Although external radiation from ⁵⁵Fe is minimal, internal exposure is hazardous due to its long biological retention, which can affect blood constituents and bone marrow, increasing the risk of leukemia².

The measurement of ⁵⁵Fe is particularly challenging due to its low-energy emissions and the presence of multiple interfering species. The conventional determination process involves an initial precipitation at pH 9 using NH₄OH or NaOH solutions to preconcentrate iron, during which other elements such as nickel and cobalt also precipitate. The sample, once reconstructed in 9M HCl, is then passed through an anion exchange column for sequential iron isolation before radiometric measurement. The primary detection technique employed is Liquid Scintillation Counting (LSC); however, in real samples where iron content exceeds 200 mg, the maximum detection efficiency achieved is only ~15%³. Furthermore, existing isolation procedures are suboptimal, as no resins with selectivity for iron currently exist due to the absence of iron-specific extractants.

To address these limitations, this study presents the development of a novel scintillating material specifically designed for ⁵⁵Fe determination, offering enhanced selectivity over conventional methods while mitigating color quenching in LSC measurements. This material is based on ion-imprinted polymers, marking the first application of scintillating ion-imprinted polymers for radioactivity determination. The developed material demonstrates detection efficiencies for ⁵⁵Fe exceeding 10% and an iron binding capacity of up to 20 mg·g⁻¹, providing a promising alternative for the accurate quantification of ⁵⁵Fe in nuclear decommissioning and waste management.

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YO-57**DEVELOPMENT OF RAPID ANALYTICAL PROTOCOLS FOR PLUTONIUM AND AMERICIUM DETERMINATION IN ENVIRONMENTAL SAMPLES FOR RADIOLOGICAL EMERGENCY RESPONSE**

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The rapid and accurate determination of transuranic elements such as plutonium (Pu) and americium (Am) is essential for radiological emergency response and environmental monitoring. In emergency scenarios, rapid assessment of radioactive contamination is crucial for decision-making and public health protection. This study presents the development and optimization of rapid analytical protocols for the quantification of ^{238}Pu , $^{239/240}\text{Pu}$ and ^{241}Am in environmental samples, including water, soil and biota, using a combination of extraction chromatography resins and alpha spectrometry. (1–3)

The proposed methodology utilizes TEVA® and DGA® resins (Triskem International) for sequential separation of Pu and Am, ensuring high selectivity, minimal matrix interferences, and reduced analytical time. The procedure begins with sample pre-treatment, where an initial co-precipitation step using Fe(III) hydroxide allows the efficient pre-concentration of actinides. After dissolution in nitric acid, the sample is loaded onto a chromatographic system composed of a TEVA® column connected in series with a DGA® cartridge. Pu are selectively retained in the TEVA® resin, while Am is retained in the DGA® resin. Successive washing and elution steps allow the isolation of the target radionuclides, which are subsequently microprecipitated as cerium fluoride onto stainless steel planchets for measurement by alpha spectrometry. (4,5)

The methodology has been validated through intercomparison samples provided by the Spanish Nuclear Safety Council (CSN) and the International Atomic Energy Agency (IAEA), demonstrating excellent accuracy and reproducibility. For example, chemical recoveries for Pu and Am exceed 80% for water samples, with detection limits suitable for environmental monitoring, making the protocol applicable to low-level contamination scenarios. Additionally, the optimized procedure significantly reduces processing time compared to conventional methods, enabling rapid radiological assessment in emergency situations.

One of the key advantages of this approach is its adaptability to different sample matrices and can be applied either sequentially for the full actinide separation or selectively for the determination of specific radionuclides based on the incident scenario. Moreover, the use of extraction chromatography minimizes reagent consumption and radioactive waste generation, contributing to safer and more sustainable radiochemical analysis. (3)

This work represents a significant contribution to the field of environmental radiological protection by providing a fast, reliable, and validated methodology for the determination of Pu and Am in environmental samples. The ability to rapidly assess transuranic contamination is essential for emergency preparedness, regulatory compliance, and environmental impact assessment. These findings support decision-making in radiological crisis situations, helping authorities implement timely protective actions to mitigate risks to human health and the environment.

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YO-58

LABEL-FREE CHEMICAL SPECTROSCOPY OF CANCER CELLS: A COMPARATIVE STUDY OF O-PTIR AND FTIR SPECTROSCOPY

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Cancer is a major global public health issue and one of the most frequent causes of death around the world, with both incidence and mortality rates rapidly increasing. [1] Early detection and intervention significantly improve treatment outcomes, making the early identification of metastasis crucial for determining the most effective treatment. [2]

Offering label-free chemical analysis, FTIR spectroscopy has been successfully used to identify cancer marker bands in cell and tissue samples [4,5]. However, applications in water demand a different approach due to the broad IR absorption band of water. Relying on the refractive index contrast between the sample and its environment, Optical Photothermal Infrared (O-PTIR) spectroscopy is considered a promising alternative to traditional chemical-staining-based pathology methods. [3]

Our custom O-PTIR setup, was specifically designed to study cancer cells in aqueous media. In addition to the acquisition of spectra in several milliliters of water and the identification of biomarker bands, our system also allows us to perform chemical imaging based on these marker bands. By combining our findings with classification models, we can distinguish between healthy, cancerous and metastasizing cells. Moreover, we are able to analyze the chemical composition of individual cells. Additionally, we are collaborating with our partners in the Tumor-LN-oC project to build a lab-on-a-chip system which enables us to monitor the metastasis of cancer cells.

Here, we present a basic overview of O-PTIR spectroscopy and imaging, comparing it to conventional FTIR spectroscopy. Furthermore, we present our supervised learning approach towards differentiating between cancerous and healthy cells, comparing spectra obtained through conventional FTIR microscopy with those acquired using our custom O-PTIR instrument.

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YO-59

IN SILICO MODELLING OF THE INFRARED SPECTRUM OF URINE FOR OPTIMIZED DISEASE DIAGNOSIS

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Infrared (IR) spectroscopy, coupled with multivariate analysis, has become a valuable tool for clinical analysis. However, there is a strong limitation in the development of vibrational methodologies for the analysis of complex biological samples: the information about the samples composition is generally encrypted in a set of overlapped bands. The use of complex machine learning and chemometric tools, although extremely effective in extracting this information, always requires the laborious and expensive phase of the collection of a large representative set of samples to be measured by both the vibrational and the reference methodology. Moreover, changes in samples pretreatment steps or measurement parameters would require measuring the entire dataset with a new protocol, making it completely unaffordable to achieve optimal values through multiparametric optimization. Thus, optimization becomes exceptionally challenging in the case of label-free and multivariate approaches. In this study we propose developing *in silico* based optimizations to solve these limitations and anticipate the outcomes of vibrational spectroscopy-based models of biofluids. A bottom-up approach [1], which requires the measurement of the pure components of the sample, is followed to develop simulated spectra through the combination of the individual spectra of the sample components. We apply this methodology to develop *in silico* urine spectra as a complement in design of experiments in order to reduce time and resources during multiparametric optimization in the diagnosis of diabetic kidney disease (DKD). This pathology is characterized by albuminuria and its detection requires the determination of the UACR (urinary albumin to creatinine ratio) with efforts focused on the early detection of the disease (microalbuminuria, 30-300 mg_{albumin}/g_{creatinine}) [2]. The sample pretreatment is designed so that an increase in albumin prediction diminishes creatinine prediction [3]. Therefore, if we want to quantify both, we have to find the optimal pretreatment that yields the best compromise between the two analytes. Different parameters such as infrared spectrometer type, number of scans, albumin preconcentration factor, and low-weight metabolites dilution factor were tested with the help of *in silico* spectra. Artificial urine (prepared by mixing the standards of the main urine components followed by experimental measurement of the resulting spectra) was pretreated and used for comparison with the machine learning models obtained with *in silico* samples. Then, real urine samples were measured following the selected optimal parameters. Obtained quantification and classification errors were comparable to the *in silico* dataset ones, as well as spectral visualization. Thus, simulated urine spectra have proven to be a valuable tool in early stages of experiments design when few or no real urine samples are available.

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YO-60**DETECTION OF PESTICIDES IN LIGHT CANNABIS INFLORESCENCE USING RAPID EXTRACTION METHODS AND AN EXTRACTIVE-LIQUID SAMPLING ELECTRON IONIZATION-MASS SPECTROMETRY SYSTEM**

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Extractive-Liquid sampling Electron Ionization-Mass Spectrometry (E-LEI-MS) is a real-time technique that combines ambient sampling with the high electron ionization (EI) identification power. An advantage of using the E-LEI-MS system is the possibility of simultaneously analyzing volatile and low-volatile compounds. The E-LEI-MS system has already been tested in various applications, including detecting food pesticides [1]. In this study, the system is employed as a new analytical approach for detecting pesticides in *Cannabis sativa* L. inflorescences. The use of pesticides in the cultivation of crops is a customary practice, and cannabis crops are not an exception. The use of pesticides in cannabis crops is not regulated in Europe; hence, developing analytical methods for detecting pesticides in cannabis inflorescences is acquiring great interest. However, these techniques require laborious sample preparation procedures and chromatographic separations. In this context, the E-LEI-MS is proposed as a direct MS technique for rapidly detecting pesticides in this complex matrix using a targeted approach in Multiple Reaction Monitoring acquisition mode.

The E-LEI-MS setup includes a solvent-release mechanism for extracting the analytes from the sample surface and a capillary system for transferring the solution to the EI source by the suction force of the MS high vacuum. The vaporizing process takes place in the vaporization microchannel using the high temperature of the transfer line, where the analytes reach the gas phase before entering into the EI source.

65 pesticides were selected as targeted compounds for pesticide detection in *Cannabis sativa* L. according to California and Washington regulations concerning the Maximum Residue Levels of pesticides in cannabis crops. Standard solutions were analyzed in direct aspiration mode using the E-LEI-MS coupled with an Agilent 7010 GC/QqQ-MS. Source and Vaporization Micro-Channel (VMC) temperatures were optimized according to the analyte molecular weights, boiling points, and degradation temperatures. 52 pesticides were correctly identified using the NIST library, 7 were identified through a comparison with predicted EI spectra of online databases, and 6 were not amenable to E-LEI-MS analysis.

The MRM transitions of 59 pesticides were applied to analyze fortified Cannabis inflorescence samples. The challenge associated with the direct analysis of pesticides in cannabis inflorescences is the high complexity of the matrix, mainly attributable to the presence of cannabidiols, phenols and terpenoids. Rapid extraction procedures, e.g., solid-liquid extraction and QhEChERS, were evaluated for the extraction of pesticides before the E-LEI-QqQ-MS analysis.

The E-LEI-MS system has already been employed for qualitative and semi-quantitative applications, the results obtained from the analysis of fortified demonstrate its promising approach for the rapid screening of several pesticides in a complex plant matrix.

ACKNOWLEDGMENTS

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YO-61**ENHANCED ARSENIC DETERMINATION IN TIRE PYROLYSIS OILS USING A HIGH-TEMPERATURE TORCH INTEGRATED SAMPLE INTRODUCTION SYSTEM (hTISIS) COUPLED WITH AN ICP-MS/MS**

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Accurate determination of arsenic in tire pyrolysis oils is critical due to its potential impact on fuel stability, environmental safety, and catalytic activity in refining processes. While inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) is widely recognized for its sensitivity and low detection limits, the analysis of organic matrices remains challenging. Challenges such as plasma thermal degradation, spectral interferences, and soot deposition often complicate the analysis when organic samples are introduced into the spectrometer. Interfering ions such as $40\text{Ar}35\text{Cl}^+$, $38\text{Ar}37\text{Cl}^+$, $59\text{Co}16\text{O}^+$, $40\text{Ca}35\text{Cl}^+$, $\text{Sm}2^+$ and $150\text{Nd}2^+$ overlap with 75As^+ peaks, thereby complicating the quantification. Furthermore, the complex matrix of pyrolysis oil samples could influence the entire analytical process from nebulization to ionization, leading to matrix effects and varying arsenic chemical forms, which could degrade the accuracy of determinations.

To overcome these challenges, we propose an advanced analytical strategy that combines the benefits of high-temperature sample introduction with ICP-MS/MS. Specifically, the so-called high-temperature torch integrated sample introduction system (hTISIS), operated at 400°C , was employed following a 1:10 dilution of samples in xylene. This configuration significantly improved arsenic transport, reduced matrix-induced interferences, and enhanced overall method sensitivity. The hTISIS system improved analyte transport efficiency, yielding signal intensities up to 20 times higher than those achieved with conventional introduction systems. As a result, the method reached arsenic detection limits as low as 2 ng kg^{-1} in xylene, significantly outperforming the 20 ng kg^{-1} detection limit obtained using conventional setups. Additionally, the procedural limit of quantification (pLOQ) with the hTISIS system was established at 60 ng kg^{-1} , underscoring its suitability for trace-level arsenic determination in complex organic matrices.

The hTISIS configuration not only achieved an order of magnitude greater sensitivity than conventional systems but also enhanced quantification robustness by effectively mitigating the impacts of complex matrices and the effect of arsenic chemical forms. The utilization of external calibration with standards prepared in xylene showcases the method's adaptability and precision in handling complex organic matrices. As proof of concept, thirteen tire pyrolysis oil samples were analyzed, yielding arsenic concentrations ranging from 41 ± 4 to $924 \pm 80\text{ }\mu\text{g kg}^{-1}$.

YO-62

HERITAGE GLASS ANALYSIS: NOVEL MULTIVARIATE APPROACHES AND UNCERTAINTY ESTIMATION FOR ELEMENTAL DETERMINATION BY PORTABLE X-RAY FLUORESCENCE

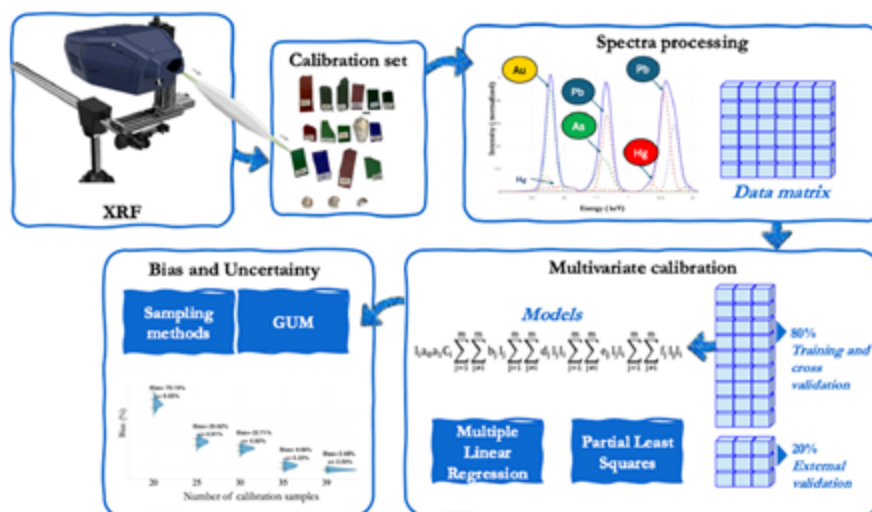
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Historical glasses provide invaluable insights into the technological and cultural evolution of past societies [1]. While portable X-ray fluorescence (p-XRF) enables non-destructive elemental analysis, accurate quantification faces challenges due to surface heterogeneity, complex curvatures, and matrix effects in heritage glass objects [2,3]. In this context, the implementation of chemometric methods has become increasingly relevant for elemental quantification using p-XRF. However, these methods have significant challenges because they require many samples for model training, which is challenging in heritage object analysis. Additionally, the models often involve numerous terms, leading to high prediction uncertainties [4].

This study evaluates different multivariate calibration strategies to address these challenges by using 40 in-house reference materials and focusing the application on heritage glass objects. External validation was conducted using four certified reference materials. The Figure shows the integrated analytical strategy applied. For the calibration method, the first approach involves a novel multiple linear regression (MLR) incorporating genetic algorithms for influence coefficient estimation. The second approach employs partial least squares (PLS) regression. Both methods were assessed for the quantification of 19 elements. Uncertainty estimation was conducted using various resampling procedures, including jackknife and bootstrap methods. These were then compared with an analytical solution derived from the Guide to the Expression of Uncertainty in Measurement (GUM) and its supplements [5].

PLS models achieved errors of ca. 1% for major components such as Na, K, Ca, and Si. The MLR models demonstrated comparable performance for specific elements, notably Fe and Pb. Historical elements, including Mn, Pb, Fe, Cu, Sn, As, and Sb, were quantified with errors ranging from 0.8% to 12%. Furthermore, uncertainty estimation revealed that, depending on the element, the number of training samples could be reduced by up to 20% while maintaining acceptable uncertainties and biases for the application.



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YO-63

BENCHTOP LED-NMR A PROMISING TOOL FOR THE MONITORING OF PHOTOCHEMICAL REACTIONS

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In the last decade, high-field nuclear magnetic resonance (NMR) has been increasingly employed in the field of photochemistry, thanks to the association with a fiber-coupled light emitting diode (LED). This innovative analytical technique, known as LED-NMR, enables a direct irradiation in the NMR spectrometer. It can be used as a real-time monitoring of photoinduced reaction such as photoswitches and photopolymerization [1] or photocatalysis [2].

Recently the emergence of Low-field NMR (45-100 MHz) spectrometers has opened up many applications, especially as a low-cost method of screening or quality control [3]. Those spectrometers are compact benchtop devices, equipped with a permanent magnet, designed for easy setup and installation. Furthermore, they are highly user-friendly and can be operated without specific NMR knowledge. Despite the loss of sensitivity and resolution compared to high-field NMR, benchtop NMR has been successfully employed in several fields such as the monitoring of degradation of the persistent organic pollutant PFOA [4]. With direct access to the top part of the NMR tube, the development of an illumination system can be achieved on a compact NMR spectrometer.

In this work, we will present the instrumental methodology developed for monitoring photochemical reactions by ^1H and ^{19}F low-field NMR, using irradiation outside (ex-situ) and inside (in-situ) the spectrometer. The first step was to miniaturize the photochemical reactor into the NMR tube and standardize the ex-situ irradiation with a 3D printed setup. For the in-situ irradiation, instrumental modifications have been made.

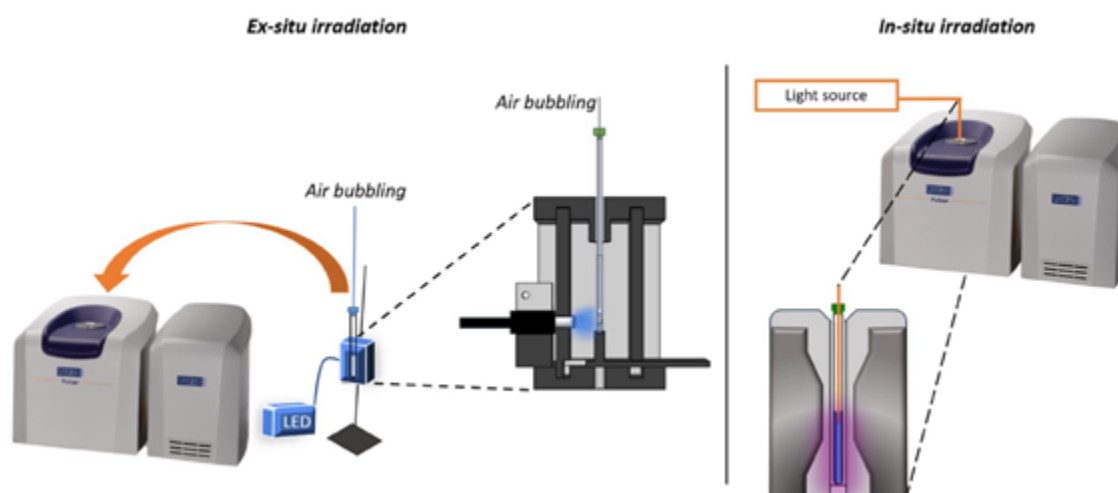


Figure : Ex-situ and in-situ irradiation setups used for the monitoring of photochemical reactions by Low-field NMR.

With those illumination setups, several applications have been explored: the study of photochromic properties of azobenzene-type photoswitches and the monitoring of photodegradation through photocatalysis of organic pollutants. We will focus on three contaminants: 5-fluorouracil, an anti-cancer agent; 2,4-dihydroxybenzoic acid, a degradation product of salicylic acid found in effluents from chemical industries; and perfluorooctanoic acid, a persistent pollutant. The advantages of low-field NMR in the study of photoinduced reaction, as well as its limitations, will also be discussed.

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YO-64

UV-Vis AND NIR SPECTROSCOPY FOR THE DETECTION AND QUANTIFICATION OF ADULTERATION IN EXTRA VIRGIN OLIVE OIL

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ABSTRACT: Extra virgin olive oil is one of the cornerstone foods of the Mediterranean diet. To be classified as "extra virgin," it must meet strict chemical parameters and be free from organoleptic defects. This study aims to evaluate and compare the effectiveness of UV-Vis and NIR spectroscopy, combined with different chemometric models (PLS, SVM, and RF), in detecting and quantifying the adulteration of extra virgin olive oil with virgin olive oil at various concentrations. These two analytical techniques are characterized by their simplicity, low cost, speed, and minimal sample preparation, making them attractive tools for food analysis. These high-quality standards significantly increase its market value, making it particularly vulnerable to adulteration. To this end, 14 levels of adulteration were prepared, ranging from 0% to 30% in 5% increments, and from 30% to 100% in 10% increments. The main challenge of this study lies in the high compositional similarity between extra virgin olive oil and the adulterant.

The results demonstrate the strong potential of both techniques, coupled with the proposed chemometric models, to accurately predict the degree of adulteration.

Keywords: extra virgin olive oil, adulteration, UV-Vis, NIR, regression, PLS, SVM, RF.

YO-65**VISUALIZATION OF SPACE STRESS UNDER LOW GRAVITY USING HAIR SAMPLES****Kuwaba, Yuya; Taira, Shu.***Graduate school of Agricultural Science, Fukushima, Japan.***Introduction**

The Artemis program is NASA's initiative to return humans to the Moon and establish a sustainable presence there within 50 years. However, it is expected that we take unknown stress under low gravity what is called space stress. To understand new type stress through assessment and confirmation based on objective indicator, we need to develop novel techniques to replace common methods such as interview and blood test. Moreover, we should avoid invasive blood correction in zero or low gravity from a safety standpoint.

Our research group choose hair as new sample to reveal and understand biological condition. Hair contains an enormous amount of molecular information that is sensitive to chemical and physiologic influences. Because hair grows, it is possible to evaluate biological changes over time by analyzing hair samples from the root to the shaft. Thus, hair samples can be used to obtain daily information non-invasively.

In this research, we analyzed mouse fur that raised in International Space Station (ISS) for 30 days under 1/6 G (FLT) to find space stress marker by comparison with grand mice (GL) as control. In addition, we successfully image the time-course changes in candidate of space stress marker by imaging mass spectrometry (IMS)(1).

Method

The fur of FLT mice (n=12) was crushed and extracted with methanol. A non-targeted analysis was performed using LC-MS to explore biomarkers. For visualization of the fur, longitudinal cross-sections were prepared using a hair sectioning device (KATANA) and analyzed by nanoparticle-assisted imaging mass spectrometry (Nano-PALDI-IMS). As a control, the same analysis was conducted on the fur of GL mice (n=12).

Results

A differential analysis revealed significant differences in 40 compounds between the two groups (FLT and GL) ($p < 0.05$). Notably, certain amino acids tended to increase significantly in the mice fur reared under microgravity conditions. Enrichment analysis showed significant differences particularly in metabolites related to the BCAA metabolic pathway. Extracting the top 25 compounds with differences between the two groups from the non-targeted analysis yielded an interesting result: compounds highly expressed in FLT mice were lowly expressed in GL mice, while those highly expressed in GL mice were lowly expressed in FLT mice.

Discussion

In microgravity, muscle atrophy occurs, leading to an increase in blood amino acid levels. A similar increase in amino acids was observed in body hair, suggesting that BCAAs, in particular, may be transferred to hair instead of being metabolized into proteins.

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YO-66

EFFECTS OF VITAMINS ON CATECHOLAMINES IN A PARKINSON'S DISEASE MODEL MOUSE

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Introduction

Vitamins are not merely essential nutrients; they perform diverse functions in vivo and play a crucial role in maintaining health. Their multifaceted physiological functions have gained increasing attention in recent years, expanding the possibility of nutritional approaches for neurodegenerative diseases. Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases, with a growing number of patients worldwide, posing a significant challenge in an aging society. Currently, symptomatic treatment centered on L-DOPA is widely used; however, long-term use leads to motor complications and treatment resistance, which remain major issues. Therefore, the development of new treatment strategies is urgently needed. Some vitamins promote neurotransmitter synthesis and possess antioxidant and anti-inflammatory properties, making nutritional interventions utilizing these characteristics a promising approach for PD prevention and treatment. However, most previous studies have focused on the neuroprotective effects of individual vitamins, and few have analyzed their combined effects or clearly demonstrated potential synergistic benefits.

In this study, we investigate the effects of combined administration of retinol (vitamin A₁:VA₁), thiamine (vitamin B₁:VB₁) and pyridoxine (vitamin B₆:VB₆) on catecholamine metabolism in brain using a unilateral PD mouse, exploring the potential of new nutritional interventions. VA₁ promotes neuroplasticity and has antioxidant properties, VB₁ supports energy metabolism and exhibits neuroprotective effects, and VB₆ facilitates dopamine synthesis and has anti-inflammatory effects. Each vitamin may help maintain neural function through distinct pathways. This study aims to clarify the neuroprotective and functional restorative effects of a novel therapeutic approach by using these vitamins in combination.

Method

We established a unilateral PD model mouse by injecting 6-hydroxydopamine (6-OHDA) into the right striatum. Vitamins were administered orally. To assess the progression of PD symptoms, a rotation test was performed once per week for seven weeks following subcutaneous injection of apomorphine. After seven weeks of treatment, the brains were extracted and analyzed using imaging mass spectrometry (IMS)¹ with MALDI-Q-TOF to examine the distribution of catecholamines in the striatum and substantia nigra. Additionally, the number of tyrosine hydroxylase (TH)-positive cells was evaluated by immunostaining and c-Fos staining was performed to assess changes in neuronal activity.

Results

Rotation experiment showed vitamin-treated group had fewer rotations. This indicated a delay in PD symptom progression. For IMS results, oral vitamin administration effectively preserved dopamine levels in the striatum and substantia nigra of PD model mice. In the untreated group, dopamine levels in the right striatum dropped to 0.01%, whereas 90% of baseline levels were maintained in the vitamin-treated group. Furthermore, c-Fos staining showed significant changes in the expression of c-Fos-positive cells in the striatum. The number of c-Fos-positive cells in the untreated group was increased 1.25 times compared with that of vitamin-treated group ($p < 0.05$ *). These findings suggest that vitamin treatment may suppress c-Fos expression and be associated with changes in neural activity. Collectively, these results indicate that combined vitamin administration may exert neuroprotective and symptom-modifying effects in PD through both biochemical preservation and functional modulation of neural circuits.

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[1] Taira S, Kaneko D, Shikano H, Miyama Y, Kurosawa T, Kuwaba Y, Miyabayashi A, et al. Comparative Localization Analysis of Immature Soybean (Edamame) Content via Mass Spectrometry Imaging. *Sensors and Materials*. 2024;36(3):841-8.

YO-67**MULTISCALE AND MULTIMODAL IMAGE FUSION. JOINING CHEMICAL AND MORPHOLOGICAL INFORMATION OF RAMAN/FLUORESCENCE IMAGES OF LABELLED SAMPLES**

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Nowadays, researchers employ simultaneously different spectroscopic imaging platforms to study biological samples and obtain more comprehensive information about the nature of their sample constituents. However, the vast amount of data and the complexity of the interpretation present significant challenges for suitable analyses.

Image fusion actively exploits the complementary information among spectroscopic techniques by combining data from different HSI modalities but faces often difficulties linked to spectroscopic and spatial differences among the platforms to be combined. Unmixing analysis techniques offer a good option to obtain concentration maps and spectral fingerprints of the image constituents from the sole information in the raw measurement, but current approaches experience some limitations in image fusion scenarios.^{1,2}

In this work, a challenging example of images of HeLa cells labelled with external fluorophores were measured with fluorescence and Raman microscopies is presented. In this context, the high spatial resolution of fluorescence images provided detailed spatial and morphological information of the cellular compartments labelled while Raman images provided spectral profiles with a very rich chemical information. The differences in area scanned and spatial resolution among the two imaging techniques used required the use of a so-called incomplete multiset structure, which concatenated all available imaging information at the cost of leaving empty blocks when a part of image information did not have an equivalence between platforms.³

To handle this complex data structure, a recent variant of the Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) unmixing algorithm, able to analyse incomplete multiset structures, was used. In doing so, the simultaneous analysis of fluorescence and Raman images could be carried out via a single factorization model that kept the high spatial resolution of fluorescence and exploited the rich chemical information of Raman spectra.⁴

The pure distribution maps and pure spectral profiles of cellular components such as cytoplasm, nucleolus, nucleus, lipid droplets, protein regions, and actin cytoskeleton were successfully recovered, allowing a full chemical and spatial characterization of the main cellular constituents.

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YO-68

BIOMARKER FROM A SINGLE HAIR TO PREDICT PRENATAL AND POSTPARTUM DEPRESSIVE SYMPTOMS IN PREGNANT FEMALES AND THEIR PARTNERS

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Introduction

Postpartum depression affects 10–15% of females and 7–10% of males. Studies have shown that parents influence each other's mental health.

In recent years, there has been growing interest in biomarkers for assessing and predicting stress-related mood disorders. The endocannabinoid system (ECS), including anandamide (AEA), protects mental health from stress. However, the role of peripheral cannabinoid-related compounds in predicting perinatal mood disorders remains poorly understood, and objective biomarkers are still lacking. Although blood-based biomarkers have been investigated, their invasive nature limits their feasibility, particularly for partners. Recently, we demonstrated that a single strand of hair can serve as a retrospective biochemical archive when analyzed using nanoparticle-assisted laser desorption/ionization imaging mass spectrometry (Nano-PALDI IMS) (1).

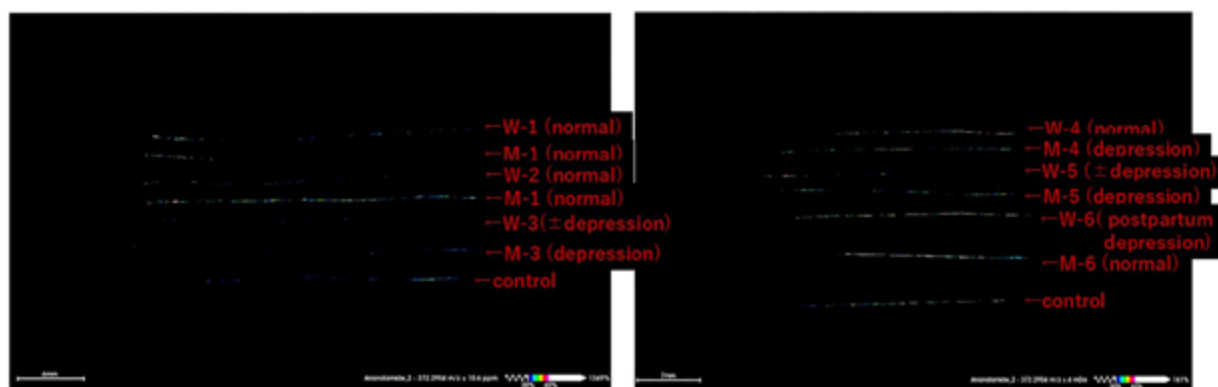
Therefore, in this study, we aimed to investigate whether cannabinoid-related compounds in hair are associated with prenatal and postpartum depressive symptoms in pregnant females and their partners and to identify novel biomarkers using a combination of omics and imaging approaches.

This is the first report to propose cannabinoid components and their related compounds in the hair during pregnancy as potential biomarkers for predicting current or future depressive symptoms in pregnant females and their partners.

Methods

Twelve participants (six pregnant females and their male partners) were included. Each female was assigned an identifier from W1 to W6, and each male was assigned an identifier from M1 to M6. They were recruited as volunteers by the Ministry of Education, Culture, Sports, Science, and Technology, Society 5.0 Project PJ 3-1 Watching Over the First 1000th Day of Life consortium. Additionally, approximately 5–10 strands of hair were collected from females during pregnancy (at 35 weeks of gestation or later, but before delivery) and from their partners, and a 3 cm lateral hair section from the root was analyzed using Nano-PALDI IMS. Biomarker candidates were identified using omics software (MetaboScape; Bruker). This study was reviewed and approved by the Ethics Committee of Osaka University.

Fig. Imaging of Anandamide (AEA) in Pregnancy-Period Hair Sections of Participants With and Without Depressive Symptoms



Results

Imaging of longitudinal hair sections using Nano-PALDI IMS suggested a potential association between endocannabinoid components and depressive symptoms.

AEA, a key endocannabinoid, was found at lower levels in the hair of W3 and W5, and they exhibited mild depressive symptoms during pregnancy and in M3, who showed moderate depressive symptoms. In contrast, M6 did not show low AEA levels during pregnancy but developed depressive symptoms one month postpartum.

O-Arachidonoyl Ethanolamine (O-AEA), another endocannabinoid, was successfully detected in measurable amounts in the pregnancy period hair from W1 to W3 and M1 to M3. Among them, M3, who exhibited moderate depressive symptoms, had the highest O-AEA levels.

Melatonin, which is involved in sleep regulation and ECS activation, was observed at higher levels in the W5 and M5. They both exhibited depressive symptoms. Although the detection of melatonin in hair has not been widely established, similarities in imaging results between couples suggest that the sleep environment may influence melatonin production. Cortisol, a well-known stress biomarker, shows no clear correlation with psychological stress.

These findings suggest that low AEA levels are associated with the onset of depressive symptoms. Further research is needed to explore the relationship between the ECS components, melatonin, and related compounds. Although this study has not yet established a method for predicting postpartum depression, increasing the sample size in future studies is essential to further validate these findings.

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[1] Taira S, Shikano H, Takahashi N. Analysis of hair components by nanoparticle-assisted laser desorption/ionization mass spectrometry imaging. *Arabian Journal of Chemistry*. 2022;15(11):104205

YO-69**HYPERSPECTRAL IMAGING COMBINED WITH CHEMOMETRIC ANALYSIS FOR CHARACTERIZATION AND VISUALIZATION OF ISCHEMIC INTESTINAL NECROSIS****Peng, Chenxi¹; Tauler, Roma²; De Juan, Anna¹.**¹University of Barcelona, Barcelona, Spain; ²IDAEA-CSIC, Barcelona, Spain.

Ischemic intestinal necrosis is a life-threatening pathological condition closely associated with various severe diseases such as necrotizing enterocolitis (NEC) and acute mesenteric ischemia (AMI). Accurate and timely identification of necrotic intestinal segments is crucial for clinical diagnosis and treatment decisions. Hyperspectral imaging (HSI), as a rapid, non-destructive, and real-time imaging technology, has demonstrated great potential for detecting ischemic necrosis of intestinal tissue due to its ability to acquire both spectral and spatial information. However, the complex pathological environment and subtle spectral differences between normal and necrotic tissues pose significant challenges for the precise analysis of ischemic necrosis using HSI.

Among these challenges, the identification of characteristic spectral bands plays a key role in enhancing the accuracy and interpretability of necrotic tissue analysis. Indeed, most conventional methods for spectral band selection often suffer from empirical dependency and lack of objectivity, limiting their application in practical scenarios.

To address this problem, a preliminary study involved the analysis of hyperspectral images with cell-wise outlier detection techniques to automatically and accurately identify characteristic spectral bands for ischemic necrotic intestinal tissue [1]. Using a rabbit small intestine model to simulate intestinal ischemia, the study successfully identified the band near 763 nm — closely related to the absorption of deoxyhemoglobin — as a robust spectral marker for distinguishing necrotic tissues. This result not only provides valuable spectral indicators for the early detection of intestinal necrosis but also offers theoretical guidance for the selection of optical filters in clinical spectroscopic devices.

In a subsequent recent study [2], it was recognized that beyond spectral band identification, there is an urgent need for real-time discrimination and visualization of the progression of intestinal ischemia and necrosis. This second study was aimed to develop advanced chemometric methods combined with HSI to achieve comprehensive analysis of ischemic intestinal tissue from spectral, spatial, and temporal dimensions. Multivariate Curve Resolution (MCR) has been proposed as a powerful chemometric tool capable of resolving pure component contributions from complex imaging datasets [3]. The feasibility of integrating MCR with HSI for visualizing the dynamic evolution of intestinal necrosis will be shown, allowing a deeper understanding of occurring pathological process and providing new insights for clinical monitoring and decision-making.

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YO-70

DESIGN OF A MICROFLUIDIC PAPER-BASED DEVICE (μ PAD) FOR THE DETERMINATION OF TOTAL PHENOLIC CONTENT BY FBFB METHOD

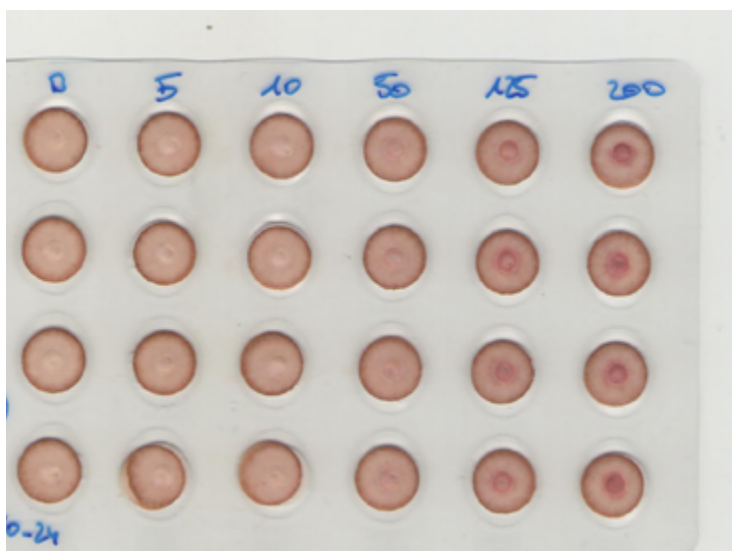
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In this work, the design of a microfluidic paper-based analytical device (μ PAD) for the determination of total phenolic content (TPC) by the Fast Blue BB (FBFB) method [1] is described. This method is being used due to the advantage of not reacting with non-phenolic compounds, unlike the Folin-Ciocalteu method traditionally used for the determination of TPC. In addition, there are already μ PADs reported employing the traditional method.

Paper devices are cheaper than traditional spectrophotometric methods, more accessible, and portable [2]. Small amounts of reagents are used, and they are easily disposable. For the device construction, a vertical flow 3D arrangement was chosen, and filter paper discs aligned within perforated laminating pouches was the procedure used to establish hydrophilic and hydrophobic areas [3]. The lamination technique used represents a good alternative to the wax printing technique. The latter is one of the more used procedures, but it is no longer easily accessible as commercial wax printers have been discontinued due to their reported harmful and toxic consumables [4].

For the optimization of the TPC determination μ PAD, several variables associated with the design of the device were studied, namely filter paper porosity, paper treatment, and paper disc size, among others. Furthermore, different standards were tested (gallic, caffeic and trans-ferulic acids, catechin and epicatechin) for potential application to different samples such as wine and herbal infusions. Stability studies under different conditions were also performed. The studies were carried out in Gallic Acid Equivalents (GAE) for tea/infusion samples and in Catechin equivalents (CaE) for wine samples. The developed μ PADs achieved a linear range of 5-200 mg GAE/L and 25-125 CaE/L with a limit of detection of 6.21 mg GAE/L and 4.93 mg CaE/L, and a limit of quantification of 21.63 mg GAE/L and 16.44 mg CaE/L.



Precision and accuracy below 11% were obtained. The designed device was stable for 7 days when stored at room temperature in vacuum atmosphere, and the color reaction stability depended on the standard, up to 30 min for GA and up to 60 min for CaE. A μ PAD, with which 24 determinations can be made, consumes 2.16 mg of FBFB reagent, 24 mg of NaOH and 60 μ L of each sample.

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YO-71

DEVELOPMENT AND APPLICATION OF AN AUTOMATED METHODOLOGY FOR THE DETERMINATION OF TETRACYCLINES IN ENVIRONMENTAL SAMPLES

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Antibiotics are by design difficult to remove in the biological treatment of wastewater and are becoming ubiquitous in aquatic environments, which bears the risk for the formation of resistant bacteria strains. Concentrations in aquatic environments and sediments have been reported from the ppt to ppb range [1]. The presence of antibiotics in surface water, groundwater, and even drinking water is increasingly addressed, too. Many harmful effects of their presence in the aquatic environment can already be observed, including drug resistance in many animal species, as well as in humans. Due to the new EU Urban Wastewater Treatment Directive, it is necessary to develop fast, cheap, efficient and environmentally friendly methods for determining antibiotics. However, for their analysis, there is general the need for sample preparation and analyte preconcentration. For this purpose, liquid phase extractions remain reliable and often approaches while simultaneously, they show important drawbacks in terms of procedural greenness.

Recently, the use of deep eutectic solvents (DES) for microextraction has attracted the attention of researchers. They are considered an environmentally friendly alternative to conventional organic solvents due to their negligible vapor pressure, miscibility with other solvents, low toxicity, and high thermal stability [2,3]. Due to their ability to undergo various types of analyte interaction, they allow to extract efficiently also moderately polar analytes.

Moreover, automation of the preparation step, often allowing simultaneous downscaling and enhancement of procedural reproducibility and reliability, can further improve method greenness. Here, an early attempt to automate such process using DES from 2022 should be highlighted [4].

We present an automated dispersive liquid-liquid microextraction method, which was automated using the Lab-In-Syringe technique and coupled only to high-performance liquid chromatography. The method was developed for the determination of selected antibiotics namely tetracycline, oxytetracycline, doxycycline, chlortetracycline, sulfamethoxazole, and trimethoprim in environmental waters. The extraction procedure including intermediate system cleaning is accomplished within 8 minutes using only 200 μL of a hydrophobic DES and 1.7 mL of sample. The method showed excellent linearity, detection limits ranging from 9.9 to 30.3 $\mu\text{g L}^{-1}$, high precision with RSD values typically below 4.3% and analyte recoveries similar to previously reported preparation processes. Optimization of key parameters including DES type, sample and solvent volumes, pH, salting out, stirring speed, and critical times is reported. The method was successfully applied to the determination of selected antibiotics in surface waters demonstrating its practical application. These results highlight the potential of DES-based extractions as well as procedural automation in improving analytical methods for pharmaceuticals in environmental samples.

Acknowledgments:

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YO-72**ARDUINO BOARD FOR THE DETERMINATION OF COPPER IN WATERS**

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A new colorimetric detection system is being studied to prove its effectivity, and the possibility to its implementation to analysis in situ. For this purpose water from Acequia Real del Júcar is being analysed to determine the quantity of Cu(II) by undergoing a reduction to Cu(I) with ascorbic acid. Cu(I) undergoes a complexation reaction with bicinchoninic acid (BCA) giving a purple solution easily detected naked eye.

This system is based on an Arduino motherboard which connects a 566 nm emitting LED with a cuvette holder leading to a photodetector which gives emitted radiation data. For the reference system, Flame Atomic Absorption Spectroscopy (FAAS) was used as it gives the total copper composition without taking into account the oxidation state of the element in the samples. UV-Vis spectroscopy was used also as a reference method as the development of the colour in the copper samples depends on the concentration of the reagents, including the reduction of Cu(II) to Cu(I).

The calibrations were prepared to study the reach of the linearity in the reference system of UV-Vis and compare it to the Arduino board, for this purpose, a calibration line with concentrations ranging from 0 to 12 mg/L improving 2 mg/L in each step was prepared. It was studied the effect of the concentration of the BCA and ascorbic acid in the reaction by fixing the content of one of the reagents and changing the concentration of the other. In the end, the most appropriate volumes of reagents were 30 µL of ascorbic acid (9.2 g/L) and 60 µL of BCA (1 %).

In the UV-Vis system it was also observed that the linearity was lost at the 6 mg/L breakpoint, meaning that the system worked better in more diluted concentrations (from 0 to 6 mg/L), which could be due to the saturation of the colour of the calibration, but with the Arduino Board it could be achieved linearity till 12 mg/L of copper.

As the samples were obtained straight from the irrigation ditch of the Acequia Real del Júcar, it was needed to study the matrix effect of the water. In order to determine this parameter, it was used ultrapure water and water from the ditch to prepare a matrix matched calibration. Once prepared the calibrations and measured, UV-Vis spectroscopy and the Arduino Board system, both gave out non-significant variations in the slope of the calibration line.

Once all the parameters for the preparation of the samples were settled, a calibration line ranging from 0 to 5 mg/L with improvements of 0.5 mg/L was prepared and measured in UV-Vis and Arduino Board, the concentrations of the interpolated samples were compared in between the two reference methods, FAAS and UV-Vis, and then Arduino Board Results was compared against the UV-Vis, which gave out comparable results.

As an addition, image colour processing is being studied as an alternative to obtain analyte concentrations taking into account for this study the parameter C* which is dependant to the concentration of the BCA-Cu (I) complex.

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YO-73**ENHANCING LIGNIN RECOVERY FROM COCONUT HUSK: INNOVATIONS WITH DEEP EUTECTIC SOLVENTS FOR SUSTAINABLE BIOMASS PROCESSING****Todolí Carbonell, Mar; Sánchez Romero, Raquel; Beltrán Sanahuja, Ana.***Universidad de Alicante, Alicante, Spain.*

Currently, approximately 1.3 billion tons of food are wasted globally each year, with fruit being the most wasted category. (Informe Del Consumo de Alimentación, Spain, 2016). In the case of coconuts, a tropical fruit of the palm tree, only 17% of the fruit is used, while the remaining 83% is considered waste, despite being a lignocellulosic biomass rich in lignin, cellulose, and hemicellulose. (Obeng et al., 2020)

Conventional lignin extraction methods are typically time-consuming (up to 6 hours) and require a large amount of reagents, achieving extraction yields of up to 70%. (Saini et al., 2024) These limitations highlight the need for more efficient and sustainable alternatives. This study aims to develop and optimize green methods for lignin extraction from coconut husk using Deep Eutectic Solvents (DES), with a focus on a ternary DES system. Key extraction parameters were optimized (pH, sample amount, extraction time, and ultrasonic power) using a Box-Behnken experimental design, which enables efficient multivariable optimization while minimizing the number of experimental runs. One of the developed methods was based on the use of an ultrasonic bath, achieving a 100% extraction yield in one and a half hours. Under the same time frame, an ultrasonic probe yielded 80%. Notably, a novel combined ultrasonic approach reduced the extraction time to just 30 minutes while maintaining an 80% yield. These yields were calculated relative to the total lignin content in the coconut husk. Compared to conventional and other modern extraction methods—which typically require about one hour and achieve 70–80% yields—this approach demonstrates superior time efficiency and, in some cases, higher extraction yields. (Salgado-Ramos et al, 2022.; Ma et al., 2022)

After extraction, lignin recovery was performed by acidifying the solution to a specific pH, resulting in a 60% recovery yield. This process allows the lignin to precipitate in solid form, making it readily available for various applications. The recovered lignin was subsequently characterized to assess its potential value. Thermogravimetric Analysis (TGA) revealed a decomposition onset at 180 °C (5% weight loss). This result indicated good thermal stability, an essential property for applications requiring heat resistance. Additionally, Total Phenolic Content (TPC) test showed values of up to 300 mg/kg, highlighting the lignin antioxidant potential and its applicability to industries requiring antioxidant-rich materials. The high phenolic content suggests its suitability for applications in food preservation, cosmetics, or pharmaceutical formulations, where antioxidant properties play a crucial role extending shelf life and enhancing product stability. Overall, the results obtained have important implications for the biomass industry and contribute to the transition toward a circular and sustainable economy.

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YO-74**HYDROGEL-BASED STOCHASTIC SENSOR FOR SIMULTANEOUS DETERMINATION OF VCAM-1, IL-6, AND NPPC - AN INNOVATIVE APPROACH IN THE EARLY DIAGNOSIS OF CARDIOVASCULAR DISEASES****Stefan, Andrada-Ioana; State, Ramona; Stefan-Van Staden, Raluca-Ioana.***National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania.*

Cardiovascular diseases (CVDs) represent a significant global health threat, closely linked to obesity and characterized by key biomarkers such as vascular cell adhesion protein 1 (VCAM-1), interleukin 6 (IL-6), and natriuretic peptide precursor C (NPPC). Rapid and accurate detection of these biomarkers is crucial for early diagnosis and effective disease management. However, conventional detection methods are often costly, time-consuming, and require complex procedures. Stochastic sensing has emerged as a promising alternative due to its affordability, rapid response, and straightforward sensor fabrication. In this study, a novel carbon paste based sensor was developed, incorporating a chitosan hydrogel (CS), multi-walled carbon nanotubes decorated with synthesized gold nanoparticles (AuNPs@MWCNT), and β -cyclodextrin (β -CD) to enhance sensitivity and selectivity. The sensor was applied to both healthy and cardiovascular patient blood samples for qualitative and quantitative biomarker assessment. The hydrogel matrix optimized sensor performance by facilitating bioactive substance monitoring and controlling electrochemical reactions. Electrochemical characterization via cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and stochastic method confirmed that the proposed sensor (β -CD/AuNPs@MWCNT/CS/CPE) has exceptional sensitivity, fast response, and selective biomarker detection capabilities. This study highlights the potential of hydrogel-based electrochemical sensors for biomarker detection in CVD diagnosis. The integration of hydrogel properties with advanced electrochemical sensors paves the way for biocompatible, efficient, and innovative diagnostic technologies.

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YO-75

SUSTAINABLE VALORIZATION OF BIOACTIVE COMPOUNDS FROM CHESTNUT HEDGEHOGS BIOWASTE: A BIOREFINERY APPROACH

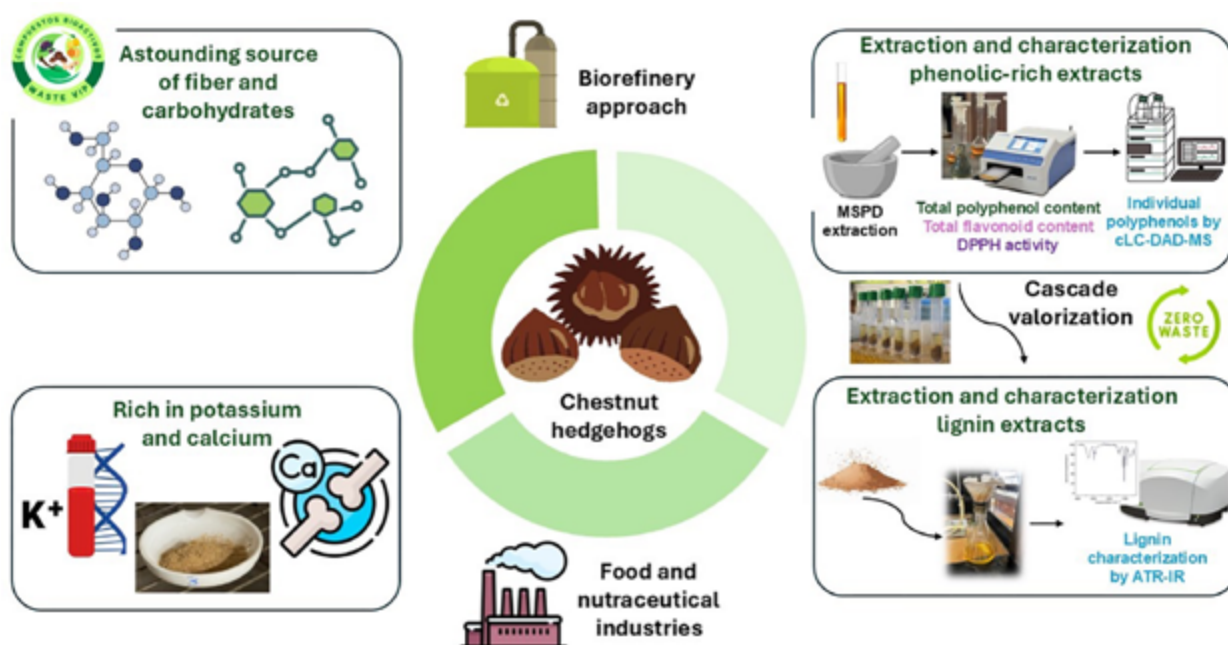
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In the last few years, owing the global environmental concerns, sustainable waste recovery to obtain high value-added compounds has become an issue of utmost importance in the world [1]. Chestnut hedgehogs are poorly explored by-products of agricultural and food industries; however, they are an underutilized source of bioactive compounds, mainly lignin and polyphenols [2-4].

In this line, the present study proposes a cascade biorefinery process to obtain bioactive compounds (polyphenols and lignin) from chestnut hedgehog residues of Avila geographic origin. Firstly, a nutritional composition analysis was carried out using the official AOAC standard methods for the determination of moisture, ash, fat, crude fiber, protein and carbohydrates. Subsequently, the content of metals (Ca, Fe, Zn, Cu and K) was determined individually by flame atomic absorption and atomic emission spectroscopy, respectively. Following, an effective and sustainable matrix solid-phase dispersion (MSPD) extraction procedure, using a mixture of ethanol and water and sand as dispersant, has been developed and optimizing employing a multi-level experimental design and response surface methodology, for the recovery of polyphenols from chestnut hedgehogs. The phenolic extracts were characterized in terms of total flavonoid content (TFC) and DPPH antioxidant activity. Furthermore, the profile of individual polyphenols was determined by cLC-DAD-MS. Additionally from the solid residue generated in the polyphenol extraction step, a second lignin extraction was conducted in cascade with the previous one; and the lignin content was characterized by ATR-FTIR. Complementarily, the bioactive activities of the obtained extracts were evaluated. The findings showed that chestnut hedgehogs waste constitute a biomass rich in fiber, carbohydrates, potassium and calcium, also exhibiting bioactive properties.

Finally, the results underline the prospective of chestnut hedgehog's by-products for recovering phenol-rich and lignin bioactive extracts, which could be utilized in the food and nutraceutical industries. This approach offers a feasible and potential biorefinery approach for the cascade valorization of chestnut agri-food waste [2,5].



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YO-76**DEVELOPMENT OF A DLLME-BASED METHOD USING NADES IN COMBINATION WITH LC-MS/MS FOR THE DETERMINATION OF ILLICIT DRUGS IN URINE SAMPLES****Clivillé-Cabré, Pol; Borrull, Francesc; Aguilar, Carme; Calull, Marta; Fontanals, Núria.***Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Sescelades Campus, Marcel·lí Domingo 1, 43007, Tarragona, Spain.*

In recent years, one of the major social issues has been the increased consumption of illicit drugs. As a result, the scientific community has shown growing interest developing of new methods for their determination. Cocaine and amphetamines are some of the most commonly consumed drugs, followed by opioids and benzodiazepines [1].

As a consequence of their consumption, these compounds can be usually found at low levels of concentration (in the ng/L or µg/L range) in urine samples. Since urine is a highly complex matrix, a sample pre-treatment step is required. This step plays a significant role on the overall greenness of the analytical method. In recent years, new miniaturised versions of traditional extraction techniques have been developed, alongside the introduction of alternative solvents to replace conventional hazardous organic solvents. Among the most promising alternatives are natural deep eutectic solvents (NADES), which are mixtures of naturally derived, non-toxic compounds [2–4].

In the present study, dispersive liquid-liquid microextraction (DLLME) was selected for the extraction of cocaine and benzoylecgonine, four amphetamine-type stimulants, five benzodiazepines and six opioids, using a NADES as the extractant. NADES usually are synthesized from natural compounds such as menthol or thymol in combination with organic acids [3]. In the present study, thymol and octanoic acid in a molar proportion of 1:1, was selected as the extractant phase. For the extraction, were used 200 µL of NADES as the extractant and 500 µL of ethyl acetate as the dispersant. The method achieved recoveries in the range between 8% and 100% for all the compounds, with limits of detection between 0.1 and 0.5 µg/L.

The method was validated using urine samples and then applied to 12 urine specimens from women who were starting a detoxification program, confirming the suitability of the developed method in toxicological and forensic analysis. The analysed urine samples revealed that polyconsumption was a common trend among the patients. The most frequently detected substances were cocaine and its main metabolite benzoylecgonine, and amphetamine-types stimulants (amphetamine and MDMA).

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YO-77**MICROWAVE-ASSISTED EXTRACTION OF PROTEINS FROM CITRUS PEELS. A COMPARATIVE STUDY OF DIFFERENT PROTEASES TO OBTAIN BIOACTIVE HYDROLYSATES**

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The agri-food industry is responsible for the generation of a substantial amount of waste during production processes. That waste may contain high-value bioactive compounds that, if properly utilized, can significantly contribute to circular economy and to the sustainability of the food chain. The use of food residues as sustainable sources of value-added products is a growing area of interest, in response to the global demand for environmentally friendly practices in the food industry. Residues from fruits and vegetables, such as citrus peels, are often discarded despite their potential to provide bioactive compounds (proteins, antioxidants, and other functional compounds). The valorization of these residues through green techniques, such as microwave-assisted extraction (MAE), enables to obtain compounds with applications in various fields, including food, pharmaceutical, and cosmetic industries. In the case of protein extraction, MAE offers advantages in terms of efficiency and reduced environmental impact when compared with the conventional techniques usually employed for this purpose [1].

The aim of this work was to develop an analytical methodology based on MAE for the sustainable extraction of proteins from citrus peels (lemon and mandarin) and to evaluate different protease enzymes to obtain bioactive peptides from protein extracts. A Box-Behnken experimental design was employed to determine the most favourable conditions (percentage of ethanol, temperature, and time) for the extraction of proteins. The optimal conditions were established by maximising the protein content and minimising the formation of Maillard compounds. The protein extracts obtained were then characterized and hydrolysed to obtain bioactive peptides. The experimental conditions used for different protease enzymes were optimized and their potential to obtain bioactive peptides was compared. The peptide content and degree of hydrolysis were determined in the hydrolysates, and different bioactivities (antioxidant, antihypertensive, and antimicrobial) were evaluated. Finally, most active hydrolysates were analysed by UHPLC-MS/MS for peptide identification.

Acknowledgements

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YO-78**A NOVEL SORBENT STRIP MICROEXTRACTION (SS μ E) TECHNIQUE FOR OPIOIDS AND LOCAL ANAESTHETICS DETERMINATION IN URINE****Maria, Marisa¹; Berg, Thomas²; Neng, Nuno³.**

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The issue of drug abuse extends beyond the consumption of illicit substances. Medications like analgesics, opioid substitution drugs, sedatives and hypnotics are often used to induce psychoactive effects or to modify the effects of other substances, potentially resulting in dependence. Misuse, such as increasing dosages without medical supervision, may also lead to serious health consequences [1]. Therefore, effective monitoring and regulation of these substances are essential for safeguarding public health. In forensic and clinical laboratories performing routine analyses of biological matrices, techniques such as protein precipitation, liquid-liquid extraction, and solid-phase extraction are commonly employed [2]. While these methods are fast and effective, some are not cost-efficient, despite efforts to reduce the use of organic solvents by minimizing the volumes of both the biological matrix and extraction solvent.

Sorption-based techniques, such as solid phase microextraction and stir bar sorptive extraction, have become essential tools in modern sample preparation for analysing complex matrices. Our group has also developed bar adsorptive microextraction technique with several applications for different matrices [3]. These techniques offer simplicity, speed and minimal sample volume requirements, and align well with green analytical chemistry (GAC) principles, promoting more sustainable and eco-friendly practices [4].

To further advance sorption-based techniques, this study entailed the development, optimization, validation, and application of a novel analytical approach, using an innovative extraction technique entitled Sorbent Strip Microextraction (SS μ E). This novel method determines three opioids (buprenorphine, tapentadol and tramadol) and two local anaesthetics (articaine and bupivacaine) in urine.

Simple microextraction strips coated with a sorbent phase were employed for the first time in combination with high-performance liquid chromatography with diode array detection (HPLC-DAD). Various parameters that could influence SS μ E were evaluated and optimized to enhance the analyte recovery from urine. These included sorbent selectivity, desorption conditions and microextraction parameters affecting SS μ E efficiency. Under optimized conditions, recoveries ranged from 78 % (bupivacaine) to 108 % (tramadol). Additionally, trueness ($\leq \pm 7$ %) and precision (≤ 13 %) were achieved.

The validation assays of the proposed methodology demonstrated appropriate linear dynamic ranges (5–500 ng/mL for the local anaesthetics and 50–5000 ng/mL for the opioids) and good determination coefficient ($r^2 \geq 0.9922$), as well as, excellent selectivity, robustness and accuracy.

The developed and validated methodology (SS μ E/HPLC-DAD) was successfully applied for screening the opioids and local anaesthetics in urine samples. The proposed analytical methodology proved to be an eco-user-friendly approach to monitor these five compounds in complex urine matrices, offering a distinguished analytical alternative compared to other microextraction-based techniques.

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YO-79**VALIDATION OF AN ANALYTICAL METHOD FOR RESIDUAL PESTICIDE QUANTIFICATION IN HDPE AGROCHEMICAL CONTAINERS USING UPLC-MS/MS**

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Pesticide remains contained in agrochemical packaging waste are a source of uncontrolled risk for human health; they are also a quality feedstock for the plastic recycling industry. Many governments have recently started to establish laws and regulations to develop systems for recovering and recycling the polymeric packages used for pesticides. There is also a demand in having a procedure to control the suitability of the pesticide packages to be reused [1].

This study presents a validated analytical method for the determination of pesticide residues in high-density polyethylene (HDPE) agrochemical containers. The method was developed to quantify 88 diverse pesticides, including both non-ionic and ionic compounds, using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). For this purpose, 88 pesticides were classified into 8 different groups based on their chemical stability, and their standard solutions were prepared separately. Two solvent systems were optimized for preparing standards and the extraction process: TBME-acetone-methanol (50:25:25) for non-ionic pesticides and methanol-water (50:50) for ionic pesticides.

For sample preparation, unused high-density polyethylene (HDPE) containers were manually cut into 1 × 1 cm pieces. 10 gr of the pieces were fortified by 20 ml of the standard solutions. These were subjected to ultrasonic-assisted extraction using 80 mL of fresh solvent at 50°C for 2 hours. After extraction, the solution was transferred to a 250 mL volumetric flask. The remaining plastic pieces were then air-dried at room temperature and ground into a fine powder (particle size ~500 µm) using a Retsch ZM 200 centrifugal mill. The pulverized material was further extracted using the same procedure—80 mL of solvent, sonication for 2 hours at 50°C—followed by an additional rinse with 80 mL of the solvent. The final solution were brought to a volume of 250 mL.

Sample preparation for UPLC analysis involved evaporating 2 mL of each extract solution to dryness under nitrogen. The dry residue was subsequently dissolved in 0.5-5 mL of mobile phase, with the dilution optimized according to concentration. The standards and samples were analyzed separately by Ultra Performance Liquid Chromatography (UPLC), acquity, from Waters Chromatography (Cerdanyola del Vallès, Spain), coupled mass spectrometer with a triple quadrupole analyzer XEVO-TQ-S (Waters).

To validate the analytical method, the HDPE samples were fortified with selected pesticide standards at three concentration levels (0.2×, 1×, and 2× the acceptable contamination level, ACL). The fortified samples were stored at room temperature in the dark for three specific times (24 hours, 48 hours, and 5 days) to evaluate the stability and recovery of the target analytes over time. Method validation included assessment of identity (retention times, MS/MS spectra), specificity, linearity, accuracy, and precision.

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YO-80**COMPARATIVE ELECTROCHEMICAL STUDY OF GRAPHENE-BASED NANOMATERIALS TOWARD DOPAMINE****Mikhralieva, Albina¹; Kruei, Ana²; Zaitsev, Volodymyr².***¹Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro; Tallinn University of Technology, Tallinn; ²Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Carbon-based materials have been used as surface modifiers for glassy carbon electrodes; however, not all of them necessarily improve electrochemical properties. One of the challenges with modern carbon-based materials is classification, which affects their final properties and applications. For instance, carbon-based nanodots can be synthesized from small molecules (bottom-up approach) or etched from larger precursors like graphene oxide (top-down). Researchers were confident about achieving the same final product until 2015, when they discovered the influence of synthesis types, precursors, and purification methods on the morphology and properties of the obtained materials. In 2024, we demonstrated that hydrothermal treatment of graphene oxide in the presence of NH_3 and H_2O_2 leads to the preparation of two absolutely different materials: a small fraction containing graphene quantum dots (GQDs) and meso-hole graphene oxide (MHGO). They have distinct structural, optical, and electrochemical behaviors that confirm the differences between these materials. Perforation of graphene can enhance the specific surface area of the nanosheet, ensuring effective wetting and penetration of electrolytes to the electrode surface, facilitating rapid charge transfer, and boosting the electrocatalytic efficacy of the transducers. Due to their certain degree of similarity regarding oxygen-containing groups, solubility, and photoluminescence, the mechanisms involving both their photoluminescence and electrochemical activity are still debated. There is a need for systematic and comparative studies addressing the electrochemical properties exhibited by these different carbon-based nanodots obtained with different doping elements and their subsequent roles in electron transfer kinetics. MHGO demonstrated a fourfold increase in electrochemical enhancement compared to GQDs. We decided to investigate further into the behavior of nanoparticles and analyzed the influence of doping graphene structures with boron towards dopamine. With this aim, three series containing GQDs and MHGO (without B-doping, using boric acid, and using aminophenylboronic acid) were synthesized. Modification of the electrode was carried out directly or by mixing with Nafion through drop-casting. The electrochemical capabilities of the modified electrode were evaluated against dopamine using cyclic voltammetry.

Albina Mikhralieva, Adriano RS Lima, Cristiane L Jost, Michael Nazarkovsky, Yutao Xing, Volodymyr Zaitsev. Mesoporous Nitrogen-Doped Holey Reduced Graphene Oxide: Preparation, Purification, and Application for Metal-Free Electrochemical Sensing of Dopamine. *Small* 20 (40), 2400650, 2024

YO-81**HPLC-PAD: A USEFUL AMPEROMETRIC DETECTION APPROACH FOR THE DETERMINATION OF ORGANIC MOLECULES WITH COMPLEX ELECTROCHEMICAL BEHAVIOUR**

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Liquid chromatography with amperometric detection (HPLC-EC) is a powerful analytical technique that combines the separation capabilities of liquid chromatography with the sensitive and selective detection of amperometry. This method involves measuring the current produced by the oxidation or reduction of the analytes at an electrode through the application of a constant (negative or positive) potential. HPLC-EC is particularly effective for analyzing electroactive compounds, such as, for instance, the bioactive compounds used for cinnamon authentication: cinnamic acid (CA), cinnamaldehyde (CD), eugenol (EU) and coumarin (CU). However, initial attempts to detect them through HPLC-ED using a screen-printed carbon electrode (SPCE) yielded no results as no compounds were detected by the application of a reductive potential (i.e. -1 V) and only eugenol provided signal in oxidative potentials (i.e. $+1$ V). Thus, the electrochemical behaviour of the four compounds was studied by means of differential pulse voltammetry in static conditions, concluding that the electrochemical detection of CD, CA, EU and CU using a SPCE can be achieved by means of reduction as far as analytes are previously oxidized.

In this context, pulsed amperometric detection (PAD) was considered as an alternative detection technique because it allows the application of a repeating sequence of potential steps with specific durations. PAD has been demonstrated to enhance detection sensitivity and to overcome problems related to electrode fouling and, in this context, it could also be implemented for the electrochemical determination of organic compounds that require an oxidation or reduction step prior to their electrochemical detection.

Thus, this study aims to evaluate the possibilities of HPLC-PAD for the detection of compounds that require a preoxidation or prereduction stage prior to their detection, such as CD, CA, EU and CU. For this purpose, the shape of the PAD wave was first optimized considering two potential values: (i) $+1$ V to preoxidize the species, and (ii) -1.5 V to reduce the previously oxidized species. In this sense, oxidation and reduction times (t_{ox} , t_{red}) were evaluated considering several values and ratios, which leads to distinct shapes of the PAD wave. For analytical performance evaluation, two extreme situations ($t_{ox} \gg t_{red}$ and $t_{ox} \ll t_{red}$) were considered. The results indicated that the PAD wave with $t_{ox} \gg t_{red}$ provides a better analytical performance for most of the tested compounds, i.e., CD, CA and EU. In contrast, CU, which is the less electroactive compound, shows a better analytical performance at the PAD wave with $t_{ox} \ll t_{red}$.

The applicability of HPLC-PAD was validated through the determination of CD, CA, EU and CU in cinnamon samples. The obtained results are comparable to those achieved by means of HPLC-UV, which was considered as reference analytical technique. Thus, the results presented in this work demonstrate the usefulness of HPLC-PAD in the determination of organic molecules that require a two-step electrochemical process and cannot be directly determined through standard HPLC-EC.

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YO-82**INTEGRATION OF PAPER-BASED ELECTRODES INTO LATERAL FLOW ASSAY FOR ELECTROCHEMICAL DETECTION**

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Lateral flow assay (LFA) is a widely used diagnostic tool due to its rapid response time, ease of use, and cost-effectiveness [1]. However, traditional LFA methods primarily rely on colorimetric detection, which often suffers from low detection limits and limited quantitative capability. To overcome these challenges, we propose an electrochemical LFA system incorporating paper-based electrodes to enhance sensitivity and quantification [2]. In this study, we are developing a novel LFA platform by integrating carbon and silver ink-based paper electrodes, which we are fabricated using a customized printing process [3]. We are developing a sensing system where antibodies are immobilized on the working electrode while the reference and counter electrodes are printed on the same substrate to ensure a compact and efficient design. Our idea is to design the LFA strip to facilitate the interaction between antibody-modified gold nanoparticles and the electrode surface, leading to a measurable electrochemical response. We aim to use a portable potentiostat (PalmSens) to perform precise and sensitive measurements. Our platform aims to enable rapid and quantitative biomarker detection, making it a promising alternative for point-of-care applications, particularly in resource-limited settings. We expect this approach to highlight the potential of electrochemical LFA as a next-generation diagnostic tool, offering a cost-effective and scalable alternative to conventional methods. In future studies, we plan to focus on multiplex detection and real-world sample validation to broaden the application range of this innovative platform.

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YO-83**COMPARATIVE FABRICATION AND ELECTROCHEMICAL CHARACTERIZATION OF Ni₃HITP MOFs VIA SOLVOTHERMAL SYNTHESIS AND ANODIC DEPOSITION****Lamminmäki, Suvi¹; Länne, Aino²; Vepsäläinen, Mikko¹.**¹VTT - Technical Research Centre of Finland, Espoo, Finland; ²LUT University, Lappeenranta, Finland.

Two-dimensional conductive metal–organic frameworks (MOFs) such as Ni₃(HITP)₂ (nickel–hexaiminotriphenylene) are promising materials for electrocatalysis, sensing, and energy storage applications. Their conjugated π -systems and metal–ligand coordination afford high electrical conductivity and chemical tunability, making them attractive as functional electrode coatings. However, forming stable, adherent, and electrochemically accessible MOF films remains a challenge, particularly when targeting device integration or long-term performance.

In this study, we investigate two complementary routes to produce Ni₃(HITP)₂ films and evaluate their structural and electrochemical properties. First, Ni₃(HITP)₂ was synthesized in bulk under aqueous conditions. The resulting microcrystalline powders were characterized using nitrogen physisorption (BET) and powder X-ray diffraction (XRD), confirming a high surface area of 370 m²/g and the expected crystalline phase. XRD analysis of the Ni₃(HITP)₂ films showed clear reflections corresponding to the (100), (200), and (001) planes, which are characteristic of the expected layered structure. The (100) and (200) peaks reflect the in-plane periodicity of the hexagonal lattice, with the (200) appearing at approximately half the d-spacing of the (100) due to crystallographic symmetry. The presence of the (001) reflection around 3.3 Å confirms regular interlayer stacking, indicating that the films possess both lateral crystallinity and out-of-plane order. These bulk materials were then deposited by drop-casting onto rod-shaped electrodes from an ethanol–water suspension containing perfluorosulfonic acid (PFSA) as a binder. Film uniformity, adhesion, and electrochemical response were optimized.

In parallel, we investigated an in situ anodic deposition approach to directly grow Ni₃(HITP)₂ films on conductive planar substrates from a DMSO–methanol (3:1) solvent system containing the respective metal salt and organic ligand. This method allows film formation and MOF growth to occur simultaneously at the electrode interface, eliminating the need for binder additives. Anodic deposition was found to produce smooth, conformal coatings with varying adhesion to both nickel and glassy carbon substrates. The effect of electrode surface quality was investigated. The deposited films were analyzed using scanning electron microscopy (SEM) and XRD to confirm structural integrity and surface coverage.

Electrochemical characterization of both drop-cast and anodically deposited films was carried out using electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), linear sweep voltammetry (LSV), and chronoamperometry in 0.1 M KCl and 1 M KOH electrolytes. These experiments assessed redox behavior, stability, and electrochemically active surface area under both acidic and alkaline conditions.

To gain deeper insight into surface composition and spatial uniformity, time-of-flight secondary ion mass spectrometry (TOF-SIMS) was investigated as a complementary technique. TOF-SIMS imaging revealed uniform distribution of metal ions and organic fragments across anodically deposited films and provided early-stage information on possible inhomogeneities or interface-related features not evident in SEM or XRD. Although still exploratory, the use of TOF-SIMS in this context underscores its potential for high-resolution MOF surface diagnostics.

This work presents a comparative strategy for fabricating and characterizing Ni₃(HITP)₂ films via both bulk and electrodeposition methods, offering insight into how processing routes influence film adhesion, structure, and electrochemical behavior. By integrating film preparation, structural analysis, and electrochemical testing across different electrode types and environments, we highlight the versatility and tunability of Ni₃(HITP)₂ for future use in a wide range of electrochemical technologies.

YO-84**VOLTAMMETRIC STUDIES OF ALKALOID ATROPINE ON BORON-DOPED DIAMOND ELECTRODES FOR FORENSIC APPLICATIONS****Dushna, Olha¹; Dubenska, Liliya²; Vojs, Marian³; Bulska, Ewa⁴.**

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Alkaloids are biologically active compounds found in plants, fungi, and synthetic substances, with significant pharmacological applications. Still, they can also be found in food and beverages, making their detection crucial in various forensic and toxicological investigations. Due to their potent biological activities, identifying and quantifying alkaloids are important for medical and forensic purposes, including drug abuse monitoring, poisoning cases, and toxicological screenings. In forensic chemistry, accurate and rapid detection of alkaloids in complex matrices like soft drinks, biological fluids, and pharmaceutical products is essential for legal and medical investigations. Therefore, there is a growing need to develop new, reliable, and efficient analytical methods. Voltammetric techniques offer a sensitive, rapid, and cost-effective approach for detecting trace alkaloids in such matrices, with their real-time analysis capability making them valuable tools in forensic toxicology and legal investigations.

In this work, special attention was paid to the use of a screen-printed sensor with a working boron-doped diamond electrode (SP/BDDE) for the voltammetric measurements in Cola matrix, focusing on the detection of the alkaloid atropine (ATR) [1]. Different BDDE diameters, namely 1, 2, and 3 mm, were tested to optimize the detection process. ATR exhibited an irreversible oxidation peak at 1.5 V in 2 M HClO₄. The developed procedure employed differential pulse voltammetry (DPV) and square-wave voltammetry (SWV), generating calibration curves for ATR on BDDE. The method demonstrated a limit of detection (LOD) as low as 0.08 µM with the 3 mm diameter electrode and a detection capability of 4.1 µM ATR using the 1 mm diameter electrode. The proposed analytical procedure with voltammetric detection, enables the determination of ATR in beverages like Cola without the need for extensive dilution or sample preparation. Furthermore, the method is applicable for detecting ATR at lethal doses or below, using just a single drop of the aliquot. The validation of the developed method showed excellent linearity, sensitivity, precision, accuracy, stability, and selectivity, making this approach rapid, portable, and highly relevant for forensic and toxicological investigations. The proposed method aligns with current forensic and medical chemistry trends, emphasizing the development of miniature, cost-effective tools for real-time, reliable assessments.

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YO-85**MEMBRANE-EXTRACTION ASSISTED ELECTROCHEMICAL APTASENSOR FOR ANTIBIOTIC RESIDUE DETERMINATION IN RAW MILK SAMPLES****Jakab, Kristóf¹; Horváth, Viola²; Keresztes, Zsófia¹.**

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The careless administration of antibiotics to dairy cows may lead to health risks in the form of residual pharmaceuticals in the raw milk. This can further contribute to the emergence of antimicrobial resistant bacteria, which is already of the leading causes of deaths worldwide. To combat this issue, fast, easy-to-use and reliable analytical methods are in need.

Aptamer-based electrochemical sensors are promising alternatives to the non-specific microbial test kits as well as to the expensive and laborious liquid chromatography. Aptamers are such DNA or RNA molecules that are able to bind to a specific protein or small-molecule target. During this interaction, the aptamer undergoes a conformational change that can be exploited for electrochemical sensing, usually by using appropriate redox tagging.

Aptamer-functionalized electrode surfaces are prone to fouling, when they come into direct contact with complex biological fluids such as blood, urine or milk. This electrode fouling is mostly due to the presence of proteins through non-specific binding and enzymatic cleavage [1]. To alleviate this, a polymer inclusion membrane (PIM) based pre-treatment method was developed for raw milk. PIMs are a sort of liquid membranes that contain a polymer matrix, carrier molecules and usually some plasticizer as well [2]. The carrier is responsible for the extraction of the compound of interest from the donor phase to the acceptor phase. This process is facilitated by the high salt concentration in the acceptor phase, which means that the extraction can proceed against concentration gradient, so pre-concentration is also feasible with this technique.

In this work the development and the characterization of a complex analytical system for the detection of oxytetracycline (OTC) residues in raw milk is presented. This system is composed of a PIM-based extraction system that is combined with an electrochemical aptasensor based on a screen-printed graphite electrode (SPGE).

The extraction system was optimized in terms of acceptor phase composition and the extraction characteristics were determined. Furthermore, the extract obtained via the PIM-based method was investigated with circular dichroism spectroscopy to prove that it is a viable medium for aptamer-based detection. Aptamer solution was added to OTC-containing PIM-extracts and a similar response was observed as in buffer [3].

The electrochemical aptasensor fabrication includes multiple steps. After the electrode printing, gold was electrodeposited from solution onto the surface of graphite screen-printed electrodes. The thiolated aptamer with methylene blue tagging was immobilized onto the nanostructured gold electrode surface, which was followed by the backfilling with mercaptohexanol.

The optimized SPGE-based sensor was used to measure the OTC concentration of model solutions and real raw milk samples as well. The detection limits, the sensitivity and the selectivity of the analytical system was also determined.

The developed analytical system is made via simple fabrication processes with the use of inexpensive components. The sensor is easy to operate and on-site antibiotic residue detection is feasible with the use of a portable potentiostat.

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YO-86

A NON-INVASIVE SENSING PLATFORM FOR CREATININE, CYSTATIN C, AND UREA DETECTION IN HUMAN SWEAT FOR KIDNEY DISEASE MONITORING

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Monitoring levels of urea, creatinine, and cystatin C in body fluids is critical in clinical practice for the early detection, diagnosis, and management of kidney diseases. Urea is a key indicator of renal function, as its accumulation in blood is often associated with kidney dysfunction [1]. Serum creatinine is considered the gold-standard marker of kidney function, with elevated levels indicating impaired glomerular filtration rate (GFR) [2]. Cystatin C has also emerged as a sensitive biomarker for early-stage kidney disease and subtle functional changes, and it can improve the estimated GFR, which may be inaccurate when relying on creatinine levels alone [2,3].

Traditional biosensors for creatinine and cystatin C typically use antibodies for detection in serum, while urea biosensors generally rely on urease-catalysed hydrolysis. However, the growing demand for non-invasive wearable sensors and patient monitoring outside specialized laboratories fosters the development of complementary sensing strategies, designed to operate reliably over extended periods and in diverse environmental conditions, alongside established approaches.

As part of the European project “Kermit”, our research focuses on measuring these biomarkers in sweat to offer a non-invasive and convenient alternative to blood testing. Sweat analysis can improve patient comfort and facilitate continuous monitoring, which is essential for individuals with chronic or acute kidney conditions. However, detecting these biomarkers in sweat is challenging due to their significantly lower concentrations compared to blood.

In this work, we present a nanomaterial-based three-sensor system for the electrochemical measurement of cystatin C, creatinine, and urea levels. In the cystatin C sensor, an aptamer-based recognition approach is combined with the high conductivity of $\text{Ti}_3\text{C}_2\text{T}_x$ MXene to achieve a low signal-to-noise ratio. MXene nanosheets were delaminated and functionalized to enhance aptamer anchoring. Titanium carbide is also used for the creatinine sensor, where it promotes the electro-oxidation of copper-creatinine complexes, following a strategy previously reported in the literature [4]. For urea detection, a mix of metal hydroxide nanoparticles was developed to interact with urea, increasing the overall impedance of the system. The nanoparticles were synthesized through controlled urea-assisted precipitation, with optimized conditions to ensure high surface area and control the element speciation. Our goal is to achieve simultaneous monitoring of creatinine, urea, and cystatin C in sweat using an integrated, wearable, three-sensor platform.

Electrochemical impedance spectroscopy was used to assess the performance of the biosensors within clinically relevant concentration ranges in sweat. Levels above 20 mM and 40 mM for creatinine and urea, respectively, can be considered dangerous [5,6], whereas, to the best of our knowledge, data about levels of cystatin C in sweat are not available in scientific literature. The creatinine biosensor was tested in the 10–100 μM range and had a limit of detection (LOD) of 1 μM , with a root mean square error (RMSE) of 10%. For urea detection, a LOD of 36 mM and a RMSE of 15% over a 50–200 mM range were obtained. The cystatin C biosensor was tested within a concentration range of 10–20 ng/mL, and achieved a LOD of 3.1 ng/mL and a RMSE of 8%.

These promising results support the sensors’ integration into a wearable lab-on-chip platform for remote, point-of-care kidney function monitoring, potentially improving patient outcomes.

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YO-87

A GREEN APPROACH IN DACTYLOSCOPY: CURCUMIN DEPOSITION ON METAL SURFACES WITH FINGERPRINTS AND THEIR CHARACTERIZATION

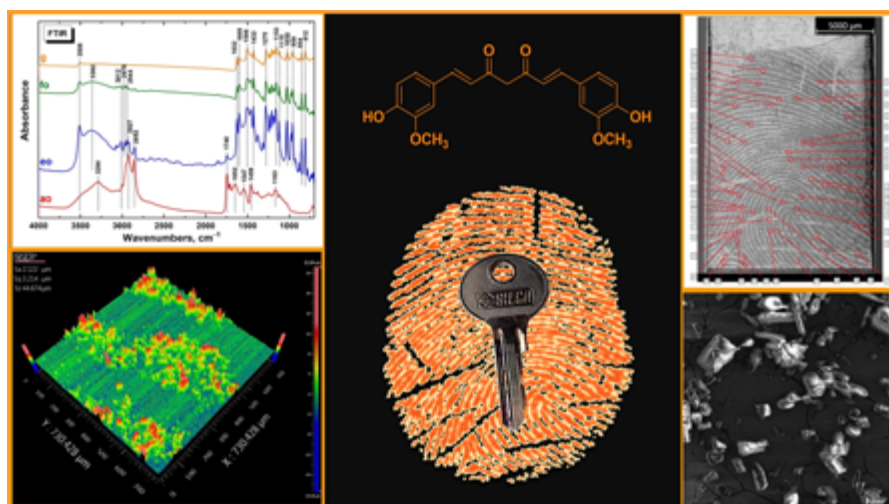
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With the increasing emphasis on green chemistry principles [1], adapting dactyloscopic techniques to meet 21st-century sustainability standards is highly desirable. Commercial methods for visualizing latent fingerprints often rely on toxic and environmentally harmful chemicals. A promising eco-friendly alternative is curcumin (CUR), a natural dye that has been successfully applied not only in powder form [2] but also via chemical deposition from a water-ethanol solution [3].

In our study, the chemical deposition of CUR was tested on steel, brass, aluminum, and copper plates. The deposition time was optimized to 10 or 30 minutes, depending on the metal type. The visualized fingerprints were first assessed under a stereomicroscope for a basic evaluation of ridge quality. A detailed surface characterization was then conducted using multiple methods. Profilometry provided insight into the microstructure and height differences of the fingerprint surface, evaluating CUR's effectiveness in enhancing fine ridge details. Raman spectroscopy analyzed the dye's chemical interactions with the fingerprint surface, identifying compositional and structural changes at the place of dye application. FTIR characterized the absorption properties of organic fingerprint components and their interaction with the dye. SEM, combined with EDS analysis, enable high-resolution visualization of fingerprint morphology, revealing details not captured by other methods. Additionally, it confirmed the competitive sorption of the dye onto sebaceous fingerprint components.

After optimizing the procedure on model metal plates, the method was tested on real objects, including a key, Czech coins (2, 5, 10, and 20 CZK), and a dining knife. The initial deposition time was determined based on prior experiments, but it was further adjusted for each object based on its specific metal composition and impurities. The practical applicability of CUR was validated using the DactyScope fingerprint comparison device.



YO-88**CUSTOMIZED GOLD-SILICA NANOCONJUGATES FOR SENSITIVE ELECTROCHEMICAL IMMUNOSENSING OF CHEMOKINE LIGAND-12 IN COLORECTAL CANCER SCENARIOS****Cabrero-Martín, Andrea¹; Santiago, Sara¹; Serafin, Verónica¹; Montero-Calle, Ana²; Pingarrón, José M.¹; Barderas, Rodrigo²; Campuzano, Susana¹.***¹Universidad Complutense de Madrid, Madrid, Spain; ²Chronic Disease Programme, UFIEC, Institute of Health Carlos III, Madrid, Spain.*

The development of highly sensitive, reliable, and rapid analytical technologies is crucial for the advancement of precision medicine through the accurate and reliable detection of clinically relevant biomarkers. Electrochemical immunoplatfroms have emerged as powerful tools in this field, especially when combined with advanced surface functionalization strategies, such as click chemistry, and nanomaterials that enhance signal transduction.

This work presents a novel electrochemical immunotechnology for the determination of chemokine ligand-12 (CXCL12)[1], a key biomarker associated with colorectal cancer (CRC) progression. The platform employs gold-silica nanoconjugates (Au@SiO₂NCs) functionalized with both recognition and signaling elements biotinylated detector antibody and horseradish peroxidase molecules, respectively. Its exploitation as recognition and signaling nanolabel into an amperometric sandwich immunoassay was shown to significantly improve analytical performance by increasing enzyme loading capacity, improving antibody orientation, and ensuring high signal reproducibility. To fully exploit the full potential of this nanotag, key parameters—including composition, biomolecules immobilization, and electrochemical detection conditions were systematically optimized. The nanolabel-based platform exhibited a remarkable linear response in the range of 72–1000 pg mL⁻¹, achieving a 4 times higher sensitivity than the conventional labeling-based immunoplatfrom (using biotinylated detector antibody conjugated to streptavidin-horseradish peroxidase), with a low detection limit (LOD) of 22 pg mL⁻¹. Additionally, the immunotechnology demonstrated excellent stability, maintaining its analytical performance for at least one month.

The immunoplatfrom applicability was evaluated by determining CXCL12 in plasma samples both healthy individuals and CRC patients. The results not only confirmed significant alterations in CXCL12 levels in CRC patients but also reinforced its potential as a plasma biomarker for early cancer detection and disease monitoring.

The integration of this point-of need compatible platform into clinical practice could significantly improve the management of CRC patients. In addition, both the immunoplatfrom and the Au@SiO₂NCs can be easily modified with other recognition and/or signaling molecules, enabling the detection of different targets or multiple targets in a single assay, which would add precision to clinical diagnosis and prognosis.

Acknowledgments

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YO-89**ENHANCING DETECTION SENSITIVITY IN GAS CHROMATOGRAPHY BY COOLING A NANO-GRAVIMETRIC DETECTOR DOWN TO -100°C**

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In recent years, quantification of volatile compounds has become increasingly critical for process monitoring, quality control and environmental and health protection. The ever-lower detection thresholds required for impurities and pollutants necessitates the development of analytical instruments capable of detecting and quantifying trace amounts. Achieving this goal typically requires either the integration of a preconcentration step in the analytical process, often impractical for on-line analysis, or the use of highly sensitive instruments, which are generally lab apparatus requiring sample transportation.

Recently, nano-gravimetric detector (NGD) based on nano-electromechanical systems (NEMS) technology [1] has been introduced. Such compact, non-destructive system operating without any additional gas is particularly well-suited for on-line and in-situ analysis. NGD is ATEX-compliant, making it safe for use in hazardous environments, and can be used to quantify a wide range of compounds. Its detection principle relies on an adsorption/desorption mechanism on detector beam, and the response signal increases with the adsorbed mass of analyte on the beam. This feature makes it highly advantageous for analysis of heavy compounds [2], where traditional detectors such as the thermal conductivity detector (TCD) are limited.

Since the adsorption equilibrium between the gaseous phase and the adsorbent layer covering the beam is temperature-dependent [3], reducing the temperature favors adsorption, thereby enhancing sensitivity. Thus, cooling systems for NGD have been designed to lower the limits of quantification (LOQ) to trace or even ultra trace levels.

Cooling the NGD to sub-zero temperatures has demonstrated a substantial improvement in detection capabilities, leading to a significant reduction in quantification limits. Tetrahydrothiophene (THT) response across a wide temperature range revealed that peak height increases exponentially as temperature decreases, while baseline noise does not vary significantly. As a result, the LOQ of THT was reduced by a factor of 100, from 18 ppm at 50°C to 0.17 ppm at -65°C, thus fully meeting regulatory requirements for THT levels in natural gas in France [4], which range between 4,2 and 11,1 ppm.

LOQ of BTEX compounds were estimated at an NGD temperature of -35°C : 1,7 ppm (benzene) – 0,23 ppm (toluene) – 0,1 ppm (ethylbenzene) – 0,12 ppm (ortho-xylene) using 10 µL injection volume at atmospheric pressure without preconcentration step. By decreasing the NGD temperature down to -45°C, the analysis showed that toluene, ethylbenzene and ortho-xylene were detected without preconcentration at a concentration of 20 ppb in nitrogen.

Various compound families — including aromatic molecules, sulfur compounds, permanent gases, and hydrocarbons— have been tested with detector temperatures as low as -100°C. For all analytes, detection sensitivity was significantly increased with the temperature reduction, confirming both the feasibility and the benefits of cooling the detector. For heavier compounds, which are already easily detected by the NGD under conventional GC conditions, trace and even ultra-trace detection is now achievable through temperature reduction.

Finally, ongoing study aims to better understand the thermodynamic and kinetic phenomena occurring withing the NGD at negative temperatures.

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YO-90**SUSTAINABLE RECOVERY OF ANTIMICROBIAL COMPOUNDS FROM GINGER AND TURMERIC WASTES USING SUBCRITICAL WATER EXTRACTION AND HIGH INTENSITY FOCUSED ULTRASOUNDS: IDENTIFICATION BY RP-UPLC-ESI-QTOF**

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Ginger and turmeric are spices increasingly employed in the production of juices, due to their outstanding composition and association with healthy benefits. This practice results in wastes that, like the whole spice, may constitute rich sources of compounds with antimicrobial properties. Indeed, the replacement of synthetic substances with antimicrobial activities (e.g., sodium benzoate, sorbitol, calcium propionate, nitrates, etc.) by natural compounds is a clear trend for obtaining *clean label* foods. The exploitation of these wastes requires the development of efficient and environmentally friendly methods. This work aims to evaluate low environmental impact techniques to make the most of these wastes: high-intensity focused ultrasounds (HIFU) and subcritical water extraction (SWE). In all cases, water has been used as extracting solvent and design experiment chemometric tools (Box-Behnken and factorial 2³ design) were employed to optimize the recovery of antimicrobial compounds, while minimizing the formation of potentially harmful Maillard products during extraction.

The antimicrobial activity of the extracts was evaluated against two pathogenic bacterial strains, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). Under optimal extraction conditions, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Extracts obtained by SWE from turmeric waste were highlighted over those from ginger due to outstanding antimicrobial activity, especially against *Staphylococcus aureus* gram-positive bacteria. The extracts showing the highest antimicrobial activity were analyzed by reversed-phase ultrahigh-performance liquid chromatography coupled to high-resolution tandem mass spectrometry (RP-UPLC-ESI-QTOF) for the identification of the main compounds responsible for the observed activities. Many phenolic compounds were detected in the extracts.

YO-91**ANALYSIS OF COMPLEX METABOLOMIC ABNORMALITIES OF PATIENTS SUFFERING FROM GENITOURINARY CANCER USING UNTARGETED HPLC-MS-BASED METABOLOMICS****Frolova, Anastasiia; Ikhmalainen, Yuri; Vokuev, Mikhail; Rodin, Igor.***Lomonosov Moscow State University, Moscow, Russian Federation.*

Malignant tumors of the genitourinary system remain one of the most important problems in the structure of oncological diseases. Diseases of this type (mainly bladder cancer (BCa) and prostate cancer) occupy the second place among malignant neoplasms in men after lung cancer and are among the ten most prevalent cancers in the world. The high mortality rate from this type of pathology is also due to the detection of the disease at terminal stages. The disease in its initial stages is mostly asymptomatic, or produce similar symptoms as other diseases of the urinary system. BC is one of the most widespread urologic cancers worldwide, characterized by high cancer-specific mortality and high cost of treatment. Traditional diagnostic methods allow to determine the diagnosis, however have certain disadvantages, among which, depending on the type of investigation, we can highlight for invasive methods - the painfulness and initiation of inflammatory processes, and in case of of non-invasive ones - low sensitivity for some types of tumors.

Obtaining characterization profiles of biological samples containing a large number of metabolites with different physical and chemical properties and a wide range of concentrations is a major analytical challenge. Mass spectrometry coupled with chromatography, due to its high sensitivity, is a key approach for metabolomic studies, allowing the detection of multiple metabolites from different biochemical pathways and at different concentration levels. The broadest metabolomic profile for patients with BCa can be obtained using untargeted urine profiling due to its proximity to the tumor.

Our study deals with sample preparation, selection of conditions for HPLC-MS analysis, and metabolomic profiling of urine samples by high performance liquid chromatography-high resolution mass spectrometry to obtain a maximum possible number of metabolites. Urine samples from cancer patients, as well as from healthy patients and patients with urinary tract diseases, served as the objects of analysis. The analysis also evaluated the effect of storage conditions on the number and content of metabolites found in final samples under study.

Mass chromatograms were used to obtain tables of signals, for most of which prior annotation by comparison with library (NIST) fragmentation spectra allowed to get and identify preliminary lists of marker compounds. The obtained peak tables further underwent reproducibility filtering, filling of missing values, and normalization. Among the obtained biomarkers identified on the basis of ion-fragment spectra, compounds involved in regulatory pathways associated with inflammatory processes are observed, including those previously considered in the literature as key in the course of BCa disease.

The results obtained with the developed approach can further complement clinical studies and be used in the laboratory diagnosis of genitourinary cancer together with the search for markers, the detection of which in biological materials could be considered as highly effective diagnostic indicators contributing to the accurate diagnosis of the disease.

YO-92**AN INSIGHT INTO EUROPEAN TEA (*CAMELLIA SINENSIS*) INFUSIONS: POLYPHENOLS PROFILE, ANTIOXIDANT ACTIVITY, AND SENSORY ANALYSIS****Fanesi, Benedetta; Lucci, Paolo; Pacetti, Deborah; Carloni, Patricia; Damiani, Elisabetta.***Università Politecnica delle Marche, Ancona, Italy.*

Tea is one of the most consumed beverages all over the world, highly appreciated for its high content of health-promoting compounds [1]. The increasing demand of tea has led Europe to start the cultivation, transformation and commercialization of tea in recent years [2]. Therefore, the aim of this study was to unravel the potential of European tea, providing a complete characterization of phenolic compounds, as well as antioxidant and sensory properties. Black (n=6) and green (n=6) tea were obtained from 5 European gardens (Netherlands, Switzerland, Portugal, Azores, Germany) and infusions were prepared using boiling water at a sample to solvent ratio of 1:50. All samples were analyzed for their polyphenol/flavonoid content (TPC, TFC), antioxidant activities (ABTS, FRAP, ORAC), as well as sensory attributes. Moreover, phenolic compounds and caffeine content were determined by means of ultra-high performance liquid chromatography coupled with photo diode array detector and mass spectrometer (UHPLC-PDA-MS) operated in negative electrospray ionization mode. Tea samples were mainly characterized by the presence of catechins and their derivatives and phenolic acids. Green tea showed a higher number of polyphenols (approximately 1200 mg/L) compared to black ones where the concentration varied between 70 and 325 mg/L. Accordingly, the antioxidant potential of green tea samples was almost double that of black tea. As regard alkaloids, the concentration of caffeine varied according to the origin of tea, with samples from Germany showing the lowest values (~300 mg/L). Black tea samples were characterized by fruity, woody and spicy notes, and a sweet taste. A prevalent vegetable scent and umami flavor distinguished green teas, and those from the Netherlands and Germany were the most appreciated ones for taste and flavor. In conclusion, European tea represents a valuable alternative to other commercial teas from other continents, given its high quality expressed both in terms of polyphenolic content and antioxidant capacity.

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YO-93**ION MOBILITY SPECTROMETRY AND MACHINE LEARNING FOR RAPID DETECTION AND QUANTIFICATION OF ADULTERATION IN COCOA POWDER**

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Cocoa powder, a widely consumed cocoa-derived product, is appreciated for its distinctive flavor and nutritional properties. It is extensively utilized in the food industry, particularly in baked goods, beverages, confectionery, and pastries. However, the increasing demand for cocoa powder has led to frequent adulteration practices, which compromise both product quality and consumer safety. Although chromatographic and spectroscopic techniques are currently employed for authentication purposes, these methods are often expensive, time-consuming, and lack portability and ease of use. Thus, there is a growing interest in developing faster, cost-effective, portable, and reliable alternatives. In this study, an innovative approach based on the analysis of the volatile composition of cocoa powder has been developed by combining ion mobility sum spectra (IMSS) derived from headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) with various machine learning algorithms. The method was applied to detect and quantify adulteration in pure cocoa powder using four common adulterants: cocoa shell, carob, soybean, and foxtail millet. Laboratory-prepared adulterated samples were generated at seven concentration levels ranging from 10% to 40% (w/w). The headspace conditions for volatile extraction were optimized using a Box-Behnken design and response surface methodology (BBD-RSM). The developed method demonstrated high precision, with coefficients of variation (CV) below 5% (1.8% for repeatability and 2.0% for intermediate precision). Coupling IMSS data with machine learning techniques enabled accurate classification of adulterants, with linear discriminant analysis (LDA) achieving 92% accuracy on the test set. For quantification, support vector regression (SVR) yielded the best performance ($R^2 = 0.88$; RMSE = 3.60) on the test data. Furthermore, a user-friendly web application was developed, integrating the best-performing LDA and SVR models for automated adulteration detection. The proposed IMS-machine learning approach is fast, economical, and environmentally friendly, which could also be applied to portable systems. Therefore, it can be used as a screening tool for quality control and authentication of cocoa powder.

Keywords: Cocoa powder, ion mobility spectrometry, adulteration detection, quality control, machine learning.

YO-94

SYRINGE RESIDUE ANALYSIS AS INDICATOR FOR TRACKING INTRAVENOUS DRUG USE IN SPAIN

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Syringe residue analysis is a relatively new drug-use indicator, developed by Néfau et al. in 2015 (Néfau et al., 2015), to identify the substances consumed through intravenous administration (Brunt et al., 2021), overcoming the bias introduced by other indicators such as surveys, police seizures or hospitalization data. Moreover, it is a fast alternative to detect the eventual appearance of new substances (e.g. fentanyl, nitazenes, etc.) as an early warning indicator.

In this work, almost 1,000 syringes were collected in two different sampling campaigns, in 2023 and 2024, in collaboration with several organizations (NGOs, public entities, etc...) which provide syringe exchange services as a harm reduction service in supervised drug consumption facilities, drug addiction treatment centres or field mobile units throughout the Spanish geography. Collection was performed in separated containers by gender and a specific sampling point of people who inject drugs related to sexual practices (commonly known as *chemsex*) was included.

A simple sample preparation protocol established by ESCAPE Network (EMCDDA, 2021) was employed. Briefly, syringes were rinsed with 1 mL of methanol. Subsequently, a 1:10 dilution was performed and, finally, extracts were filtered through a 0.22 µm syringe filter prior to the injection into a liquid chromatography equipment coupled to a quadrupole-time-of-flight high-resolution mass spectrometer (LC-QTOF-HRMS). Acquisition was done in auto MS/MS mode (data dependent acquisition, DDA). Data were treated with a suspect screening approach, searching for the compounds into a tandem mass spectrometry (MS/MS) library created *ad hoc* from different sources and containing 203 drugs, metabolites, adulterants, etc (EMCDDA, 2021). Identification was performed by accurate mass and MS/MS spectra matching and confirmed against standards whenever possible.

The most frequently detected substances were the ones related to cocaine and heroin consumption (metabolites and degradation products), such as e.g. 6-acetylmorphine, benzoylcegonine, morphine... Additionally, other some other opioids (codeine, tramadol, etc.), fentanyl, pregabalin, were occasionally detected. Adulterants were also frequently found, among which phenacetin, piracetam, noscapine and papaverine can be highlighted. Furthermore, cocaine and heroin co-consumption signs were frequently encountered.

In the case of *chemsex* related syringes, the pattern was, however, totally different, as synthetic cathinones and methadone was the most prevalent substances, which also points to a shift in the market of methylmethcathinone (MMC) and chloromethcathinone (CMC), as regards the isomers detected, and self-reported drug use.

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YO-95

INTEGRATION OF OMICS AND SPECTROSCOPIC APPROACHES FOR NOVEL POTENTIAL BIOMARKER DISCOVERY OF HCC

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, with late diagnosis due to the initial absence of symptoms, significantly impacting survival rates [1].

In this study, plasma samples from 101 hepatitis C virus (HCV)-infected individuals, of which 69 were diagnosed with HCC, were analysed using mass spectrometry and infrared spectroscopy. For the mass spectrometric analysis, plasma was processed via the Matyash protocol to partition polar and non-polar components, whereas the Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopic analysis was conducted directly without prior sample preparation.

The generated Metabolomics, Lipidomics, and spectroscopic datasets underwent pre-processing and were evaluated using both univariate and multivariate approaches in Matlab R2024b (MathWorks Inc., Natick, MA, USA). Metabolomics dataset was normalised using Total Ion Current (TIC), whereas Lipidomics dataset was normalised relative to the internal standards specific to each lipid class. The spectroscopic dataset required various steps of pre-processing, including baseline correction via Asymmetric Least Squares algorithm [2], Multiplicative Scatter Correction [3] and External Parameters Orthogonalization [4].

Prior to classification analysis, the dataset was partitioned into training (80%) and test (20%) sets using the Kennard-Stone algorithm. Although initial classification models based on Partial Least Squares-Discriminant Analysis (PLS-DA) [5] with cross-validation yielded suboptimal performance on the test set, a subsequent data fusion strategy employing Sequential-Orthogonalized Covariance Selection (SO-CovSel) [6] combined with Linear Discriminant Analysis (LDA) [7] was implemented. All possible two and three dataset combinations were evaluated, ultimately leading to the selection of two classification models: one integrating metabolomics and spectroscopic data, and the other combining lipidomics and spectroscopic data, both achieving accuracies exceeding 90% on the test set.

The variables selected by these models underscored consistent metabolic alterations associated with tumor development, including decreased levels of lysophosphatidylcholines, acylcarnitines, and ceramides, as well as an upregulation of the kynurenine pathway, a process implicated in immunosuppression. Additionally, the spectroscopic data provided critical insights into modifications in protein content.

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OPTIMIZATION OF A QuEChERS-BASED LC-HRMS ANALYTICAL METHOD TO DETERMINE CECs IN ORGANIC LETTUCE IRRIGATED WITH RECLAIMED WATER AND ITS POTENTIAL RISK

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Since water scarcity is one of the main challenges nowadays, circular economy routes are proposed as a sustainable solution. The reuse of wastewater is an increasing practice in, for example, the irrigation of herbaceous crops. However, several studies indicate that reclaimed water introduces contaminants of emerging concern (CECs) into the ecosystem [1]. Pesticides, pharmaceuticals, and personal care products are the chemical families most studied in crops [2–4]. In contrast, there is a lack of studies focused on compounds with industrial applications, as well as methods capable of evaluating them. Thus, this study aimed 1) to optimize a method based on QuEChERS extraction and subsequent analysis by LC-HRMS for analysis of CECs, with focus on industrial chemicals, in lettuce using a factorial fractional experimental design, 2) to validate the optimised method following SANTE 11312/2021 guidelines, 3) to apply this method in field crops irrigated with surface water partially sourcing from reclaimed wastewater, and 4) to assess their associated risk to human health.

The optimized QuEChERS extraction conditions allowed the detection of 10 CECs in lettuce samples partially irrigated with reclaimed water: 9 compounds with industrial applications and 1 pharmaceutical. None of them are regulated and thus subjected to maximum residue levels (MRLs) established by the European Commission. Therefore, in this study, to evaluate the potential health risks associated to the presence of some CECs in the lettuce samples analyzed, the threshold of toxicological concern (TTC) approach was applied [5,6]. The detected compounds were classified into Class I (least toxic, TTC = 30 µg/kg/day), Class II (TTC = 9 µg/kg/day), and Class III (most toxic, TTC = 1.5 µg/kg/day), according to Munro et al. (1996) [7], and this classification was used to calculate the daily consumptions (DC) required to reach the corresponding TTCs. Five out of the 10 detected contaminants were classified as Class III. However, none of them represents a human health risk on the basis of amount of lettuce normally consumed and that necessary to pose a risk (DC). For instance, in the case of the plastizier diethyl phthalate, a person would have to eat 32 kg of lettuce in a single day for it to pose a risk.

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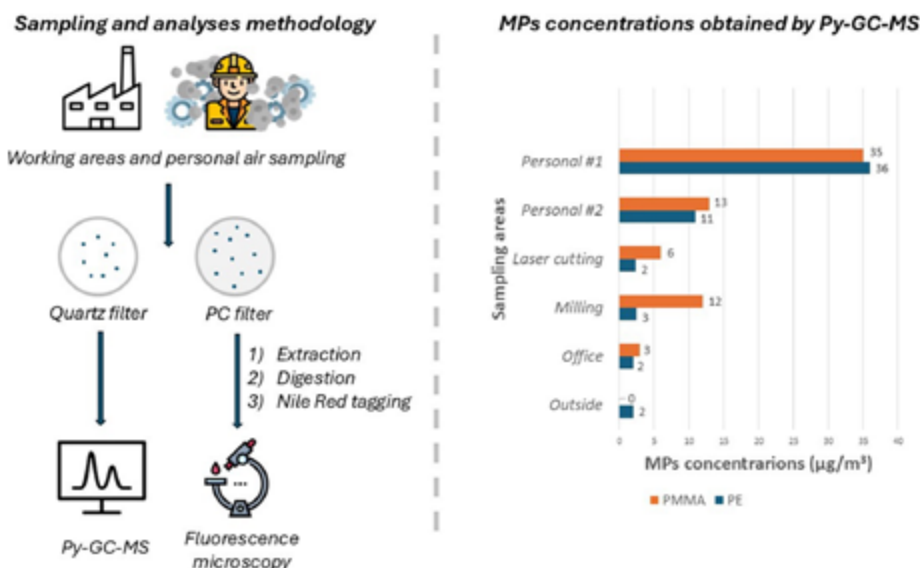
NOVEL APPROACH OF ESTIMATING AIRBORNE MICROPLASTICS CONCENTRATION IN PLASTIC PROCESSING WORKPLACES

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The widespread use of synthetic polymers has led to a significant environmental crisis caused by plastic pollution, with microplastics (MPs) being found in various environments and posing risks to both human health and ecosystems [1]. The potential for microplastic items to disperse in the air as particulate and to be inhaled by humans potentially causes harm to the respiratory and other systems [2]. Therefore, there is a particular need to study MPs as air pollutants and to quantify their presence in outdoor and indoor environments [3]. In this study, we tested a combination of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and fluorescence microscopy to quantify both the mass and number of microplastics in airborne particulate matter and settled dust from different factories and treatment plants in central Italy, where different plastic materials are mechanically processed. Air samples from various working areas across three different workplaces, as well as from personal air samplers worn by workers during their working hours, were collected through active sampling using quartz and polycarbonate (PC) filters for subsequent Py-GC-MS and fluorescence microscopy analyses, respectively. Quartz filters were punched and directly analysed by Py-GC-MS, while PC filters were extracted and digested using Fenton's reagent. MPs were subsequently filtered onto cellulose esters filters, stained with Nile Red, and finally counted by fluorescence microscopy (Figure 1).

By using this combined approach, we were able to quantify the mass and number concentrations of airborne MPs in different areas of the working environments accurately. Py-GC-MS analysis enabled the determination for eleven different polymers: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polymethyl methacrylate (PMMA), acrylonitrile butadiene-styrene (ABS), styrene-butadiene rubber (SBR), polyethylene terephthalate (PET), PC, nylon 6 (N6), and nylon 66 (N66). Preliminary results of Py-GC-MS quantification at one factory revealed varying PMMA and PE MPs concentrations, ranging from 2 $\mu\text{g}/\text{m}^3$ to 36 $\mu\text{g}/\text{m}^3$ in different sampling areas.



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POSTERS



P1-001

BLACK CARBON IMPACTS ON SNOWPACK: A COUPLED MODELING FRAMEWORK OF OPTICAL ALBEDO, SNOW METAMORPHISM, AND DEPOSITION OF BLACK CARBON OBTAINED FROM SATELLITE DATA

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Black carbon (BC) deposition on snow significantly alters its albedo, accelerates melt rates, and impacts hydrological cycles. In mountain regions like the Chilean Central Andes (Chica)—where seasonal snowpack is a key water resource—understanding how BC influences snow dynamics is vital for water management and climate projections. However, quantifying BC deposition and its effects remains complex due to uncertainties in atmospheric BC concentrations and their interaction with snow surfaces.

This study evaluates BC deposition on snow using two widely used reanalysis datasets—Copernicus Atmosphere Monitoring Service (CAMS) and the Modern-Era Retrospective analysis for Research and Applications (MERRA)—and presents a new coupled model that integrates satellite-derived BC deposition, an optical albedo module, and a snowpack evolution model. The framework accounts for time-dependent processes involving BC accumulation, snow morphological changes, and albedo feedbacks.

Calibration was performed across six sites along latitudinal gradient of Chile, capturing regional variability in snow processes. CAMS dry deposition data, combined with a low diffusivity coefficient ($10^{-7} \text{ m}^2 \text{ s}^{-1}$) and a high atmospheric scavenging coefficient (10^4), enabled the model to closely match observed BC concentrations at four sites. One of the remaining locations required a higher diffusivity coefficient ($10^{-5} \text{ m}^2 \text{ s}^{-1}$) to reach observed deposition levels. The final site, located at higher latitude, exhibited larger discrepancies—likely due to known distortions in reanalysis data at high latitudes.

MERRA, in contrast, underestimates atmospheric BC concentrations by approximately one order of magnitude. Nevertheless, it captures the overall temporal trend more accurately, as seen at Portillo, and better reproduces the latitudinal profile of BC deposition on snow. To offset the underestimation bias of MERRA, the best model fit required medium to high diffusivity values (10^{-6} – $10^{-5} \text{ m}^2 \text{ s}^{-1}$) and elevated scavenging coefficients (5×10^4 – 10^5).

For detailed validation, two sites in the Chica were selected: Tapado (Coquimbo Region, -30.16 , -69.90 , 4200 m a.s.l.) and Portillo (Valparaíso Region, -32.84 , -70.13 , 2800 m a.s.l.). At Tapado, the model showed strong agreement with observed snow water equivalent (SWE) data under stable roughness conditions ($z_0 = 2 \text{ mm}$), achieving $R^2 = 0.981$, RMSE = 18.37 mm, and BIAS = -0.63 mm . Even under unstable conditions ($z_0 = 8 \text{ mm}$), performance remained high ($R^2 = 0.943$ – 0.963 , RMSE = 22.85–28.45 mm). Simulations showed that, under low and medium diffusivity values, snow duration decreased by 4–13 days in stable conditions and 1–8 days in unstable ones.

At Portillo, model outputs were compared against in-situ data from NUNATAK-1, a field-based environmental laboratory that functions as a natural refuge and records diverse variables such as meteorology, solar radiation (albedo), and atmospheric pollutants. Among these, BC concentrations are particularly relevant to this study. CAMS overestimates BC levels here, leading to additional snowpack reduction by 1–5 days, or up to 12 days under medium diffusivity values.

Overall, results indicate that low to medium diffusivity values (10^{-7} – $10^{-6} \text{ m}^2 \text{ s}^{-1}$) yield the most reliable outcomes. CAMS provides BC magnitudes closest to observations, particularly in CHICA, although with an inverse temporal trend. MERRA, while underestimating atmospheric BC, better captures its temporal evolution and latitudinal deposition profile, provided that appropriate diffusivity values are applied to correct for bias.

This work improves the understanding of radiative effects of BC on snowpack evolution in arid, high-altitude environments and enhances the accuracy of snow models used for climate projections and water resource management in the Andes.

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P1-002**ELECTROCHEMICAL SENSORS BASED ON PHENYL ISOTHIOCYANATE DIAZONIUM SALTS FOR TOXIC COMPOUNDS DETECTION IN COMPLEX SAMPLES****Bucur, Bogdan; Radulescu, Maria-Cristina; Bucur, Madalina-Petruta; Radu, Gabriel Lucian.***National Institute of Research and Development for Biological Sciences, Bucharest, Romania.*

There is a need to develop rapid and simpler methods to detect the presence and the concentration of numerous toxic compounds among heavy metals which are released into the environment, particularly into water sources, with negative impact on human health [1]. From numerous existing methods for the electrodes functionalization a special attention is attributed to the diazonium moieties produced in situ with acid nitrous from aromatic amine [2]. Also, it is known the affinity of the isothiocyanate moieties for metals and the superior conductive properties [3]. Thus, in this work, it is reported the design of the electrodes functionalized with isothiocyanate moieties by using diazonium chemistry for heavy metals detection. 4-nitrophenyl isothiocyanate (4-NITC) was used as coordinative compound that has also nitro groups for immobilization on the electrode surface. The electrode modification procedure was performed in three steps in the same solution: (1) the reduction of nitro groups of 4-NITC to amino moieties by chronoamperometry at constant potential of -0.6 V for 30 s, (2) formation of diazonium salts in situ in the presence of nitrous acid and (3) the electrografting of diazonium salt by chronopotentiometry at constant current of -0.025 mA for 30 s. The analytical performances of the modified electrodes depend on the thickness of the electrodeposited organic layer. Higher amount of diazonium salts grafted on the electrode surface led to the electrode passivation and the minimization of the electron transfer. To prevent this, the thickness of the obtained organic layer electrodeposited on the electrode surface was directly controlled by varying different parameters from the reduction of the nitro groups and the diazonium grafting steps. A solution of 1 mM ferricyanide/ferrocyanide couple in PBS pH 7.4 supplemented with 0.1 M KCl was used for the electrochemical characterization of the modified electrodes and to investigate the stability of the grafted organic layer. By the proposed method, it was obtained an optimal thin layer with minimum surface fouling without the use of the radical scavenger (DPPH) required for the amino modifiers [2]. The developed electrodes were used for the Pb(II) and Fe(II) determination with a limit of detection of $1.2 \mu\text{g L}^{-1}$ for Pb(II) and of $2.0 \mu\text{g L}^{-1}$ for Fe(II), respectively. Also, the studies could be extended to detect other metal ions due to the strong interaction with thiol group.

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P1-003**FIRST APPROACH TO THE CONSUMPTION OF PSYCHOACTIVE SUBSTANCES IN SANTIAGO DE CHILE BASED ON WASTEWATER ANALYSIS****Calzadilla, Wendy¹; Salazar-González, Ricardo².**

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Urban wastewater reflects the lifestyle of a population, as it represents anonymous urine samples of thousands of people [1]. The consumption of psychoactive substances can be estimated by measuring human excretion products or the unaltered substance itself in untreated wastewater, known as wastewater-based epidemiology (WBE). In the last decade, the application of wastewater-based epidemiology to monitor illicit drug loads as a new indicator of drug use has increased and WBE is now applied on a global scale, complementary to the established drug monitoring tools [2].

This study used WBE to assess the temporal profile of the consumption of a group of psychoactive substances in the Metropolitan Region of Santiago, Chile. Composite samples of raw wastewater were collected in May and July 2024 at the WWTP Santiago Poniente, which covers a population of about 20.000 inhabitants. An analytical method based on direct injection and solid-phase extraction and liquid chromatography-tandem mass spectrometry was developed and validated for the analysis of psychoactive substances in wastewater. The study revealed the daily presence of cocaine, ketamine, MDMA and phentermine in the influent samples in the days studied. According to retrospective calculations, the highest mass loads were found on May 1, which coincides with a public holiday. For cocaine, MDMA and phentermine, the highest values were 4286, 36 and 374 mg/day/1000 inhabitants, respectively. In addition, the highest value found for ketamine was 595 mg/day/1000 inhabitants.

This research propose the analysis of wastewater for epidemiological purposes in Chile. It remains a limited study, which highlights the importance of increasing analytical capacities, as well as increasing the number and category of psychoactive substances. In addition, WBE monitoring campaigns should be conducted on a larger scale to obtain temporal and spatial patterns of psychoactive substance use that are more representative of the Chilean population.

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P1-005**A SELECTIVE ELECTROCHEMICAL APPROACH TO DEFLUORINATED ACTIVE PHARMACEUTICAL COMPOUNDS IN AQUATIC ENVIRONMENTS****Dushna, Olha¹; Gawor, Andrzej²; Bulska, Ewa³; Bilewicz, Renata¹.**

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Fluorinated pharmaceuticals, including fluoroquinolone antibiotics like levofloxacin and ciprofloxacin, are extensively used in medicine for their broad-spectrum antibacterial properties. However, their widespread use has resulted in their accumulation in aquatic environments, which persists due to the strong carbon-fluorine (C–F) bond. This persistence poses environmental risks, including bioaccumulation, ecological toxicity, and the promotion of antibiotic resistance. Conventional wastewater treatment methods are insufficient to effectively degrade these compounds, creating a need for new, efficient, and selective defluorination strategies.

This study presents an innovative electrochemical approach for the selective defluorination of pharmaceuticals containing C–F bonds, addressing a significant gap in environmental and analytical chemistry. The method utilizes hydrogen-boosted electrochemical reduction at a glassy carbon electrode to achieve complete defluorination under mild, environmentally friendly conditions. The electrochemical process was validated through mass spectrometry and fluoride ion detection using an ion-selective electrode. Mass spectrometry data confirmed the formation of a single defluorinated product, while potentiometric monitoring of fluoride ions allowed real-time tracking of fluoride release during the reaction.

A comparative evaluation of the performance of vanadium-based catalysts was conducted to assess the electrochemical efficiency. The results revealed that our developed approach is more efficient and selective, producing fewer byproducts than conventional methods, often leading to multiple, uncontrolled degradation products. In contrast, the electrochemical process ensures a selectively well-defined product, showcasing its potential for controlled defluorination.

The novelty of this work lies in its systematic investigation of electrochemical defluorination under controlled conditions, demonstrating high selectivity, scalability, and environmental sustainability. The efficiency of the developed method was demonstrated using fluoroquinolone antibiotics (levofloxacin and ciprofloxacin) and the kinase inhibitor ripasudil. Its broad applicability to other fluorinated pharmaceuticals further highlights its potential as a transformative tool for environmental remediation and pharmaceutical analysis.

The method was also verified using natural water samples, confirming its robustness and practical applicability for real sample analysis. The ability to monitor defluorination in real time adds significant value, making the method suitable for tracking persistent pollutants in aquatic environments. With its high selectivity, precision, and reproducibility, this electrochemical approach presents an effective solution for pharmaceutical contamination, offering a green and sustainable strategy for environmental analysis and pollutant degradation.

P1-006**DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN HOUSEHOLD DUST OF BREASTFEEDING MOTHERS BY UHPLC-HRMS****Fuentes-Ferragud, Esther¹; Miralles, Pablo¹; López, Antonio¹; Ibáñez, María²; Coscollà, Clara¹.***¹FISABIO, Valencia, Spain; ²Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain.*

Per- and polyfluoroalkyl substances (PFAS) comprise a large family of synthetic compounds characterised by their highly stable chemical structure, which provides them high resistance to degradation. While this stability makes them valuable for a wide range of applications, it also contributes to their persistence in the environment generating a potential human exposure. PFAS exposure has been linked to harmful effects on human health, such as cardiovascular diseases, impaired fertility, and an increased risk of cancer (1). In fact, exposure during pregnancy and lactation has raised significant concerns, since these compounds can cross the placenta, reaching the foetus, and be transferred to infants through human milk (2). Despite dietary intake is considered the main exposure route, ingestion and inhalation of household dust are also important contributors, especially for pregnant and lactating women.

In this work, an analytical method has been developed and validated for the determination of PFAS in household dust from breastfeeding mothers' homes. The method is based on ultrasound-assisted extraction (UAE) followed by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS), using a Thermo Scientific™ Orbitrap ID-X™ Tribrid™ system. A total of 54 household dust samples were collected from homes of breastfeeding mothers in the Valencian Community (Spain), representing a pioneering investigation in this region. PFAS exposure levels were determined, and a risk assessment was conducted. Additionally, statistical analyses were performed to identify predictors of exposure based on household characteristics and maternal lifestyles, exploring potential correlations with the detected PFAS levels.

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P1-007**THE INTERACTION OF PERFLUORINATED CONTAMINANTS WITH PEPTIDES AND PROTEINS****Jarashneli, Andrei; Vetere, Alessandro; Schrader, Wolfgang.***Max-Planck-Institut für Kohlenforschung, Mülheim a.d. Ruhr, Germany.*

PFASs (per- and polyfluoroalkyl substances) are a wide and diverse group of emerging contaminants, with more than 7800 compounds identified to date and production volumes of over 46000 tons per year. They possess at least one carbon atom, on which all the hydrogen substituents were replaced with fluorine atoms. Due to the exceptional stability of the C-F bond, they are widely used in firefighting foams, cosmetics and surface coatings. The stability of the perfluorinated chain makes it resistant to biodegradation and leads to accumulation of these substances in nature. PFASs are detected in human blood, other biota, water and soil. Adverse effects such as hepatotoxicity, disturbances in lipid and protein metabolism and even carcinogenesis are attributed to these compounds. Most of studies until now focused on the detection of accumulated PFAS in the environment, including living organisms. However, few studies tackle the interaction of PFAS with the biological material on the molecular level.

In this project, interactions between proteins, peptides or amino acids of different size and function, and PFASs belonging to different classes, including PFCAs (perfluorocarboxylic acids) and PFSA (perfluorosulfonic acids), were studied. Each pair of compounds was incubated in aqueous medium for several hours, then the resulting solution and precipitate (when present) were measured by HRMS. The samples were analyzed by ultrahigh resolution mass spectrometry using both a 7-T FT-ICR-MS and an Orbitrap Elite-MS (Thermo Scientific) with ESI ionization.

The results show that interactions happen between the PFASs and proteins/peptides and they depend both on the PFAS and the biomolecule present. Most of the tested proteins and peptides interact with the PFSA compounds, as well as with perfluorophosphinic acids, but only a few exhibit interaction with PFCAs. Each protein or peptide can interact with several PFAS molecules.

P1-008**URBAN SANDBOXES: A MICROPLASTIC MENACE?****Krzciuk, Karina.***Jan Kochanowski University, Kielce, Poland.*

While the widespread presence of microplastics in the general environment has been extensively documented, there's a significant gap in our understanding of their distribution within specific environments, particularly children's sandboxes, which are a common feature of playgrounds. To address this, a novel study investigated the factors influencing microplastic contamination in sandboxes located in three urban residential areas in Kielce, Poland. Twenty-seven sand samples collected from nine sandboxes were analysed for the presence of microplastics using a simple quality control approach developed by the researchers. Each sample contained microplastics, with concentrations ranging from 60 to 5540 pieces per kilogram of sand. The main types of microplastics identified were fragments and fibres, accounting for almost 95% of the total particulates. Within these categories, transparent fibres were most common among fibres (63%) and red particles were most common among fragments (57%). A robust, direct and statistically significant relationship was observed between the amount of microplastics and the height of the sandboxes ($r_{\text{Spearman}}=0.90$). Conversely, no significant associations were found between microplastic levels and the population density of residential areas ($r_{\text{Spearman}}=0.03$), the distance of areas from the city centre ($r_{\text{Spearman}}=0.02$), or their proximity to major roads ($r_{\text{Spearman}}=0.43$). Given that sandboxes are a potential source of microplastic exposure for children, and assuming that children's sand ingestion is comparable to estimated daily soil ingestion rates, our results suggest that children could potentially ingest up to 1106 microplastic particles per day. The potential for children's health to be adversely affected by inhalation, skin absorption and ingestion of microplastic particles from sandboxes is a concern. However, more research is needed to better understand the health risks associated with this particular source of microplastic pollution.

P1-009**TRACE ANALYSIS OF AMISULPRIDE, CITALOPRAM, METOPROLOL, CANDESARTAN AND IRBESARTAN IN WASTEWATER SAMPLES BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

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In recent years, there has been a relevant increase of emerging contaminants (ECs) occurrence in natural environments, which has the potential to cause detrimental ecological effects. Wastewater treatment plants (WWTP) have been identified as the main route for ECs to enter water bodies since they are permanently released through treated wastewater (TWW). For this reason, European legislation is progressively imposing stricter requirements for wastewater reclamation and reuse, encouraging the identification of potentially hazardous persistent agents in TWW. Pharmaceuticals such as amisulpride, citalopram, metoprolol, candesartan and irbesartan are some of these ECs of greater concern, since they can be considered micropollutants (potentially hazardous substances even in low concentrations). Amisulpride is an antipsychotic drug prescribed to ease the symptoms of schizophrenia. Citalopram is a type of antidepressant used to treat depression and panic attacks. Metoprolol (beta-blocker), candesartan and irbesartan (angiotensin receptor blockers) are applied to treat hypertension and heart failure. Consequently, it seems to be necessary the development of faster, selective and sensitive analytical methodologies for the monitoring of micropollutants and emerging contaminants such as pharmaceuticals in waters.

In this work, a rapid and sensitive analytical method has been developed for the simultaneous determination of 5 ECs (amisulpride, citalopram, metoprolol, candesartan and irbesartan) in wastewater. The procedure involves an initial single-phase extraction of water samples with acetonitrile by sonication, followed by liquid-liquid partition aided by the "salting out" process using sodium chloride (SALLE). Then, 5 µl of the final extract was injected into a HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1200, Agilent Technologies, Santa Clara, CA, USA) at a flow-rate of 0.6 ml min⁻¹. The separation of the selected ECs was achieved using a reversed phase C8 analytical column of 150 mm × 4.6 mm and 5 µm particle size (Zorbax Eclipse XDB-C8), maintained at 25 °C. Mobile phases A and B were acetonitrile and 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A after 35 min. A 10 min post-run time was used after each analysis. The mass spectral analysis was performed on a G6410A triple quadrupole (QQQ) mass spectrometer from Agilent equipped with an ESI interface source operating in positive ion mode, using the following operation parameters: capillary voltage, 4000V; nebulizer pressure, 40 psi; drying gas, 9 L min⁻¹; drying gas temperature, 350 °C. Mass spectra were recorded across the range 50–1000 m/z. A preliminary study of the optimal SRM transitions for every compound was carried out by injecting individual analytes at a concentration level of 10 µg mL⁻¹. Several fragmentor voltages and collision energies were applied to the CEs under study.

The proposed SALLE-HPLC-MS/MS method for the extraction of these ECs from wastewater samples exhibited great validation parameters. The influence of water matrix on ECs recoveries was also evaluated. The method presented good linearity over the range assayed (10 – 500 µg L⁻¹) and the most frequent detection limits was 0.05 ng mL⁻¹. The average ECs recovery obtained for the LC-MS-MS method varied from 75 to 110% with a relative standard deviation between 3 and 8%. The proposed method was used to determine ECs levels in wastewater samples collected from a modular WWTP (AT-8) from Augustspain (Spain).

Acknowledgements

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P1-010**GREEN LEACHING APPROACH FOR THE RECOVERY OF METALS FROM SPENT LCO CATHODE MATERIALS****Mušović, Jasmina; Tekić, Danijela; Marić, Slađana; Jocić, Ana; Dimitrijević, Aleksandra.***Institute of nuclear sciences Vinča, University of Belgrade, Belgrade, Serbia.*

The exponential growth in the production and use of lithium-ion batteries (LIBs), driven by the global demand for portable electronics, electric vehicles, and renewable energy storage, has led to a corresponding increase in battery waste, raising serious concerns about resource depletion and environmental sustainability [1]. These batteries contain several critical raw materials—notably lithium and cobalt—that are finite and unevenly distributed in nature. Among the various components of LIBs, the cathode material is the richest source of these valuable metals. One of the most commonly used cathode materials is lithium cobalt oxide (LCO), prized for its high energy density and stability [1]. Efficient recovery of lithium and cobalt from spent LCO cathodes is therefore essential to reduce dependence on primary mining and to promote the circular use of materials. After dismantling and separating battery components, leaching represents the first key step in hydrometallurgical recycling, enabling the dissolution of metals into a liquid phase. Conventional leaching processes typically rely on strong mineral acids, such as sulfuric acid, often combined with oxidants, generating acidic waste that poses environmental and safety concerns [2]. To address these challenges, ionic liquids (ILs) have emerged as alternative green solvents. ILs are organic salts composed entirely of large organic cations and organic or inorganic anions, typically liquid at room temperature, with negligible vapor pressure, high chemical stability, and tunable solvation properties [3]. Their ability to coordinate metal ions makes them promising agents for selective metal recovery in aqueous systems [3]. In this study, a green leaching process was developed for the selective recovery of lithium and cobalt from spent cathode material. To reduce the use of strong mineral acids, the leaching medium was based on sulfuric acid with hydrogen peroxide as an oxidant. An ionic liquid, tetrabutylphosphonium diethylenetriaminepentaacetate ([TBP]₃[DTPA]), was introduced as a chelating agent. The addition of the ionic liquid enabled a reduction in acid concentration without compromising leaching efficiency, aligning with environmental sustainability goals. The leaching system was optimized by evaluating the effects of IL and acid concentration, temperature, and time. The most efficient extraction was achieved using 0.375 mol L⁻¹ H₂SO₄, 4 wt.% H₂O₂, and 20% IL, depending on the targeted metal. Notably, the optimized acid concentration represents a fourfold reduction compared to the conventional leaching approach using 1.5 mol L⁻¹ H₂SO₄, demonstrating the effectiveness of the IL-assisted system in minimizing acid usage. Optimal conditions also included a temperature of 45°C and a leaching duration of 60 minutes, offering a balance between efficiency and energy consumption. These results highlight the potential of IL-assisted leaching as a more sustainable alternative to conventional methods, contributing to the development of greener technologies for lithium-ion battery recycling.

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P1-011**ENVIRONMENTALLY FRIENDLY EXTRACTION OF LITHIUM AND IRON VIA IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS****Mušović, Jasmina; Tekić, Danijela; Marić, Slađana; Jocić, Ana; Dimitrijević, Aleksandra.***Institute of nuclear sciences Vinča, University of Belgrade, Belgrade, Serbia.*

With the rapid growth in the use of lithium iron phosphate (LFP) cathodes in lithium-ion batteries, the development of environmentally friendly and efficient techniques for recovering lithium and iron has become increasingly important. As these two metals are key components of LFP materials, their recovery not only supports critical raw material conservation but also contributes to the advancement of circular economy strategies in battery recycling [1]. In conventional hydrometallurgical processes, metal separation is typically achieved through leaching with strong acids followed by solvent extraction using volatile and often hazardous solvents. These approaches, while effective, raise environmental and safety concerns due to the generation of toxic waste and solvent losses. To address these limitations, ionic liquids (ILs) have emerged as a promising class of alternative solvents. ILs are low-volatility organic salts that remain liquid at or near room temperature and can be tailored for selective interactions with specific metal ions [2]. Their unique properties, such as thermal stability, tunable solvation, and low flammability, make them attractive for use in greener separation processes [2]. Among various IL-based technologies, aqueous biphasic systems (ABS) represent a particularly attractive platform. These systems consist of two immiscible aqueous-rich phases, typically formed by combining ILs with inorganic salts or polymers [3]. ABS offers several advantages over traditional organic aqueous extraction systems, including biocompatibility, low toxicity, and full water solubility, which significantly reduces the environmental footprint [3]. Furthermore, ABS can be designed to provide selective partitioning of target metals between the two phases, enabling efficient separation under mild and sustainable conditions [2]. In this study, extraction experiments were performed using aqueous biphasic systems composed of the hydrophilic ionic liquid tetrabutylphosphonium salicylate ([TBP][Sal]) and two different ammonium salts to evaluate their efficiency for lithium and iron separation. Model solutions simulating the composition of LFP cathode leachates were used to investigate metal partitioning behavior. The results demonstrated distinct phase distribution patterns depending on the salt used. In the [TBP][Sal] + ammonium nitrate system, lithium predominantly partitioned into the salt-rich phase, while iron was extracted into the ionic liquid-rich phase, indicating potential for selective separation. Conversely, in the [TBP][Sal] + ammonium sulfate system, both lithium and iron were extracted into the salt-rich phase, suggesting the possibility of simultaneous recovery when separation is not required. These findings highlight the tunability of IL-based ABS depending on the choice of salt, enabling targeted extraction strategies for either co-extraction or separation. The observed behaviors may be attributed to variations in ionic strength, hydrogen bonding interactions, and specific ion–solute affinities between the systems. Although preliminary, these results confirm the potential of [TBP][Sal]-based ABS as a flexible and environmentally friendly platform for the extraction of critical metals from LFP cathode materials.

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P1-012**SPECTROFLUORIMETRIC DETERMINATION OF TRIFLURALIN WITH CARBON NANOMATERIAL SYNTHESIZED FROM OLIVE PITS****Özbek, Nurhayat; Turgut Ocak, Ümmühan; Ocak, Miraç.***Karadeniz Technical University, Department of Chemistry, Trabzon, Turkey.*

Materials with at least one dimension between 1-100 nm are defined as nanomaterials. Nanomaterials exhibit thermal, mechanical, electronic and biological properties (1). All nanomaterials consisting of carbon atoms are called carbon-based or carbon nanomaterials (CNMs). Examples of CNMs are nanodiamonds, carbon nanodots (CNDs), carbon nanotubes, graphene, fullerenes and carbon onions. Synthesis methods of CNMs are divided into two main groups as top-down and bottom-up approaches. Top-down methods are longer processes that require difficult reaction conditions and expensive equipment. The pyrolysis method, which is one of the bottom-up methods proposed in this study, has the advantages of being simple, environmentally friendly, and not using any catalyst or passivation agent.

Pesticides are chemical substances that have been used for years in agricultural production to prevent, control or completely eliminate pests and weeds. While careful use of pesticides provides benefits, careless and poor use leads to accumulation in food, environment and animal resources. In particular, long-term exposure to pesticides potentially causes weakened immunity, hormonal disorders and drug resistance (2). Trifluralin is a moderately persistent and toxic dinitroaniline herbicide used to selectively and effectively control many annual grasses and broadleaf weeds in a wide variety of tree fruit, nut, vegetable and cereal crops (3).

Considering the threatening toxicity and trace residues of pesticides as well as direct exposure to organic pollutants, the establishment of sensitive and selective analytical strategies for the determination of trifluralin and similar pesticides is of great importance in terms of environmental, food and human safety regulations. Chromatographic techniques such as electron capture detection gas chromatography (GC/ECD), gas chromatography with mass spectrometry (GC/MS), and high-performance liquid chromatography (HPLC) with different detectors used in the determination of trifluralin are sensitive methods, but they are time-consuming, expensive and require pre-treatment. In contrast, the spectrofluorimetric method used in the proposed study is a short-term, cheap and pre-treatment-free method.

We synthesized new CNM using olive pits by pyrolysis method at 400 °C in 3 hours. In this study, a spectrofluorimetric method was developed for the determination of trifluralin in real samples of water, carrot and cabbage. The excitation wavelength is 340 nm and the emission wavelength is 383 nm. The linear range is 0.0-50.0 mg/L. The accuracy of the developed method was determined by addition/recovery tests in real samples. This method was successfully applied for the determination of trifluralin in water, carrot and cabbage samples with recovery values of 95-108%, 93-100% and 95-103%, respectively. In conclusion, the proposed method is a simple, cheap and time-saving method that can be applied for the determination of trifluralin.

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P1-013**ASSESSING TOXICITY AND CHEMICAL PROFILE OF ARTIFICIAL AND NATURAL SALT MARSH SEDIMENTS TO EVALUATE THE IMPACT OF RESTORATION ACTIVITIES IN THE VENICE LAGOON (ITALY)**

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Salt marshes are a typical environment of the Venice Lagoon (Italy) at the interface between terrestrial, marine, and freshwater habitats. They play a fundamental role in hydrodynamics by reducing tidal currents, enhancing water quality, and supporting coastal habitats and fisheries¹. However, these dynamic systems are progressively more prone to erosion due to the increased frequency of extreme weather linked to climate change, leading to their rapid deterioration². To preserve and protect these wetlands, which guarantee essential ecological functions and biodiversity of the ecosystem, protection measures have been applied (e.g., reconstruction of degraded natural marshes and creation of artificial marshes with sediments from canal maintenance). These activities have led to the formation of transitional areas, which are artificial sites undergoing a naturalization process. However, the transfer of materials from industrialized and populated areas could lead to the dislocation of known and unknown anthropogenic-related chemicals, potentially impacting the fragile natural ecosystem of salt marshes. To prevent the spread of unwanted contaminants, sediments are routinely screened for heavy metals and common organic pollutants before reallocation. Nonetheless, when focusing on environmental preservation, it is important to exclude unwanted negative effects caused by unknown emerging contaminants, not detected by routine chemical analysis⁴. The thorough identification of contaminants possibly toxic for marine organisms is challenging in sediments due to the matrix chemo-physical complexity and the presence of chemicals ranging from low to high concentrations. Furthermore, the determination of the chemical fingerprint alone excludes the possibility of evaluating synergistic interactions of chemicals, as well as the extent and the categorization (e.g., estrogenic) of the toxic effect itself. For these reasons, this work explores the possibility of monitoring the salt marshes' health by using a multi-level approach that drives the chemical analysis starting from the observed toxicity. Preliminary ecotoxicological evaluations on marine model species (the microalgae *Phaeodactylum tricornutum* and the microcrustacean *Artemia salina*) are carried out using sediment extracts collected from 6 different salt marshes of the Venice Lagoon (2 natural and 4 transitional environments of different age formation). Sediment extracts are analysed with an HPLC-HRMS non-targeted approach to identify the main chemical components characterizing different marsh types and to determine the presence of emerging contaminants. Heavy metals are also determined by ICP-MS. Multivariate data exploration will clarify the correlation between observed biological effects and identified contaminants. Merging chemical and ecotoxicological data from natural, restored, or artificial salt marshes will offer an initial characterization of these distinctive habitats, providing insights on marsh's health status and degree of restoration, laying the foundation for developing an approach to monitor the transition state of salt marshes.

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P1-014**FAST DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER BY ONLINE SPE-LC-MS/MS**

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Haloacetic acids (HAA) and trihalomethanes (THM) are two groups of disinfection byproducts (DBP) that form when water disinfectants such as chlorine or ozone react with other natural organic matter. HAAs contaminated water ingestion may have been related to adverse effects. The degree of risk for these effects depends on the DBP levels and the duration of exposure. Consumption of water with levels somewhat above their respective maximum contaminant levels for limited durations is not likely to significantly increase risks of adverse health effects for most people. However, drinking water treatment plants (DWTP) are challenging daily to reduce at the lowest possible levels the formation of DBP¹.

The European Union with the Water Frame Directive 2020/2184² and, in 2023, the Spanish Government (Royal Decree 03/2023), applied new limits in drinking water for HAAs. Besides, the precise determination of HAAs is necessary. EPA method 552.2³ was used to determine HAA in drinking water by liquid-liquid microextraction, derivatization, followed by GC-ECD analysis.

However, this is a tedious, time and solvent demanding method. For this reason, here we describe a fast Online SPE-LC-MS method for the analysis of 9 HAAs in drinking water.

Sample handling is minimal, acidified water sample is injected into the chromatographic system, passing through a double online SPE, analytes are retained and concentrated eliminating other interferences. Analytes are then eluted in backflush, separated using a C₁₈ and detected by a quadrupole MS. Finally, the validated method was used to determine HAAs concentration in samples coming from drinking water storage tanks and from distribution network. Seasonal variations including temperature and organic matter influence were studied.

This method shortens analysis time and solvent consumption. Detection (MDL) and quantification limits (MQL) achieved are lower with a MDL of 0.1 µg/l and MQL of 0.5 µg/l compared with the previous EPA method 552.3. Since the handling from the operator is minimal, precision is better. In addition, the previous method applied at our laboratory included only six (6) haloacetic acids while the new method includes bromodichloroacetic acid, chlorodibromoacetic acid, tribromoacetic acid among the other compounds included in the EPA method 552.3. In total 9 HAAs have been quantified in various drinking water samples.

DWTP water exhibits low concentration of the $\Sigma 5_{\text{HAA}}$ (0,1 - 1 µg/l) that increases along the distribution network due to the natural reaction with the organic matter enhanced by temperature. Distribution, re-chlorination and storage rise their concentration to major levels but under the legal limits ($\Sigma 5_{\text{HAA}}$ 20 - 40 µg/l).

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P1-015

COMPARISON OF THREE DIFFERENT TOTAL OXIDIZABLE PRECURSOR (TOP) ASSAY APPROACHES FOR THE DETERMINATION OF PFAS IN SOIL

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Within a project by the German Environmental Agency (Umweltbundesamt – UBA) soil samples are taken at different sites all over Germany. The concentration of 30 PFAS - 13 carboxylic acids (C4-C18), 5 sulfonic acids (C4-C10) and 12 precursor substances) - was determined in these soil samples using three different sample preparation approaches: 1) ultrasonic extraction of the soil samples with Methanol; 2) preparation of eluates to test the leaching behaviour of the PFAS and 3) TOP assays following the method published by Houtz & Sedlak ^[1].

Targeted LC-MS/MS is used to determine PFAS concentration after all three preparation steps.

For the TOP assay, the concentration of all 30 PFAS is compared prior to and after the oxidation reaction.

Besides the 'classic' TOP assay ^[1], two other TOP assay approaches, the dTOP assay ^[2] and the photoTOP assay ^[3], are tested and compared. First, all three TOP assay approaches will be tested on a reference soil spiked with a solution including all 30 PFAS measured in the project. Later, soil samples taken within the the priorly described project will were tested.

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P1-016**QUANTIFICATION AND SUSPECT SCREENING OF PLANT PROTECTION PRODUCTS IN SURFACE AND GROUND WATERS FROM INTENSIVE VITICULTURE AREAS BY LIQUID CHROMATOGRAPHY ACCURATE MASS SPECTROMETRY**

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Viticulture is the agricultural activity generating the major profit through transformation of grapes to wine and wine related drinks. Also, it permits the use of low fertility soils, which are not suitable for the growth of other crops. On the other hand, viticulture is a pesticide-intensive use sector. The repeated application of plant protection products to vines has led to the contamination of vineyard soils. The transport of these compounds from topsoil to surface water (SW), including streams, rivers and ponds in the vicinity of vineyards, depends on their physicochemical properties and those of soils, the slope of vineyards, and the frequency and intensity of rain events. Their further leaching to groundwater (GW) is controlled by the stability and mobility of each compound in soil, which are affected by the soil pH, organic matter (OM), and texture. Moreover, the position of aquifers versus vine growing areas also determines the likelihood of GW pollution.

The combination of a sample concentration with liquid chromatography (LC) tandem mass spectrometry (MS/MS) analysis permits the multiresidue, sensitive determination of pesticide residues in water samples from agricultural areas. Moreover, accurate mass spectrometry generates a paramount volume of information to be used in screening studies. In this presentation, we employed an automated methodology based on the on-line combination of solid-phase extraction and LC-MS, using a quadrupole time-of-flight MS instrument, for the quantitative determination of pre-selected pesticides and the suspect screening of pesticides in samples of surface and groundwater obtained in vineyard areas in La Rioja and Galicia during a two-year period. The study summarizes data corresponding to total residues, spatial distribution of individual compounds and their seasonal variations in more than 60 points during six campaigns conducted during years 2023 and 2024.

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P1-017**TRACKING PHARMACEUTICAL CONTAMINANTS IN TAGUS RIVER BASIN (SPAIN): A SUSPECT SCREENING APPROACH WITH UHPLC-HRMS**

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It is widely known that freshwater ecosystems are threatened by pharmaceutical pollution from wastewater treatment plant effluents. Until now, quantitative analysis with standards has been the basis for assessing the presence of pharmaceutically active compounds (PhACs) in these environments. However, this approach may fail to investigate contaminants of high relevance that may escape monitoring programmes. To address this challenge, the present study developed and implemented a screening method to identify new PhACs suspected to be present in the river ecosystem.

The suspect analysis was carried out with an ultra-high-performance liquid chromatography system coupled with a high-resolution quadrupole time-of-flight mass spectrometer (UHPLC Exion LC-QTOF X500R, SCIEX). MS/MS spectra were acquired using electrospray positive ionisation (ESI+) and operating in data-independent acquisition (DIA) mode. The sequential window acquisition of all theoretical fragment-ion spectra (SWATH) method was used.

The implemented method was based on comparison with library spectra, reaching an identification with a confidence level of 2a according to Schymanski's scale. Given the large number of spectra generated in a DIA experiment, it was essential to establish criteria to enable the reliable identification of compounds. Accordingly, only compounds whose HRMS data met the following criteria were considered as identified: 75% spectral match with the library spectra, a maximum accurate mass error of 5 ppm, an isotopic pattern difference below 20%, and an intensity at least ten times higher than the blank intensity.

This analytical method was applied to samples (n=173) from the Tagus River Basin, including 89 river waters, 28 river sediments and 24 fishes collected during 2020, 2021, and 2022. Additionally, 16 wastewater samples (influent and effluent) and 16 sludge samples from two wastewater treatment plants were also analysed to assess the influence on river contamination and water-sludge partitioning.

A total of 161 pharmaceutical compounds (140 active ingredients and 21 metabolites) were identified. PhACs used in pain treatment (analgesics, non-steroidal anti-inflammatories, opioid analgesics) were the most numerous (30 compounds), followed by those used in the treatment of cardiovascular diseases (antiarrhythmics, antihypertensives, beta-blocking agents), psychiatry (antidepressants, antipsychotics, anxiolytics) and respiratory system (antihistamines, antitussives, decongestants), accounting for almost 60% of all identified compounds. The most contaminated matrix was surface water, where 118 PhACs were identified (up to 60 in one sample), with sulpiride standing out (81% detection frequency, Df). Effluents and influents also presented a high number of PhACs, with a total of 67 compounds (losartan and caffeine, 100% Df) and 44 (telmisartan and diclofenac, 100% Df), respectively.

Principal component analysis revealed that antifungals were more frequently detected in matrices with high organic matter content such as sediments and sludge. Besides, biological matrices (fish) were dominated by compounds with some possible natural origin, such as vitamins. It also showed that samples collected in highly populated areas were more contaminated than remote sites. The transformation products resulting from drug metabolism were also investigated. Most of them, such metabolites of psychiatric drugs (zolpidem, quetiapine), antiepileptics (carbamazepine), and opioid analgesics (tapentadol, tramadol), were more frequently identified in water matrices (>50%).

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P1-018**ANALYTICAL METHOD DEVELOPMENT FOR THE DETECTION OF TIRE RUBBER-DERIVED ADDITIVES IN WASTEWATER**

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The global automobile production has experienced a remarkable growth in the last decades, leading to a significant increase in the generation of tire wear particles (TWPs) released into the environment. These particles are generated due to the friction between the tire tread and the road surface. Tires are composed of a variety of polymeric materials, including synthetic and natural rubbers, as well as a range of chemical additives (antioxidants, vulcanizers, plasticizers, and crosslinking agents) that enhance their performance and extend their durability. This group of emerging pollutants includes among others, N,N'-substituted p-phenylenediamines (6PPD, 6PPD-q, DPPD, IPPD), hexamethoxymethylmelamine (HMMM), benzothiazoles (MBTH, 2-ABTH), ureas (CPU, DCU) and guanidines (DPG, DTG).

These substances represent a significant environmental concern as they can be released into different ecosystems, be transformed into new chemical products, and generate toxic effects in the organisms. In fact, a transformation product of 6PPD, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-q) has been identified as the primary compound responsible for "urban runoff mortality syndrome" in coho salmon (*Oncorhynchus kisutch*). Additionally, in March 2025, 6PPD and its transformation product 6PPD-q were included in the 5th Watch List of the EU Water Framework Directive, highlighting the need to generate high quality data on their concentrations in the aquatic environment to support subsequent risk assessment.

The presence of tire rubber-derived additives (TRDAs) in aquatic ecosystems has been also related to wastewater treatment plants (WWTPs), which receive inputs from contaminated urban runoff. However, data on the specific environmental impacts of these emerging chemicals remain limited. Therefore, in the present study, the analytical methodology was developed to determine the occurrence of 23 TRDAs in influent and effluent wastewater samples collected seasonally during 2022 from two Spanish wastewater treatment plants.

Wastewater samples were filtered, spiked with labelled internal standards and extracted by solid phase extraction using Oasis HLB (500 mg, 6mL, Waters) cartridges. The identification and quantification of the TRDAs was carried out using high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS; ExionLC Shimadzu-SCIEX Triple Quad 3500). Several mobile phases were considered to reach the best chromatographic separation in a Luna® Omega C18 100 Å column (Phenomenex). The determination of the target compounds was performed in positive/negative electrospray ionization (ESI) using multiple reaction monitoring (MRM) mode. ESI interface and mass spectrometer parameters were also optimized in order to improve the signal-to-noise (S/N) relation for each TRDA.

The analytical methodology was developed and validated according to the SANTE/2020/12830 and SANTE/11312/2021 performance criteria in absence of current specific guidelines for these compounds. The mean recoveries obtained were within the range 70 – 120 %, with an associated repeatability RSDr ≤ 20 %, for all analytes.

The implementation of this methodology and its application to a larger number of TRDAs will significantly contribute to broaden the knowledge related to their environmental impact and associated risks in the aquatic ecosystem. Moreover, these data will help prioritize these compounds in future regulation and environmental management strategies.

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P1-019**PREPARATION AND EVALUATION OF ACTIVITY FOR IMMOBILIZED NICOTINAMIDASE BASED ON CEDAR****Ryoya, Murata; Masaki, Tsujimoto; Sumire, Tanabe; Yuko, Shikami; Toshiyuki, Miyauchi.***Chubu university, Aichi Kasugai, Japan.*

Petroleum resources are used to synthesize chemical materials, but have an environmental impact in terms of carbon dioxide emissions and resource depletion. Then, the utilization of plant-derived biomass is required to mitigate environmental impact. Nearly 70% of Japan's land area is forested; however, in recent years, timber imports have increased, and the domestic forestry industry has stagnated. Therefore, forested areas that should be protected and managed through thinning and undercutting are left unattended, resulting in environmental degradation of forests. Furthermore, forest residues pose social problems because they flow into rivers during natural disasters such as heavy rainfall, which can cause secondary disaster events. One way of mitigating this issue is to convert plant-derived biomass, specifically forest residues, into useful, chemical materials. Many studies have been performed concerning use, as chemical materials, of lignin and cellulose extracted from plant-derived biomass. However, few studies have addressed the conversion of cedar sawdust possessing a higher-order structure into chemical materials. Cedar sawdust is a biopolymer that possesses a carbon framework microstructure. Through the introduction of various functional groups with chemical processing, cedar sawdust can be converted into chemical materials possessing novel characteristics. Such materials include adsorbents for dyes and enzymes, and chelating resins with metal-ion selectivity. This study focuses on cedar sawdust-derived functional materials as supports for the adsorption of enzymes. In this study used the enzyme, Nicotinamidase (EC 3.5.1.19) is an enzyme that degrades nicotinamide and has been reported to be abundant in *Flammulina velutipes*[1]. The active site of this enzyme contains a zinc. Nicotinamidase was extracted from *Flammulina velutipes* cultivated in Nagano Prefecture. *Flammulina velutipes* was homogenized in HEPES buffer solution (pH 7.0) for 3 minutes to form a paste and then centrifuged at $12,000 \times g$ for 15 min at 4°C. The supernatant was then filtered yielding a nicotinamidase solution. The presence of nicotinamidase was confirmed by measuring the zinc in the solution using ICP-AES. In addition, the extraction conditions for nicotinamidase from *Flammulina velutipes* were optimized by investigating homogenization time. An immobilized enzyme carrier was synthesized by pretreating plant-derived biomass with hydrochloric acid[2]. The biomass-based immobilized nicotinamidase was prepared by adsorbing nicotinamidase, a biocatalyst, onto the immobilized enzyme carrier. XPS detected a zinc atom peak (1022.0 eV) derived from nicotinamidase, confirming the immobilization of nicotinamidase(Fig. 1). EDS was used to observe the surface of the immobilized enzyme and confirm the presence of zinc originating from nicotinamidase. These results confirmed that nicotinamidase was successfully immobilized on the biomass. The apparent density of the immobilized enzyme, measured using a pycnometer, was found to exceed 1.0, indicating its feasibility for use in liquid-phase reactions. The activity of the immobilized enzyme was evaluated as a catalyst for the hydrolysis reaction of nicotinamide and was compared with the enzyme extract. The results confirmed the activity of the immobilized enzyme. To summarize, we successfully prepared immobilized nicotinamidase using plant-derived biomass as a substrate.

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P1-020**ELEMENTAL COMPOSITION AND ISOTOPE RATIO ANALYSIS OF HONEYS FROM US EAST COAST**

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Honey, renowned for its natural sweetness and versatility, serves as a flavorful and healthier alternative to traditional sweeteners in various culinary applications. This worldwide used sweetener, however, is extremely affected by anthropogenic activity through extensive agriculture, industrialization or traffic, and the impact of these influences can be detected by element and isotope analytical tools. Our former studies on unique, old series of EU (Hungarian) honeys proved their excellent relevance in reflecting the environmental conditions over a longer period of time based on their inorganic constituents but also showed not-contemporary carbon contribution which amount looks random, varying honey to honey. In present work the elemental composition and the contribution of the old carbon in aged honey samples from the eastern part of the USA were analyzed to verify the previous observations (in the EU) in a very different environment and continent. We used modern isotope ratio measurements ($^{13}\text{C}/^{12}\text{C}$, $^{14}\text{C}/^{12}\text{C}$) to determine the carbon isotopic composition and radiocarbon age of different US honey samples as well as the elemental concentration, Sr and Pb isotope ratio of the received honeys was determined by different inductively coupled plasma techniques (ICP-OES, ICP-MS, and MC-ICP-MS)

The multivariate statistical analysis of the elemental content shows a separation based on both the botanical origin of the nectar producing species and on the geographical origin of the nectar producing sites. A slight latitudinal effect was observed by the $^{13}\text{C}/^{12}\text{C}$ isotope ratio analysis, but honey samples from the Eastern part of the USA showed significant, 50% 10 years or even older carbon contribution to the honey by the radiocarbon analysis; that effect is random and does not have a correlation with the stable isotope signature. In specific cases, the measured values were even 20 ‰ (D^{14}C) higher than the expected atmospheric $^{14}\text{CO}_2$ value at the given year, which shows great old carbon contribution, presumably from not nectar origin excreted plant liquids, which shifts the calibrated age of the sample by even more than 5 years. Samples where adulteration is assumed based on the isotope analytical results show no statistically significant difference either in the composition or the concentration of the inorganic components.

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P1-021

EVALUATION OF BENZOPHENONE-3 AND OCTOCRYLENE ADSORPTION ON POLYETHYLENE USING HPLC-MS/MS AND VOLTAMMETRY WITH SCREEN-PRINTED ELECTRODES

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Microplastics (MPs) are pollutants of global concern due to their accumulation and low degradation in the environment. Additionally, MPs can adsorb and transport harmful contaminants, such as heavy metals, persistent organic pollutants (POPs), UV filters (UVFs), and pharmaceuticals, further exacerbating their environmental impact. These contaminants can adhere to the surface of MPs, facilitating their spread across different ecosystems. When ingested by marine organisms, these contaminated MPs can enter the food chain, potentially affecting not only aquatic life but also human health.

This study aims to evaluate the adsorption capacity of polyethylene (PE) when exposed to benzophenone-3 (BP3) and octocrylene (OC), taking into account various parameters such as pH, temperature, and exposure duration. The adsorption kinetics of such UVFs on PE beads were assessed using both pseudo-first-order (PFOM) and pseudo-second-order (PSOM) models. The equilibrium sorption capacity was found to be higher for OC than for BP3, likely due to OC's stronger hydrophobic interactions with the PE surface.

A central composite design (CCD) was employed to optimize resource efficiency during experimentation. The materials were subjected to controlled experiments in both pure freshwater and synthetic seawater. To assess the extent of UVFs' adsorption, liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was employed. Additionally, differential pulse adsorption stripping voltammetry (DPAdSV) measurements were conducted using disposable screen-printed carbon electrodes (SPCE).

The findings indicated that the adsorption affinity of OC on PE is greater than that of BP3. This is attributed to OC's high octanol-water partition constant ($\log K_{ow}$ 6.88), which suggests a greater affinity in non-polar environments. Adsorption analyses demonstrated that employing a CCD facilitates the identification of the most significant variables in the adsorption process of BP3 and OC on PE. In aqueous solutions, exposure time emerged as the most influential variable. In contrast, pH and temperature in pure water solutions were less significant. However, in synthetic seawater, pH and temperature significantly impacted BP3 adsorption, and its affinity for PE further decreased with increasing ionic strength. The primary interactions between BP3 and OC on PE are hydrophobic. The results obtained via DPAdSV using SPCEs were comparable to those achieved by HPLC-MS/MS, establishing it as a reliable method for in situ measurement of BP3 and OC in aquatic media.

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P1-022

THE ROLE OF CARBON FRACTIONS IN THE ACCUMULATION OF TRACE ELEMENTS IN FOREST SOIL (SUB)HORIZONS

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Soil organic matter (SOM) has a key role in carbon cycling, nutrient and trace element retention, however, its composition and properties vary in the soil profile, across individual soil (sub)horizons. SOM contains about 58% carbon, which in soils is the sum of both organic (OC) and inorganic carbon (IC). Total organic carbon (TOC) can be divided into labile fraction susceptible to biodegradation (TOC_{400} – includes low-condensation humic substances, polysaccharides, fulvic acids degradable at 400°C) and stable, hardly biodegradable form (ROC – residual oxidizable carbon). The individual carbon fractions determine the chemical and biological properties of the soil, affecting the ability to store nutrients, the mobility of trace elements and the stability of soil ecosystem. Therefore, the balance between labile and stable fractions, both organic (ROC) and inorganic TIC, is of particular importance, as it affects the processes of carbon retention and metal sorption.

In the temperate climate zone, the outermost layer of soil is the organic horizon (O), which consists of genetic subhorizons, including the fermentative-humic subhorizon (Ofh). This subhorizon has a high content of organic matter and a structure resembling tangled tobacco fibers, and it shows a far-reaching degree of organic matter decomposition. This makes it a kind of "natural sponge" in relation to trace elements (e.g. Cd, Hg, Pb, Zn).

The aim of the study was (i) to determine the variability of SOM, TC and carbon fractions (TOC, TOC_{400} , ROC, TIC) in the selected soil (sub)horizons, and (ii) to analyze the role of carbon fractions in the accumulation of trace elements in these (sub)horizons.

To achieve the aim, 130 soil samples were taken from the subhorizon Ofh and horizons-A/AE from 5 forest areas located in south-central Poland. All samples were analyzed for the content of: SOM, TC, TIC carbon fractions (magnesium and calcium carbonate, hydrogen carbonates), and selected trace elements – Cd, Hg, Pb and Zn.

The examined soil (sub)horizons showed differences in chemical composition and carbon fraction content, with the following average values: Cd ($\text{mg}\cdot\text{kg}^{-1}$): Ofh/A/AE 1.41/0.694/0.255; Hg ($\text{mg}\cdot\text{kg}^{-1}$): Ofh/A/AE 0.227/0.269/0.055; Pb ($\text{mg}\cdot\text{kg}^{-1}$): Ofh/A/AE 64.7/123/41.1; Zn ($\text{mg}\cdot\text{kg}^{-1}$): Ofh/A/AE 144/83.5/43.0; SOM (%): Ofh/A/AE 58.3/20.6/4.08; TC (%): Ofh/A/AE 33.3/12.5/3.18; TOC (%): Ofh/A/AE 33.2/12.3/3.08; TOC_{400} (%): Ofh/A/AE 32.9/11.7/2.69; ROC (%): Ofh/A/AE 0.31/0.55/0.39; TIC (%): Ofh/A/AE 0.09/0.15/0.07.

The obtained results indicate significant differences in the distribution of trace elements and carbon fractions in the studied soil (sub)horizons. In the subhorizon Ofh, which is rich in organic carbon forms, trace elements accumulate as a result of sorption and complexation with humic and fulvic acids (especially Zn and Cd). In the mineral horizon (A), further degradation of organic matter leads to a reduction in TOC content, and the retention of more stable fractions (ROC). The observed accumulation of Pb and Hg in this horizon suggests their retention by more humified organic matter, probably in association with the mineral soil fraction. In the humic-eluvial horizon (AE), eluvial processes promote the leaching of labile carbon forms (TOC_{400}), reducing the soil's ability to retain metals.

The observed differences in both the content and properties of carbon fractions in the examined (sub)horizons have an important role in soil biogeochemical processes, including metal transport, sorption dynamics and, retention of organic compounds in forest ecosystems. These findings are crucial for a better understanding of the accumulative properties of the individual soil (sub)horizons.

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P1-023**SUSTAINABLE REGENERATION OF ACTIVATED CARBON SATURATED WITH ORGANIC POLLUTANTS USING IONIC LIQUID SYSTEMS****Tekić, Danijela; Mušović, Jasmina; Marić, Slađana; Dimitrijević, Aleksandra; Jocić, Ana.***Institute of nuclear sciences Vinča, University of Belgrade, Belgrade, Serbia.*

Adsorption is widely recognized as an effective and versatile method for the removal of organic pollutants from wastewater, playing a crucial role in environmental remediation. Among various adsorbents, activated carbon (AC) stands out due to its availability, high surface area, porosity, and strong affinity for a broad range of organic contaminants, including dyes, pharmaceuticals, and pesticides. This makes AC one of the most commonly employed materials for water purification processes [1].

However, a significant challenge associated with adsorption lies in the inevitable saturation of the adsorbent over time, which leads to reduced efficiency and necessitates either disposal or regeneration of the material. Traditional regeneration methods — such as thermal treatment, chemical washing, or solvent extraction — are often energy-intensive, involve the use of hazardous chemicals, or lead to the release of secondary pollutants [2]. These drawbacks render conventional approaches inconsistent with the principles of green chemistry, which emphasize minimizing environmental impact, reducing energy consumption, and avoiding toxic reagents.

In recent years, ionic liquids (ILs) have emerged as promising alternatives to traditional solvents in various fields. ILs are salts composed out of organic cations, and organic or inorganic anions, which remain in liquid state at temperatures below 100°C. Characterized by their low volatility, thermal stability, and tunable chemical properties, ILs are increasingly investigated as green solvents. Additionally, the designer nature of ionic liquids — where specific cation-anion combinations can be tailored for targeted pollutant removal — further enhances their potential in sustainable wastewater treatment systems. A particular advantage of using ILs for these purposes is their ability to form aqueous biphasic systems (ABS) with the addition of salting out agent. This would allow for further concentration and separation of contaminants, and also the recycling of components of ABS used [3].

In this study, the regeneration of AC adsorbed with organophosphate pesticides chlorpyrifos and malathion, as well as synthetic dyes Acid Blue 9 and Acid Yellow 23, was investigated to apply the regeneration process to a broad spectrum of adsorbed molecules. A 20% wt water solution of the ionic liquids 1-butyl-3-butyl-imidazolium salicylate and 1-butyl-3-butyl-imidazolium bromide was used for this purpose. By utilizing salicylate-IL under standard conditions and room temperature, regeneration of up to 70% to a 100% was achieved in a single cycle. The salicylate IL proved to be more effective regardless of the contaminant adsorbed on the material, potentially due to specific interactions between this anion and the contaminants tested in the study.

This work highlights the potential of ILs for the sustainable removal of organic pollutants from aqueous solutions. The application of ILs would enable the development of a regeneration technology for AC used in water purification, regardless of the type of contaminant. Furthermore, it would facilitate a technology more in line with the principles of green chemistry, while also allowing the recycling of the components used, promoting a more sustainable and circular approach to water treatment.

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P1-024

INFLUENCE OF pH AND TEMPERATURE ON IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS WITH POTASSIUM PHOSPHATE: POTENTIAL FOR TARGETED MOLECULE SEPARATION

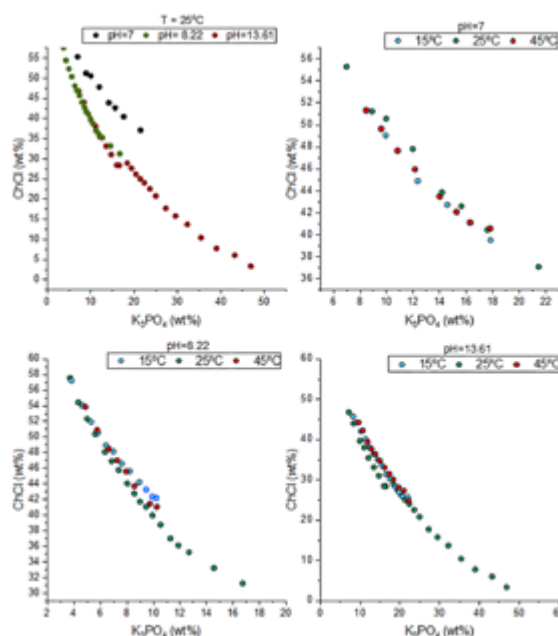
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Aqueous biphasic systems (ABSs) represent an efficient and eco-friendly separation technique, characterized by the formation of two immiscible aqueous phases. Typically, ABSs consist of two compounds soluble in water, such as polymers, salts, or ionic liquids (ILs) at specific conditions and concentrations of ABS compounds. ILs are salts composed out of organic cations and organic or inorganic anions, that remain in a liquid state below 100°C, characterized by their unique combination of physicochemical properties, including negligible vapor pressure, high thermal stability, and designer nature [1]. Choline-based ILs, stand out due to their low toxicity and biodegradability [2]. Among them, choline chloride ([Ch][Cl]) is not only distinguished by these advantages but is also commercially available, simplifying its application by eliminating the need for prior synthesis. ABSs with ILs are widely utilized for the isolation and selective separation of target compounds of interest, including biomolecules, pharmaceuticals, and environmental contaminants. Their tunable phase composition and enhanced extraction efficiency make them a promising alternative to conventional separation techniques [3].

Separation of certain molecules in ABS sometimes requires modifications of experimental parameters, such as pH and temperature, to achieve optimal partitioning. Given that many molecules exhibit pH-dependent ionization states, controlling pH can enhance their preferential distribution into the desired phase. Therefore, it is crucial to investigate the influence of these parameters on ABSs formation. Limited number of studies have focused on examining these effects, highlighting the need for further research in this area [4].

In this study, the impact of pH (7, 8.22, 13.61) and temperature (15°C, 25°C, 45°C) on the formation of [Ch][Cl]-based ABS with K_3PO_4 or phosphate buffer was investigated. Phase diagrams of these systems were determined using cloud point titration method, and represented as a function of mass percentage of the IL to the mass percentage of the salt/buffer. The results indicate that at room temperature (25°C), a higher amount of phosphate buffer is required to achieve phase separation at pH 7 compared to pH 8.22 and 13.61, where no significant difference in the biphasic region was observed. This behavior suggests that pH plays a critical role in shifting the binodal curve, likely due to changes in the ionization state of the the salt, reducing the salting out efficiency. Additionally, temperature had slight effect on the biphasic region. At lower temperatures, biphasic region decreased, which may be attributed to the decreased solubility of the salt, therefore decreasing salting out capabilities.



This study emphasizes the importance of pH and temperature in modulating the behavior of [Ch][Cl]-based ABSs. By adjusting these parameters, it is possible to fine-tune the partitioning of target molecules, enhancing the selectivity of the system. The results suggest that optimizing pH and temperature conditions can provide a more effective and adaptable approach for the extraction of pH-sensitive compounds, such as pesticides, pharmaceuticals, dyes etc.

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P1-025**FATTY ACID-BASED DEEP EUTECTIC SOLVENT-INDUCED COACERVATION IN MICELLAR SOLUTION OF ALKYL POLYGLYCOSIDE SURFACTANT: SUPRAMOLECULAR SOLVENT FORMATION AND APPLICATION IN FOOD ANALYSIS****Vakh, Christina.***Gdańsk University of Technology, Gdansk, Poland.*

The concept of green chemistry has driven the development of innovative, environmentally friendly approaches to sample preparation. Efforts have focused on replacing organic solvents in chemical analysis with more sustainable alternatives. Among the sustainable solvents, supramolecular solvents (SUPRAS) were considered and found widespread application in the determination of a variety of analytes. The term “supramolecular solvent” was first introduced in 2009 by D. Pérez-Bendito and S. Rubio and refers to a water-immiscible liquid consisting of supramolecular assemblies dispersed in a continuous phase.

SUPRAS are formed in the micellar solution and involve two self-assembly processes: aggregation of amphiphiles and coacervation, which leads to the formation of nanostructured, water-immiscible phases. SUPRAS have great potential for application in sample preparation and multiresidue analysis due to their diverse interactions (e.g. ionic, hydrogen bonding, hydrophobic) with the analytes and the possibility to adjust the polarity by varying the type of amphiphile. Depending on the type of amphiphile and the trigger that causes the coacervation process, both polar and non-polar analytes can be separated with SUPRAS.

This work focuses on the application of the biodegradable surfactant alkyl polyglycoside (APG) and its interaction with fatty acid-based deep eutectic solvents (DESs) to form SUPRAS. In this case, DESs based on hexanoic, heptanoic, and octanoic acids, as well as quaternary ammonium salts, were investigated as coacervation agents to induce phase separation in APG micellar solutions. DES precursors integrated into the micellar structure, enhancing interactions with target compounds and promoting phase separation by increasing micellar aggregate size. Computational prediction was used to determine which type of DES would be most suitable for a given analytical task in terms of extraction recovery of target analytes.

This green chemistry approach was successfully applied to extract capsaicinoids from chili sauce samples, achieving recoveries above 75% and detection limits of $1.7 \mu\text{g g}^{-1}$ for capsaicin and dihydrocapsaicin. The proposed extraction system could be applied to many other analytical tasks, especially in the analysis of solid and heterogeneous samples.

Challenges, advances, and tips for efficient extraction and reliable analyte determination will be discussed. This topic will provide insights into novel SUPRAS and demonstrate their potential for chemical analysis.

Vakh Christina, University Professor, Faculty of Chemistry, Gdańsk University of Technology. She is working in the field of sample preparation and application of green solvents such as supramolecular solvents, deep eutectic solvents and terpenoids for the extraction of target analytes from biological fluids, food and environmental samples. Her research interests also include miniaturization and automation of chemical analysis.

P1-026**EFFECT OF UV-LED IRRADIATION ON BACTERIAL DISINFECTION AND VIABILITY, CHEMICAL COMPOSITION, AND SENSORY EVALUATION OF BLACK PEPPER**

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Thermal treatments are widely used for food disinfection and have become the standard for microbial inactivation. However, these treatments can be detrimental to the nutritional composition and sensory qualities of certain foods, such as spices (Rifna et al., 2019), where these attributes are crucial. Therefore, alternative nonthermal treatments are necessary to ensure food safety. One promising alternative is ultraviolet (UV) light, which damages cellular DNA and prevents reproduction (Xu et al., 2018). UV disinfection is energy-efficient, has low installation and maintenance costs, and does not require chemical reagents. Black pepper, the most consumed spice globally, is near-spherical, allowing UV light to disinfect its surface effectively. Given that the efficacy of UV disinfection is wavelength-dependent, various wavelengths must be tested, which can be achieved through tailored wavelength LEDs.

The irradiation system employed consisted of an LED lamp positioned directly above the samples, which were constantly shaken. Samples were inoculated with *Escherichia coli*, *Salmonella enterica*, and *Bacillus cereus* spores, and plate counts were conducted before and after 10, 20, and 30 minutes of UV irradiation. Four different wavelengths were tested: 280 nm, 300 nm, 365 nm, and a combination of 280 and 300 nm. Results indicated that *E. coli* was the most sensitive to the treatment, showing a reduction of up to 1.6 log CFU/g after 30 minutes. *B. cereus* spores were the most resistant, with a reduction of 1 log CFU/g after 30 minutes. *S. enterica* showed a reduction of up to 1.4 log CFU/g. The most effective wavelengths were 280 nm or the combination of 280 and 300 nm in all cases.

Concerns about the permanent inactivation of bacteria after UV exposure led to the use of a special intercalating agent to estimate bacterial viability. Conventional agents such as EMA or PMA cannot accurately discern between viable and non-viable bacteria since UV light is not membrane disruptive. Therefore, DyeTox13, a recently developed agent that is membrane-permeable and metabolically sensitive, was used to assess *S. enterica* viability. No significant change in metabolically active and inactive cells was observed after the treatment, which could be due to the intercalating agent's lack of selectivity in a complex matrix such as pepper, or UV light rendering bacteria inactive rather than inactivating them.

Piperine, the main component responsible for the pungent taste of pepper, is crucial for its quality. The piperine content in black pepper did not significantly change after treatment, except for the 280 nm wavelength, which showed a 23% increase after 20 minutes, suggesting that UV treatment may have neutral or positive effects on piperine. Volatile compounds, responsible for food aroma, were also analysed. Shaking increased some volatile compounds, including linalool, myrcene, and alpha-pinene. However, UV irradiation consistently reduced most volatile compounds, with the 300 nm wavelength being the least impactful and the combined wavelength having the most deteriorating effect. No off-flavour-associated compounds were observed in any treatments.

A sensory analysis with 40 participants evaluated the treatment's effects on black pepper's colour, smell, and flavour. No significant differences were found between treated and untreated samples, indicating that chemical changes were not perceptible in the pepper.

In conclusion, UV disinfection achieved reductions of up to 1.6 log CFU/g for *E. coli*, 1.4 log CFU/g for *S. enterica*, and 1 log CFU/g for *B. cereus* spores after 30 minutes using 280 nm or 280+300 nm wavelengths. While some changes in volatile composition were observed, piperine content remained largely unchanged. Since no sensory differences were detected, longer irradiation times could be explored to achieve higher disinfection levels without compromising quality. Further research is needed to determine bacterial viability after UV exposure.

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P1-027**CHEMICAL FINGERPRINT AND SENSORY ANALYSIS OF UVC-IRRADIATED BLACK, WHITE, GREEN AND PINK PEPPERCORNS USING A SCALABLE REACTOR****Arcos-Limiñana, Víctor; Prats-Moya, Soledad; Maestre-Pérez, Salvador.***Department of Analytical Chemistry, Nutrition and Food Sciences, University of Alicante, Alicante, Spain.*

The application of ultraviolet (UV) light for the disinfection of spices has been the subject of considerable research over the past decade, with studies conducted on a range of spices, including oregano (Kucukoglu et al., 2024), paprika (Shoqairan et al., 2023), and black pepper (Gabriel et al., 2020), demonstrating the efficacy of UV treatments. One reason for the recent growth of the body of literature on this topic is the little impact UV light treatments have on the quality of spices, for which sensory attributes are their main asset. However, the quality assessment in such cases has been limited to superficial quality indices such as TPC and antioxidant activity, but irradiation can give rise to uncontrolled reactions, potentially impacting other sensory-related molecules. Therefore, a comprehensive chemical analysis combined with a sensory evaluation is necessary to better understand UV light's impact on peppers' quality. In addition, it should be noted that UV light has a limited penetration power; thus, only the surface of solid foods exposed to the light is effectively disinfected. Therefore, to improve the disinfection treatment a complete and homogeneous radiation of the sample must be ensured.

In the present study a 254 nm UV-C lamp was coupled to a hexagonal rotary drum reactor and black, white, green, and pink peppercorns were irradiated at an irradiance of 5.5 mW/cm² for 30, 60, and 90 minutes. This treatment, which resulted in a reduction of up to 2.6 log CFU/g of *Escherichia coli*, was observed to produce changes in peppers' chemical composition. Volatiles decreased significantly after the treatment, although not for more than 15%. White and pink peppers were the most affected by the treatment, potentially having consequences for their sensory attributes. Nevertheless, no off-flavour-related compound was detected in any of the samples.

Following the same trend, some phenolic compounds significantly changed their composition. The main phenolic compounds, gallic acid, cinnamic acid and succinic acid, remained unaltered, but different changes in the phenolic composition were observed. However, piperine, the main contributor to the characteristic pungency and bitterness of *Piper nigrum* pepper, was not observed to change significantly.

A sensory analysis was performed with 40 participants, evaluating three characteristics: visual aspect and colour, smell, and taste. Despite significant differences in the measured colour, no statistical differences could be stated in the sensory analysis. Visual perception of colour and texture is not two-dimensional, and the complexity of the colour palette in food can make it harder for individuals to observe differences. The same phenomenon was observed for the smell test. The complexity of the pepper volatiles prevented the participants from discriminating between the smell of the treated and untreated peppercorns.

A significant level of disinfection could be achieved with a 90-min UV-C treatment employing a scalable reactor with no perceptible change in sensory attributes despite finding changes in the chemical composition of the peppercorns. Therefore, further research could be conducted by increasing the treatment time or dose to enhance the disinfection capacity of the reactor if the sensory attributes are still indistinguishable or combined with other disinfection techniques.

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P1-028**APPLICATIONS OF CHROMATOGRAPHY FOR ANALYZING BEE PRODUCTS**

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Bee products has been consumed by humans since ancient times, and its ever-increasing consumption results from its nutritional value and health-promoting effects, like those relating to its antioxidant, anti-inflammatory, anticarcinogenic, antibacterial or anti-fungal properties. The nutritional value/quality and health properties of these products are linked to its constituents, which include proteins, amino acids, lipids, carbohydrates, phenolic compounds vitamins, or minerals, among several other compounds. However, its composition varies greatly according to several factors, like botanical and geographical origins, climatic conditions, the type of soil, or harvesting and processing conditions. This is quite important to prevent one of the main problems currently affecting the commercialization of these products, and consequently the beekeeping industry; this is the fraudulent practice of adulteration. As may be expected, studying the profile of a particular family of compounds (proteins, amino acids, lipids, or phenolic compounds) in bee products has been proposed, to specify/authenticate its origin and to evaluate the corresponding nutritional value or health-promoting effect.

Therefore, the main goal of this presentation is to show the potential of chromatography for determining bioactive compounds in bee products, which are among the most complex matrices that can be found in nature. Different families of bioactive compounds, some of them scarcely investigated in this matrices, have been determined by our group in the last years by using different chromatographic techniques (high performance liquid chromatography-HPLC, ultra-high performance liquid chromatography-UHPLC, gas chromatography-GC and supercritical fluid chromatography-SFC) and sample treatments (solvent extraction and solid-phase extraction-SPE). It should be mentioned that in all cases, new analytical methods were proposed, which fulfilled some of the principles of the green analytical chemistry, and that they were fully validated according with current legislation. Especial attention will be focused on recent results that demonstrated that bee pollen samples can be classified in most cases, by means of a canonical discriminant analysis (CDA) based on the content of the different compounds, according to the corresponding apiary of origin or the harvesting periods.

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P1-029**COMPARISON OF PLANT-BASED MEAT ALTERNATIVES AND MEAT****Cakar, Adem.***Agilent, Waldbronn, Germany.*

In recent years, there has been increasing consumer interest in meat alternatives. Reasons for this trend include negative impressions of the health impact of meat, environmental stress associated with animal meat production, and animal welfare.¹ Meat alternatives include plant-based, cell-based (in vitro or cultured meat), and fermentation-based (mycoproteins) products. Plant-based meat alternatives represent a primary sector of this industry, and their market has grown exponentially in recent years.¹ The transition towards a more plant-based diet is considered to reduce a person's environmental footprint compared with the consumption of animal-based foods. This transition appears to be supported by plant-based products that directly mimic meat, and thus do not lead to a fundamental change in dietary habits.² Most plant-based meat alternatives in development are protein-based, and considering their availability, cost, and processing functionality, soy and pea proteins as well as wheat gluten are most widely used.¹ From a nutritional quality perspective, the comparison of the amino acid profile of meat and plant-based meat alternatives is of interest.

The amino acid profile of a protein is typically analyzed following hydrolysis with 6 M HCl at 110 °C for 24 hours.³ Analysis of amino acids can be performed using various analytical methods, such as LC with fluorescence or UV detection following derivatization, LC/MS, or CE/MS.^{3,4} Precolumn derivatization with o-phthalaldehyde (OPA) for primary as well as 9-fluorenylmethylchloroformate (FMOC) for secondary amino acids overcomes the insufficient analyte retention on reversed-phase columns and the weak fluorescence and ultraviolet absorbance of amino acids.⁴ Furthermore, derivatization of amino acids followed by LC with FLD increases selectivity of the analysis.

Keywords: Amino acids analysis, automated pre-column derivatization, small molecules, fluorescence detection.

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P1-030**DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR BASED ON Ce-MOF@MIP COMPOSITE FOR SULFAMETHOXAZOLE DETECTION**

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Sulfamethoxazole (SMX) belongs to the sulfonamide antibiotics used in veterinary activities to treat gastroenteritis, pneumonia, and diarrhea in animals. However, the widespread use of SMX has led to its residue accumulation in animal food products intended for human consumption [1]. The studies show the presence of SMX residues in concentrations that exceed the maximum residual limits (MRL) in meat products (MRL for SMX is 100 µg/kg in meat) [2]. Therefore, SMX detection in food samples and the development of new antibiotic detection methods are of great importance. Today, as miniaturized devices, electrochemical sensors are characterized by simple and fast analyte detection, but also their portability, mass production, and cheapness significantly contribute to their wide application. The selectivity and sensitivity of the sensor can be fundamentally improved by their modification with different materials/composites [3]. Nowadays, metal-organic frameworks (MOFs) and molecularly imprinted polymers (MIPs) are promising materials in sensor design for pollutant detection in food. MOF materials (containing rare earth elements as a metal ion) influence sensor sensitivity. At the same time, MIP contributes to sensor selectivity due to the formation of selective binding sites that act as recognition sites for the target analytes after polymerization of the appropriate monomer [4]. This research synthesized Ce-MOF material. The obtained material was structurally and morphologically characterized by the XRD and SEM methods. Previously activated screen-printed carbon electrode (SPCE) was modified with Ce-MOF composite (thus prepared SPCE/Ce-MOF electrode). The most suitable monomer for MIP formation is selected according to the theoretical calculations (density functional theory, DFT). The SPCE/Ce-MOF electrode was subjected to an electropolymerization process using cyclic voltammetry (CV) and thus prepared the SPCE/Ce-MOF@MIP sensor. To gain the highest sensitivity during SMX detection, parameters such as monomer: template ratio, number of scans and scan rate (for the electropolymerization process), elution time, and incubation time were optimized using the CV method. As eluent in this study was used a mixture of CH₃OH: CH₃COOH (9:1, v/v). Differential pulse voltammetry (DPV) was used to develop the electroanalytical method for SMX detection. The developed electrochemical platform has a high potential for SMX detection in real food samples.

Acknowledgment

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P1-031**OPTIMIZED SAPONIFICATION FOR MOSH AND MOAH ANALYSIS IN FOOD MATRICES: ADDRESSING INTERNAL STANDARD VARIABILITY****Ferrara, Donatella¹; Albendea, Paula²; Gorska, Aleksandra²; Purcaro, Giorgia².**¹University of Turin, Turin, Italy; ²Gembloux Agro Bio Tech, Gembloux, Belgium.

Mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) are a complex mixture of food contaminants with high lipophilicity. Due to the complexity of MOH and food matrices, an efficient sample preparation method must be performed, which depends on the type of sample and the possible interferences. However, one step commonly included in the sample preparation for MOH analysis is the saponification, which allows the complete extraction of MOH from the lipids present in the matrix, improving the analytical sensitivity [1].

The official ISO 20122:2024 method for the determination of MOSH and MOAH determination in vegetable oils included a saponification step [2]. However, two major issues arise with the ISO method. First, it introduces substantial variability (15-25%) in MOAH quantification due to inconsistent partitioning of internal standards during saponification, affecting the ratio between 1,3,5-tri-tert-butylbenzene (TBB) and 2-methylnaphthalene (2-MN) [3]. Although the expected ratio is 1.00, values exceeding 1.15 have been reported, causing discrepancies in quantification. Second, the ISO saponification conditions (60°C, 30 min) was validated primarily on low-melting vegetable oils, which means that an adaptation is necessary for other type of food matrices.

This work presents an optimization of the ISO method using a microwave-assisted extraction (MASE) system for high-melting fats and different types of meat, as a more complex food sample. For the meat, unprocessed meat samples differing on the percentage of fat were studied: pig rib, beef rib and bacon (with 30%, 23% and 16%, respectively).

The performance of the saponification was evaluated in terms of internal standards distribution and recovery. The methods proposed in this work allows the complete saponification of both matrices, obtaining TBB/2-MN ratios closer to the expected value (1.00):

- i. For high melting fats: the TBB/2-MN ratio improved (from approximately 1.15 to 1.05), while maintaining high recoveries across diverse edible oils.
- ii. For meat: the TBB/2MN ratio was consistent among the three different types of meat tested and was on average 1.08 ± 0.02 and recoveries of 98% for MOSH and 96% for MOAH.

In conclusion, the MASE proposed in this work has shown to be adequate not only for MOH analysis in high-melting fats, but also for other types of matrices with a more complex composition.

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P1-032

DEVELOPMENT AND VALIDATION OF A GREEN AND PRACTICAL METHOD FOR STUDYING PESTICIDES AND RELATED CHEMICAL COMPOUNDS IN BEE POLLEN SAMPLES BY UAE-LC-QTOF-MS

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A novel analytical methodology was developed to quantify 79 pesticides and related chemical compounds in bee pollen samples using liquid chromatography quadrupole time-of-flight mass spectrometry. An optimal sample treatment was proposed (average analyte recoveries between 72% and 120%), involving ultrasound-assisted extraction with acetonitrile, followed by a 1:10 dilution. The environmental impact and practicality of the method were assessed using various analytical metrics, classifying it as green and blue. The method was fully validated in terms of selectivity, limits of detection ($0.06 - 30 \text{ ng g}^{-1}$) and quantification ($0.2 - 97 \text{ ng g}^{-1}$), matrix effect, linearity, precision, and trueness. Finally, the proposed method was applied to the target analytes in thirty Canadian multifloral bee pollen samples from different sources (commercial or experimental apiaries), but no pesticide residues were detected in any of them.

P1-033

COMPREHENSIVE ANALYSIS OF PESTICIDE RESIDUES IN EDIBLE WILD GREENS USING GC-MS/MS AND LC-MS/MS TECHNIQUES

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Ensuring food safety requires robust monitoring of pesticide residues in edible plants, especially wild greens that are commonly consumed but less regulated. This study investigates the pesticide residue levels in edible wild greens distributed in Gyeonggi Province, South Korea. A total of 233 samples were analyzed, comprising 145 leafy vegetables from 15 species and 88 stalk vegetables from 7 species.

Pesticide analysis was performed using Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), targeting 471 registered pesticide compounds. These techniques enabled the sensitive and specific detection of a wide range of pesticide residues.

Among the analyzed samples, 94 (40.3%) contained detectable pesticide residues. Leafy vegetables exhibited a significantly higher detection rate (50.3%) compared to stalk vegetables (23.9%), suggesting greater exposure or retention. Sixty-three different pesticides were identified in a total of 221 detection events. The most frequently detected compounds were fludioxonil (18 cases), azoxystrobin (16 cases), and tebuconazole (10 cases), all commonly used fungicides.

Importantly, four samples exceeded the Maximum Residue Limits (MRLs) established by Korean food safety regulations. These included pendimethalin and cadusafos in butterbur, carbendazim in aralia shoots, and dichlobenil in shepherd's purse. These findings underscore the necessity for continuous surveillance and enforcement of the Positive List System (PLS), particularly in wild plant products where agricultural practices are less controlled.

This study demonstrates the utility of combining GC-MS/MS and LC-MS/MS platforms in routine food safety evaluations, providing comprehensive residue profiles with high analytical reliability. The results support the ongoing efforts to enhance consumer protection and reinforce the importance of targeted monitoring strategies for non-cultivated edible greens.

P1-034**EXPLORING THE PHENOLIC COMPOSITION OF MATCHA TEA**

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Matcha tea has attracted significant attention due to its unique chemical composition and potential health benefits based on the bioactive compounds it contains. Unlike traditional green tea, matcha is made from tea leaves grown in the shade, which increases the concentration of certain bioactive compounds, such as chlorophyll, L-theanine and various phenolic acids. Moreover, matcha is consumed in powdered form, allowing the whole leaf to be consumed, leading to a higher intake of beneficial compounds compared to regular green tea.

The present study aimed to quantify selected phenolic compounds and caffeine in matcha tea using high-performance liquid chromatography (HPLC), a precision analytical method for identifying and measuring chemical components in complex mixtures. The results showed that among the phenolic acids analyzed, gallic acid showed the highest content (252.38 µg/g). Other detected compounds included 4-hydroxybenzoic acid, p-coumaric acid, ferulic acid, epicatechin gallate, sinapic acid, ellagic acid, rutin, resveratrol, myricetin, quercetin, kemferol and apigenin - all known for their strong antioxidant properties. In addition to phenolic compounds, matcha tea contains a significant amount of caffeine, measured at 2213.49 µg/g.

Research results confirm that matcha tea has a distinct chemical profile, rich in phenolic antioxidants and caffeine, making it a functional food with potential health benefits. Given its unique composition and the advantage of consuming whole leaves, matcha stands out as a valuable choice for those seeking both mental enhancement and antioxidant protection.

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P1-035

DETERMINATION OF ALDEHYDES IN EDIBLE OIL SAMPLES EMPLOYING 1-NAPHTHALENYL HYDRAZINE AS DERIVATIZING AGENT FOLLOWED BY HPLC-FLD

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Aldehydes are a group of compounds that contain a carbonyl group bonded to at least one hydrogen atom, aldehydes are ubiquitous and the importance compounds. In foods, aldehydes are formed by different oxidation pathways as by-products in the process maturing, deterioration, and microbial fermentation. Aldehydes cause deterioration in taste, flavor, odor, color, texture and appearance, and nutritional value decreases. Also, they have been associated to: cytotoxic, genotoxic and neurotoxic properties. One of the main principal sources of aldehydes are vegetable oil through heating processes generating mainly aliphatic aldehydes (formaldehyde to decanal), unsaturated aldehydes and oxygenated saturated and α , β -unsaturated aldehydes. Therefore, aldehydes determination in food samples is usually carried out as a quality control and food safety measure.

Consequently, different techniques have been employed for aldehydes detection and determination. Gas chromatography and high-performance liquid chromatography (HPLC) are the most used due to their versatility, capacity for analysis of multiple analytes and analytical sensitivity. Nevertheless, aliphatic aldehydes do not have chromophores, fluorophores or ionizable groups into their chemical structure. Therefore, derivatization processes are required to improve chromatographic stability, better resolution, higher analytical sensitivity and accuracy. Hydrazine, hydroxylamine, and amines have been evaluated as derivatizing agents being 2,4-dinitrophenylhydrazine (for HPLC) and O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (for GC) the most applied reagents. Even though HPLC with UV detection is used for aldehyde analysis with adequate separation and sensitivity, it is less suitable due to the complexity of the matrices and the low concentrations at which aldehydes are found, while fluorescence detection is preferred for its capacity to quantify individual aldehydes in complex matrices, with adequate sensitivity and selectivity after derivatization with fluorescent agents. Alternatively, naphthyl-derivatives have been used as derivatizing agents since the presence of a naphthyl group provides fluorescence characteristics, highly useful for liquid chromatography detection.

Therefore, the present work proposes the development of a sensitive fluorescent derivatization methodology using 1-Naphthalenyl hydrazine for pentanal, hexanal, heptanal, octanal, nonanal, and decanal. The derivatization products obtained are stable, allowing their separation and detection by HPLC-FLD. The method has been validated according to IUPAC criteria and applied in edible oil samples obtaining LODs in the interval of 4.0-6.2 $\mu\text{g L}^{-1}$, which are competitive with other methodologies. The proposed methodology is an alternative to derivatization methods with high sensitivity, adequate precision, and accuracy. The methodology allows the quantification of the total aldehyde content, observing excellent perspectives in food quality control.

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P1-036**MICROWAVE MINERALISATION AND ATOMIC ABSORPTION SPECTROMETRY (AAS) AS ANALYTICAL METHODS IN THE FIGHT AGAINST HIDDEN HUNGER****Łosak, Roksana.***Jan Kochanowski University in Kielce, Kielce, Poland.*

Hidden hunger is defined as the presence of nutrient deficiencies in the body and is currently a major problem, especially in underdeveloped countries. The most common deficiencies are iron and zinc. Various strategies are used to improve the nutritional value of food crops, including biofortification (Lowe, 2021). Plant biofortification is a plant breeding and production technique aimed at increasing the bioavailable levels of essential elements in the edible parts of plants through the use of agronomic measures or genetic selection to improve the nutritional value of the product (White and Broadley, 2005).

Microwave mineralisation is currently considered the most effective technique for mineralisation of environmental samples. It allows the determination of inorganic constituents, including heavy metals. The method uses microwave energy to rapidly and efficiently decompose organic material in the sample, releasing chemical elements in soluble form for further analysis (Ślęzak et al., 2016).

Atomic absorption spectrometry (AAS) is a technique used to determine the content of chemical elements in environmental samples by measuring the radiation absorbed by an element. The process involves analysing the spectra produced when a sample is excited with radiation. During this excitation, atoms absorb visible or ultraviolet light, causing them to move to higher energy levels. The atomic absorption method measures the amount of energy in the form of photons of light absorbed by a sample (García and Báez, 2012). Using atomic absorption spectrometry, it is possible to determine around 70 elements from the periodic table, including metals such as copper, zinc, iron, calcium, sodium, potassium and magnesium, which are necessary for the body to function properly (Ferreira et al., 2018).

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Hidden hunger, caused by nutrient deficiencies like iron and zinc, is a major issue, especially in developing countries. Biofortification enhances the nutritional value of crops through agronomic practices or genetic selection. Microwave mineralisation efficiently decomposes organic material for inorganic analysis, including heavy metals. Atomic absorption spectrometry (AAS) determines elemental content by measuring absorbed radiation, enabling the detection of essential metals crucial for human health.

P1-037**ANALYSIS OF ANTHROPOGENIC PARTICLES AND MICROPLASTICS IN PACKAGED DRINKS FROM SPAIN**

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Pollution by microplastics (MPs), generally defined as plastic particles ranging from 1 μm to 5 mm, is a growing environmental concern with potential implications for human health. MPs have been detected in various environmental compartments and food products, raising questions about unintentional dietary exposure. Packaged beverages, widely consumed worldwide, may contribute to this exposure due to contamination from processing, packaging, or raw materials. However, data on MPs in beverages remain limited, making further research necessary.

In this study, the presence of anthropogenic particles, including MPs and natural or semisynthetic cellulosic fibers, was investigated in packaged beverages from Spain.¹ A total of 73 samples, covering the most consumed drinks in the Spanish market, water, beer, wine, soft drinks, juices, energy drinks, sports drinks, and tea drinks, were analyzed. To assess the influence of packaging on MPs contamination levels, beverages packaged in PET and glass bottles, metal cans, and carton bricks were included. The entire contents of each package were vacuum-filtered through a 45 μm stainless-steel mesh, dried, and analyzed under a stereomicroscope to classify MPs based on shape, size, and color. The composition of selected particles was confirmed by $\mu\text{-FTIR}$ spectroscopy using vendor and open-access spectral libraries for polymer identification.²

A total of 1,521 anthropogenic particles were identified across all samples, with notable differences between beverage types. Additionally, phenoxy resins from can coatings were identified in all metal-packaged samples, suggesting potential migration from packaging materials. To estimate human exposure, the estimated daily intake (EDI) of anthropogenic particles through beverage consumption was also assessed. These findings contribute to the growing body of evidence on human exposure to MPs and highlight the need for further studies to assess their health implications and develop mitigation strategies. Understanding the role of packaging in MP contamination is essential for ensuring food safety and minimizing potential risks to consumers.

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P1-038**CHARACTERIZATION AND CLASSIFICATION OF HONEY THROUGH HPLC-UV FINGERPRINTING****Mostoles, Danica¹; Mara, Andrea²; Sana, Gavinno²; Sentellas, Sònia¹; Saurina, Javier¹; Núñez, Oscar¹.**¹*Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain;*²*Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy.*

Honey is the natural sweetener produced by honeybees from the nectar of plants or other non-floral secretions highly appreciated by society because of its multiple benefits including antibacterial, antiviral, and anti-inflammatory properties. These properties are influenced by multiple factors, considering mainly botanical and geographical origins. This phenomenon has given space for fraudulent practices, considering that a wide range of products with diverse quality and prices are known. For this reason, developing methodologies for characterizing and authenticating honey is crucial to ensure its botanical and geographical origins, as well as to avoid significant losses in the beekeeping sector or affect the consumer's trust.

In this study, the potential of HPLC-UV fingerprints was evaluated to address honey geographical origin authentication. For that purpose, a total of 157 samples from 9 countries (Spain, Italy, France, The Netherlands, Japan, Serbia, China, Costa Rica, and New Zealand) were subjected to a simple sample treatment consisting of dissolving 1 g of honey with 10 mL of water followed by centrifugation and a 1:1 dilution with methanol. HPLC-UV fingerprints were obtained by a C18 reversed-phase separation under a universal gradient elution. Then, the obtained fingerprints were evaluated as chemical descriptors for each sample through Principal Component Analysis (PCA) and the classification was performed through Partial Least Squares-Discriminant Analysis (PLS-DA). In addition, a classification decision tree was performed by consecutive paired PLS-DA models using a hierarchical model builder (HMB) for improving the classification performance and testing the prediction capability of the models.

Chemometric data analyses confirmed that the obtained HPLC-UV fingerprints were suitable chemical descriptors to assess honey geographical origin authentication, obtaining good classification performances when considering a multiclass PLS-DA model, with sensitivity and specificity values (for calibration and cross-validation) higher than 66.7 and 73.1%, respectively, and classification errors lower than 25%. The classification performance improved when the classification decision tree strategy was considered, obtaining values of sensitivity and specificity (for calibration and cross-validation) higher than 87.5 and 78.6%, respectively, while classification errors were reduced to lower than 10%. Additionally, the sensitivity and specificity were higher than 71.4 and 50% (excluding samples from Japan), with classification errors lower than 27.4%, demonstrating the usefulness to predict "unknown" samples. Thus, the proposed method based on HPLC-UV fingerprinting demonstrated to be a sound, cheap, and reliable methodology to be implemented in control laboratories to ensure honey geographical origin.

P1-039**MEAT AUTHENTICATION (PROTECTED GEOGRAPHICAL INDICATION, ORGANIC PRODUCTION, AND HALAL AND KOSHER PRODUCTS) BY HPLC-UV FINGERPRINTING AND CHEMOMETRICS****Santomá-Martí, Alexandra¹; Aijon, Nil¹; Núñez, Oscar².**¹*Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain;*²*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona. / Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona. / Serra Húnter Program, Generalitat de Catalunya., Barcelona, Spain.*

Meat products are worldwide consumed as one of the main dietary sources of protein leading to an increase in demand with the population growth, and generating significant challenges in the food sector. Incidents related to species substitution, adulteration and fraud-labelling on meat are common to generate illegal financial benefits, requiring new analytical approaches to assess their authenticity, quality and safety. This fraud is even more relevant in countries with cultural/religious practices prohibiting the consumption of meat products from some specific animal species such as on Muslim countries regarding Halal products, or the case of Kosher products in Jewish communities. The identification of the animal species present in meat-derived products is easily solved by genetic detection tools based on DNA determination such as the use of real-time polymerase chain reaction (PCR) among other techniques. However, several meat authentication issues not involving different meat species cannot be solved by means of genetic detection tools. These cases also involved cultural/religious practices, for instance, in Halal or Kosher products where the way in which animals permitted for consumption are slaughtered, or how the meat products are processed, is perfectly established. Other examples are related to the geographical origin of meat products, where the region where the animal has been raised becomes important, especially in those meat products with protected geographical indication (PGI) seals.

Within this context, in this work, the potential of a non-targeted HPLC-UV metabolomic fingerprinting method for the classification and authentication of meat products after a simple sample ultrasound extraction with water has been evaluated. 300 meat samples including different species (chicken, turkey, duck, quail, beef, lamb, pork, and rabbit), as well as different geographical origins (lamb with PGI label), production practices (organic chicken and beef), and ethical and religious aspects (Halal lamb and beef, and Kosher beef) were analyzed. Sample treatment consisted on a sonication (15 min) extraction with water (1 g sample with 10 mL water) followed by reversed-phase C18 HPLC separation under universal gradient elution conditions, and UV acquisition at 280 nm. The obtained HPLC-UV fingerprints were employed as chemical descriptors to address meat classification and authentication by partial least squares-discriminant analysis (PLS-DA). Results demonstrated a very good sample discrimination capability of the obtained fingerprints (when simultaneously considering the 8 groups of samples), with sensitivity and specificity values of 100% and higher than 99.3% for calibration and cross-validation, respectively, and classification errors below 0.36%. Besides, a classification decision tree consisting of consecutive dual PLS-DA models built using a hierarchical model builder was applied by using only 70% of the samples (randomly selected) for the calibration models, and submitting the other 30% of the samples (48 meat samples) as unknown samples for prediction. Excellent results were observed, with a 100% accuracy on the prediction capability of the proposed methodology.

PLS-DA discrimination was perfect when addressing organic chicken versus non-organic chicken, and lamb vs. Halal lamb and PGI lamb. In the case of beef samples, involving 5 sample groups (beef, 2 organic beefs, Halal beef, and Kosher beef), results were also very acceptable, with overall calibration and cross-validation values higher than 95.0% and 91.2% for sensitivity and specificity, respectively, and with classification errors below 6.9%. In any case, when addressing independently beef vs. organic beefs, or beef vs. Halal/Kosher beefs, the PLS-DA discrimination performance improved.

Finally, the obtained fingerprints were also very appropriate to easily detect and quantify meat adulteration levels by PLS regression. As a proof of concept, six adulteration cases involving adulterations on PGI, organic, Halal and Kosher meats were evaluated, with good PLS linearities, and with calibration, cross-validation, and prediction errors below 9%, 10% and 6.6%, respectively.

P1-040**CHARACTERIZATION, CLASSIFICATION, AND AUTHENTICATION OF COFFEE USING HS-SPME-GC-MS AND CHEMOMETRICS: A COMPREHENSIVE APPROACH FOR DETECTING ADULTERATION**

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The food industry faces increasing challenges in combating food fraud, particularly in highly consumed and economically valuable products. Coffee, due to its high global demand, is susceptible to adulteration, whether by adding flavor-enhancing substances or other materials to mimic the characteristics of high-quality coffee. These fraudulent practices not only affect consumer trust but also pose significant risks to food safety. This study presents two non-targeted methodologies for the classification, characterization, and authentication of coffee using Headspace-Solid-Phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) and chemometric analysis. For the first methodology, the chromatographic separation was carried out in a polar UF-WAX ms column; for the other method, the chromatographic separation was performed in a non-polar DB-5ms column. The proposed approach allows for the detection and quantification of adulterated coffee samples.

A total of 185 commercially available samples were analyzed, comprising 42 chicory samples (a typical coffee substitute or adulterant), 96 coffee samples from three geographical production regions (Vietnam, Cambodia, and Costa Rica) and species (Arabica, Robusta, and Arabica-Robusta mixture), and 47 samples were soluble coffee (decaffeinated and non-decaffeinated). The sample treatment consisted of a headspace-solid phase microextraction (HS-SPME) using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber. The analysis was performed directly from solid samples without pre-treatment before the microextraction.

The GC-MS fingerprints obtained from the analyzed samples were used to build data matrices for Partial Least Squares-Discriminant Analysis (PLS-DA) to classify, characterize, and authenticate the coffee samples based on their type, geographical origin, and variety. Paired PLS-DA models were employed, comparing a single sample class against all others, with a calibration set consisting of 70% randomly selected samples from each group and the remaining 30% used for prediction. The resulting paired PLS-DA models achieved sensitivity and specificity values higher than 95.7%, and classification error values lower than 2.2% when the polar column was obtained. For the non-polar column, the paired PLS-DA models achieved sensitivity and specificity values higher than 85.7% and classification error values lower than 7.1%.

Furthermore, the proposed methodologies were applied to quantify the levels of adulteration in coffee blends using Partial Least Squares (PLS) regression. Two specific adulteration scenarios were investigated: the first one involved Vietnamese Robusta coffee adulterated with chicory, and the second one Vietnamese Arabica coffee adulterated with Vietnamese Robusta coffee. The results were highly satisfactory, with R^2 values exceeding 0.993, demonstrating the excellent performance of both GC-MS techniques. Furthermore, the calibration and prediction errors, assessed through the Root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP), were below 2.9% and 7.4%, respectively.

In conclusion, the obtained results demonstrate the robustness and reliability of the proposed methodology for coffee classification, characterization and authentication, as well as its ability to effectively detect and quantify adulteration. This highlights the significant potential of these methods for enhancing quality control processes and preventing fraud within the coffee industry, ultimately ensuring the integrity of coffee products and safeguarding consumer trust.

Keywords: GC-MS fingerprinting; SPME; Coffee Authentication; non-targeted method, chemometrics

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P1-041**AUTHENTICITY ASSESSMENT AND FRAUD DETECTION OF TRUE CINNAMON BY TARGETED HPLC-UV AND CHEMOMETRICS**

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Cinnamon is a highly valued spice in cuisines around the world. One of the most well-known varieties is Ceylon cinnamon (also known as "true cinnamon"), which is obtained from *Cinnamomum zeylanicum*, a variety native to Sri Lanka and southern India, and is responsible for the characteristic mild and sweet flavour of cinnamon. Additionally, Ceylon cinnamon has many health benefits, as it is rich in phenolic and aromatic compounds such as eugenol and cinnamaldehyde. However, the higher price of Ceylon cinnamon makes it more vulnerable to substitution or adulteration by cheaper varieties of cinnamon, with cassia cinnamon being the most common. Cassia cinnamon (*Cinnamomum aromaticum*) is primarily native to southern China and Burma, and has a slightly different smell and taste. However, from a health perspective, the main concern is the higher coumarin content found in cassia cinnamon, which can cause hepatotoxic effects if consumed in amounts exceeding 0.1 mg/kg of body weight. Therefore, it is necessary to develop simple and rapid techniques to detect total or partial adulterations in cinnamon.

This work presents an analytical method based on liquid chromatography with UV-vis detection for detecting fraud in commercial powdered cinnamon. The developed method is based on the determination of four characteristic biomarkers: eugenol, cinnamaldehyde, coumarin, and cinnamic acid. The obtained data were analysed using partial least squares regression (PLS). Generally, the determined concentration of eugenol was much higher in Ceylon cinnamon, while coumarin was mainly present in cassia cinnamon. Large concentrations of cinnamaldehyde and cinnamic acid were found in both varieties of cinnamon. It is important to note that the content of these four compounds allowed not only the discrimination between Ceylon cinnamon and cassia cinnamon but also the detection and quantification of partial adulterations. This method was successfully tested with several mixtures prepared in the laboratory using different commercial samples considered 'pure' cassia or Ceylon, as well as commercial samples with a declared percentage of both cinnamon varieties.

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P1-042

COMPLEX ANALYTICAL INVESTIGATION OF NATURAL AND ARTIFICIAL SWEETENERS

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Safety is one of the key aspects of food quality, involving the type and quantity of contaminants, but it is also influenced by various additives, including a significant number of fossil-based components. Nowadays, there is an increasing demand for the labelling of product composition and ingredient origin, along with awareness of food safety regulations. The aim of our work is to identify markers and threshold values in sweeteners that can support the work of decision-makers and legislators in the long term to establish a harmonised system of certification that will benefit both producers and consumers. Therefore, we perform complex analysis on natural and artificial sweeteners.

In the course of our work, we have selected 71 different sweeteners from popular shops and webshops, aiming to investigate sugar substitutes with different consistencies within each type. When compiling the sample set, we took great care to test both natural and artificial sweeteners of different price ranges, in line with consumer preferences, so that the sample collection was highly representative. For the natural, plant-derived sweetener groups, we purchased several bio-labelled products from each representative for later comparisons, and also included some of the lesser-known, more specialised sugar substitutes in the studies. As a result, the sample set compiled includes agave, date, coconut blossom, rice, chicory and maple syrups, stevia, erythritol, fructose, cyclamate and saccharin derivatives, sucralose, xylitol and lucuma powder.

As different sugar substitutes have a very diverse matrix, we have developed a sample preparation procedure that allows a cost-effective, consistent digestion. Subsequently, the micro- and macroelemental composition of the sweeteners was investigated by inductively coupled plasma optical emission spectrometry. In addition, we have also started the bio-component determination of biological and fossil sweeteners using accelerator mass spectrometry (AMS) based radiocarbon measurement, one of the most modern methods currently available, relying on the fact that ¹⁴C is selectively incorporated at the atomic level in biogenic materials. The results obtained from the different analytical tools will be subjected to a complex statistical evaluation, with the objective of using these results to help clarify the questions and misconceptions surrounding natural and artificial sweeteners.

P1-043**OCCURRENCE AND DISTRIBUTION OF PESTICIDES IN WINES FROM TWO MAJOR PRODUCTION REGIONS IN THE NORTH OF SPAIN**

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In the current scenario of climate change, sudden variations of climate conditions (temperature and humidity) increase the pressure of pests affecting the productivity of vines. Thus, winegrowers need to achieve a balance between the economic and the environmental sustainability of their activity. The European Food Safety Agency (EFSA) maintains an updated list of authorized pesticides, with maximum residue levels (MRLs) in different vegetable commodities, including vinification grapes (*European Pesticide Database of MRLs in Food Commodities*, n.d.). However, no MRLs have been set for wine, yet. Therefore, although the control of pesticide residues in vinification grapes permits to verify their compliance with current legislation, it has a limited usefulness to predict their levels in wine, and to calculate exposure levels of wine consumers. Grape berries are a less homogeneous matrix than wine. In the latter case, a single sample is representative for thousands of bottles proceeding from same tank. Despite these facts, systematic studies assessing the presence of pesticide residues in wines are still scarce.

In this presentation, we show the results obtained during the survey of fungicides and insecticide residues in wines elaborated in two main production regions in the North of Spain: La Rioja and Galicia, with different pedologic and climate characteristics. The study involved the analysis of more than 400 samples, equally distributed among the different sub-zones, or Denominations of Origin, from both regions corresponding to four consecutive vintages. Compounds were determined by liquid chromatography tandem mass spectrometry, including determination of total residues and enantiomeric analysis of most often detected chiral fungicides. The aims of the presentation are (1) assessing total residues of pesticides in wine; (2) identifying potential differences depending on the production area; (3) evaluating the impact of regulatory policies on the residues of active ingredients, which lost the authorization of use, or that are candidates for substitution; and (4) investigating potential risks of human exposure to pesticides through a regular, moderate consumption of wine.

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P1-044**MULTI-ELEMENT ANALYSES OF YERBA MATE - AN UNIQUE SAMPLE MATRIX FROM CHEMICAL AND CONSUMER POINT OF VIEW****Różewska, Anna; Proch, Jędrzej; Niedzielski, Przemysław.***Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland.*

Yerba mate (*Ilex paraguariensis*) is a beverage prepared from leaves and twigs of the Paraguayan holly tree which grows in few South American countries [1]. In recent years, yerba mate has gained popularity beyond its traditional areas and its consumption has also spread to Europe and parts of Asia. This has led to increased interest not only among consumers, but also among researchers. Despite the reported increase in interest in the yerba mate matrix, no previous procedure for developing and/or certifying a yerba mate-based reference material has been reported in the literature. There is NIST SRM 3253 (yerba mate leaves), but it is only for the determination of polycyclic aromatic hydrocarbons (PAHs), which does not make it a useful tool in elemental analysis. Moreover, there is no standardized procedure for yerba mate brewing in scientific studies. In the literature, the water used is usually boiling [2,3] and this is not the preferred temperature for brewing yerba mate. For this reason, a novel approach was taken to the study and comparing the temperature difference (20°C, 80°C and 100°C) and the type of water used for extraction. For the first time, the effect of tap water on extractable element content was evaluated. Traditional steaming (CON) and ultrasound-assisted extraction (USN) were compared [4]. The study conducted is therefore novel and fills gaps in the literature on the leachability of elements from this matrix. What is more, a three-step assessment of the content of selected elements in yerba mate was verified by testing a novel infusion preparation procedure based on dynamic extraction. The evaluation of three stages of yerba mate consumption: dry leaves, infusion, and grounds contributed to a better understanding of the behavior of this matrix in the determination of elemental content. To evaluate the dynamic extraction (DYN) procedure, it was compared with the ultrasound-assisted extraction (USN) procedure mentioned before [5]. The proposed sample preparation procedures, i.e. microwave-assisted mineralization and USN extraction, were comprehensively tested using certified reference material (tea leaves, INCT-TL-1). Significant differences in extraction percentages were observed between both sample matrices, confirming the need for research on yerba mate as a potential new reference material. All extracts and digests were analyzed by ICP OES.

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P1-045**ONSITE TESTING FOR *BRETTANOMYCES BRUXELLENSIS* CONTAMINATIONS DURING WINEMAKING**

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World wine production is estimated at 262 million hectolitres in 2022. Long spared from the constraints of the agri-food industry due to the absence of pathogens, the oenological sector is now facing the yeast *Brettanomyces bruxellensis*, which has a high potential for organoleptic deviation due to the production of volatile phenols that make wines unmarketable. This problem is currently the most concerning regarding wine quality. It poses significant challenges in the current context of reduced inputs (sulfites) and climate change, which raises the pH of wines and, as a result, increases their sensitivity. One of the main difficulties is the lack of efficient tools to detect and quantify the presence of this yeast. Current tests only partially meet the criteria to enable effective prevention and management of contamination. Indeed, the assay should be fast, inexpensive, easy to use, and usable on-site while quantifying only living cells.

Here, a new approach was developed based on quantifying a specific ribosomal RNA (rRNA) sequence of *Brettanomyces bruxellensis*. Thanks to loop-mediated isothermal amplification (LAMP), the assay was highly sensitive, allowed easy detection, and was compatible with inexpensive commercially available readers. Furthermore, using rRNA as a target allowed for the quantification of living cells only. An analytical workflow was developed to simplify the isolation of rRNA using simple laboratory tools and commercial kits. The analysis of wines took less than 2h for a small cost per test. We will describe our latest results in this project.

P1-046**A VERSATILE, SELF-POWDERED AND SUSTAINABLE PLATFORM FOR WIRELESS ELECTROCHEMICAL MEASUREMENTS**

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Electrochemical sensors play a fundamental role in our healthcare system, due to their excellent performances for quantitative monitoring of several analytes in biofluids. Recent progress in the miniaturization and digitalization of electronic devices has enabled the transition from bulky clinical devices to handheld consumer devices – particularly for the monitoring of glucose or hormones from blood, urine, or interstitial fluids.

There is nevertheless a growing concern regarding the generation of electronic waste (“e-waste”) specific to the healthcare industry. Compared to other segments of conventional electric applications, electrochemical sensors operate in harsher conditions (e.g. extended immersion in biofluids, non-ideal testing environments, or user error due to non-professional consumer handling). A special care is therefore required to ensure that efforts in sustainability do not impact performances or usability of consumer products. Towards this objective, CSEM has worked to address several aspects of biosensor design and fabrication that lower the footprint of devices without compromising the quality required for healthcare diagnostics.

We envisioned a Point-of-Care device where electronic components have been reduced to a minimum, the device footprint has been minimized, and sensor materials and manufacturing methods have been transitioned to sustainable alternatives. The ambition is to have a solution that can reach the same level of usability seen in conventional electrochemical sensors, but in a more sustainable form factor.

First, we developed and integrated a custom monolithically integrated circuit (“IC”) that can process internally all required operation for several type of common electrochemical techniques (i.e. open circuit potential, chronoamperometry, or cyclic voltammetry), handle communications to remote readers, and operate with minimal power consumption – all without the need of any additional electrical components. IC is integrated onto a flexible substrate together with a sensing module and NFC coil (for remote communications). All components other than the IC were manufactured with sustainable and scalable screen-printing methods. By interfacing with a smartphone, the NFC coil supplies energy for device operation in addition to wireless data transfer. In parallel, key materials in the electrochemical sensor were substituted with more sustainable alternatives, such as bio-sourced substrates. We illustrate that replacing polymers like PET for cellulose does not significantly alter short-term sensor performance (i.e. sensitivity, stability, response time) for both potentiometric (K^+ ions) and amperometric (glucose) measurements.

Our final demonstrator is a fully functional glucometer that can work as effectively as a commercial solution yet with a significant reduction of environmental footprint. The versatility and low power operation of the IC chip allow to quickly integrate our solution into a variety of use cases common in clinical diagnostic or wearable devices, thus having the potential to become a new standard in remote healthcare monitoring.

P1-047**PEROXIDASE-LIKE ACTIVITY OF A SUPRAMOLECULAR NETWORK INCLUDING COPPER AND WS₂ NANODOTS AND THEIR APPLICATION FOR HYDROGEN PEROXIDE DETERMINATION****Pérez-Mirayo, Claudia¹; Sáez-Coronado, Adrián¹; Vázquez, Luis²; Petit-Domínguez, María Dolores¹; Quintana, Carmen¹; Casero, Elena¹.***¹Departamento de Química Analítica y Análisis Instrumental. Facultad de Ciencias. Universidad Autónoma de Madrid, Madrid, Spain; ²Instituto de Ciencia de Materiales de Madrid (ICMM)-CSIC, Madrid, Spain.*

Over the past few decades, various nanomaterials, which include nanoparticles, carbon-based compounds, and transition metal dichalcogenides, have been reported to function as nanozymes, exhibiting enzyme-like catalytic activities due to their unique physicochemical properties. These nanozymes mimic the functions of natural enzymes while offering several advantages such as high stability, cost-effectiveness, and adaptability to harsh conditions. As research in this field progresses, novel hybrid nanostructures with nanozyme activity continue to emerge, broadening their potential applications [1, 2].

In this study, we have synthesized a new material from melamine and cyanuric acid precursors by means of a supramolecular self-assembly simple process, giving rise to a two-dimensional network (MAC), in which a metal (Cu) and WS₂ QDots have been included during the synthesis process (MAC-Cu-WS₂). The synthesized materials were characterized by Scanning Electron Microscopy, Atomic Force Microscopy, X-ray Diffraction and Infrared Spectroscopy. These techniques confirmed that the MAC material has been successfully synthesized, presenting a network of nanofibers, which was maintained in the case of the material that includes the metal and the QDots in their structure. The as-synthesized material behaves like a natural peroxidase enzyme, catalyzing oxidation-reduction reactions between H₂O₂ and a reducing agent.

The measurement of the peroxidase-like activity was carried out by employing 3,3',5,5'-Tetramethylbenzidine (TMB) as reducing agent, which reacts with H₂O₂ in the presence of MAC-Cu-WS₂, giving the colored oxidized form of TMB, which presents an absorption band at a wavelength of 650 nm. After optimizing several parameters that influence the spectrophotometric response, such as temperature, pH and time of reaction, an analytical methodology for determination of H₂O₂ was developed.

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P1-048

ECO-FRIENDLY TWO-DIMENSIONAL STOCHASTIC ENANTIOSELECTIVE SENSOR FOR THE EARLY IDENTIFICATION OF BRAIN CANCER

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The biomedical field is progressively adopting disposable sensors for rapid and cost-effective biomarker screening and monitoring at or near the patient location. Serine is significantly linked to cancer. High levels of serine helped glioblastoma cells survive under stress, but low levels of serine led to fewer cells dividing and more cells dying when oxygen levels were low.¹

Serine is a prognostic and predictive biomarker used to monitor disease development and combat cancer.²

Two disposable stochastic sensors were developed using nanocomposite nanolayer deposition of copper (Cu) and graphene (Gr) on silk textiles,³ subsequently modified with octakis(6-deoxy-6-(2-aminoethyl)thio)-gamma-cyclodextrin hydrochloride (CysGCD) for biomedical assessment by the stochastic method.

We used scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) to look at the silk textiles that were used to make the two-dimensional stochastic sensors.

This category of affordable sensors facilitates the acquisition of essential analytical data by anybody, at any location, and at any time, without concerns of contamination and recalibration.⁴

We used the Green Analytical Procedure Index (GAPI), Analytical Greenness (AGREE), and Blue Applicability Grade Index (BAGI) to rate the method. The results showed that it was safe for the environment and could be used in many situations.

This study's results, when compared to other sensors and methodologies applied for serine enantioanalysis, demonstrate the capability to detect a wide range of working concentrations with exceptionally low limits of determination.

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P1-049**UPLC-MS/MS ANALYSIS OF OXYSTEROLS PROFILE: A POSSIBLE MARKER FOR EMBRYO DEVELOPMENT MONITORING**

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The investigation of the oxysterol profile in a suitable in vivo system could provide useful information on the amount, roles, and biological functions of these molecules that are enzymatically generated by the cytochrome P450 (CYP450) family, via autooxidation, or even via both pathways in synergy.

Zebrafish is an animal model used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences; this model presents several advantageous features such as high fecundity, rapid embryonic development (24 h), and external fertilization [1]. The lipidomic analyses in zebrafish early-life stages could represent a reliable tool for studying the global lipid profile during embryonic development, but the knowledge about the sterol derivatives in the context of embryogenesis of these vertebrates remains limited [2]. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules. Moreover, different oxysterols have been investigated in zebrafish as liver X receptor (LXR) activators of the liver and regulators in the metabolism of carbohydrates and lipids, and the cytotoxic effects of 25-hydroxycholesterol on nervous system cells in zebrafish larvae were demonstrated [3].

From an analytical point of view, oxysterols present many challenging aspects: first of all, deriving from the same precursor molecule (cholesterol), these compounds are an isomeric group, so that tandem mass spectrometry is not able to obtain univocal identification, and complete chromatographic separation is mandatory. Anyway, the separation of oxysterols is a challenging task as these compounds differ from each other only by hydroxylic moiety position (i.e., 27-hydroxycholesterol and 25-hydroxycholesterol). Moreover, the changes in composition and matrix characteristics of embryos during their growth need a robust analytical method with high versatility. Finally, the poor ionization efficiency of oxysterols, due to the high lipophilicity, is another challenging aspect to take into account. The aim of this work was the study oxysterol fingerprinting as a tool to understand the CYP450 metabolic pathway modification during embryo development employing UPLC-MS/MS [4]. The main issues were efficient chromatographic separation and robust sample preparation. The selective extraction of the target compounds was developed with suitable enrichment factors, maintaining an acceptable matrix effect during all embryonic growth stages. Furthermore, the use of multivariate analysis and a machine learning approach explored the variation of oxysterol profiles as possible markers of zebrafish embryo development. A combination of hierarchical clustering analysis (HCA) and Random Forest (RF) classification revealed distinct stage-specific oxysterols profiles. These findings provide new insight into the role of oxysterols during embryogenesis and establish a reliable platform for evaluating oxysterol dynamics in development, toxicological studies using the zebrafish model.

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P1-050**DETERMINATION OF POLYPHENOLS IN FOOD EMPLOYING UPLC-ESI-QqQ-LIT-MS STRATEGIES FOR THE IDENTIFICATION OF POLYPHENOLS**

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Phenolic compounds (PCs) are a broad and diverse group of secondary metabolites ubiquitously present in the plant kingdom, where they play a crucial role in various physiological and biochemical processes. These molecules are particularly known for their potent antioxidant properties, which are closely associated with their structural characteristics, especially the presence of hydroxyl groups that can donate electrons and stabilize free radicals [1]. In addition to antioxidant activity, PCs also exhibit anti-inflammatory, antimicrobial, cardioprotective, and neuroprotective effects, which have garnered significant interest in fields ranging from food science and nutrition to pharmacology and plant physiology. The structural diversity of phenolic compounds arises from the vast array of chemical modifications they can undergo within plant tissues. These modifications include glycosylation, methylation, acylation, esterification, and oxidation reactions, which can occur both enzymatically and non-enzymatically [2]. As a result, a single phenolic backbone may give rise to a multitude of conjugated forms with distinct chemical and physical properties. This complexity poses significant analytical challenges, especially in untargeted or semi-targeted approaches where both qualitative and quantitative characterization of a wide spectrum of PCs is required. In this context, the present work explores the analytical potential of hybrid linear ion trap-triple quadrupole mass spectrometry (HPLC-LIT-QqQ) coupled with high-performance liquid chromatography. This powerful combination offers the unique capability of performing multiple acquisition modes within the same analytical platform, thereby enabling a more in-depth exploration of PCs and their conjugated derivatives. Specifically, the method was developed to exploit advanced scan functions such as precursor ion scan (PI), neutral loss scan (NL), enhanced product ion scan (EPI), and MS³ (MS/MS/MS) [3,4], all of which were designed and optimized based on known structural features of key phenolic subclasses. These acquisition strategies were tailored to selectively detect characteristic moieties common to specific groups of PCs, such as phenolic acids (e.g., hydroxycinnamic and hydroxybenzoic acid derivatives), glycosylated flavonoids (including mono-, di-, and tri-glycosides), and oligomeric proanthocyanidins. By targeting conserved fragmentation pathways or mass losses associated with specific conjugation types (e.g., loss of hexose, deoxyhexose, galloyl or acyl groups), the method allows for the selective screening of compound families, even in complex matrices and without the need for extensive libraries of analytical standards. This approach not only enhances the detection sensitivity and selectivity for specific PCs but also facilitates the putative identification of unknown or novel derivatives based on their fragmentation patterns and precursor-product ion relationships. The MS³ capability, in particular, provides additional structural resolution for the elucidation of complex or isobaric compounds, offering a valuable layer of information for confirming the identity of metabolites that share similar fragmentation behavior in standard MS/MS analysis. Overall, the proposed methodology represents a versatile and comprehensive analytical strategy that can be applied to a wide range of biological and food matrices. It is particularly well-suited for studies aimed at profiling phenolic content, monitoring changes in metabolite composition under different environmental or processing conditions, or investigating the metabolic fate of PCs in vivo. By reducing dependence on authentic standards and enabling the targeted exploration of conjugated structures, this method provides a powerful tool for both routine screening and advanced research applications in the study of plant-derived phenolic compounds.

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P1-051

FIRST APPLICATION OF QuEChERS EXTRACTION AND GC-MS FOR PAHs DETECTION IN HUMAN ADIPOSE TISSUE

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Biomonitoring is a fundamental approach for protecting human health from exposure to hazardous substances, based on determining the presence and concentration of pollutants in the human body. It is increasingly applied to track large-scale exposure risks to pollutants within the population.

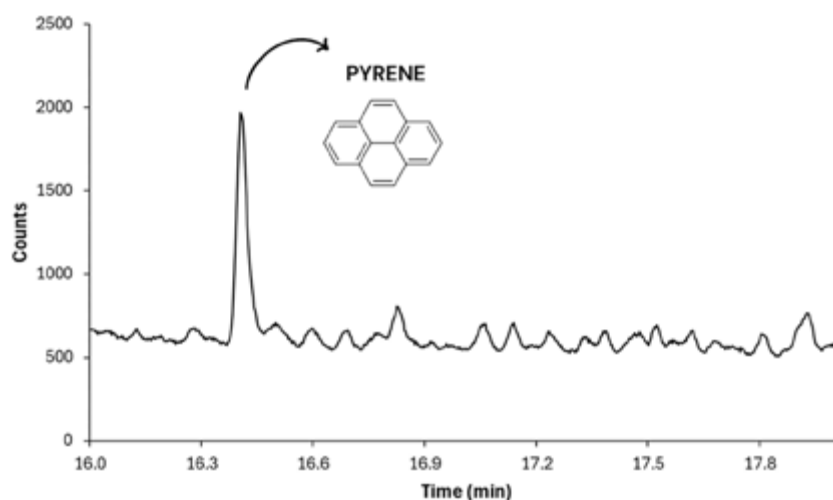
This work is part of a PRIN 2022 PNRR project titled "Integrated systemic detection of pollutants in the human body" (INSYDE-HU), which aims to obtain crucial direct data on pollutant exposure in individuals not professionally exposed. Within this project, new analytical methods are being developed and optimised for the determination of a selected set of pollutants in different target organs.

This work aims to develop an analytical methodology for the quantitative determination of polycyclic aromatic hydrocarbons (PAHs) in human adipose tissue, a complex matrix that has been scarcely investigated in biomonitoring studies. The novelty of this work lies in the fact that, partly due to the invasive nature of the sample collection procedure, very few studies in the literature assess the presence of these pollutants in adipose tissue or other human organs. Most studies focus on collecting and analysing less invasive biological matrices, such as blood or urine, which, however, provide only indirect data on internal pollutant levels.

PAHs are a group of substances known for their widespread presence in all environmental compartments, their potential to enter the food chain, their high toxicity to humans and, in particular, their lipophilic properties which make them potentially bioaccumulative in adipose tissue.

In this study, the pre-analytical phase is developed and optimised using the QuEChERS extraction and purification method and analysis is conducted using gas chromatography coupled with mass spectrometry (GC-MS). The method development and validation were conducted using a surrogate animal matrix (bovine fat), followed by application tests on human adipose tissue samples from non-exposed individuals, provided by the Complex Operative Unit of Plastic Surgery of the University Hospital of Padua.

The method was validated for 14 PAHs, with LOD and LOQ values ranging between 0.11-4.13 ng/g and 0.30-13.76 ng/g, respectively. The values obtained in this study are consistent with those reported in the literature. For instance, Sousa et al. [1] found LOD and LOQ values ranging between 0.04 and 12 ng/g and 0.1 and 39 ng/g, respectively. In the pilot application on human adipose tissue samples, phenanthrene, anthracene, and pyrene were detected. Pyrene was quantified with concentration values ranging between 2.34 and 4.88 ng/g. This is one of the few methods for the determination of these compounds in such a complex matrix and the only one, to our knowledge, that utilises the QuEChERS method and GC-MS.



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P1-052

METHODOLOGIES BASED ON THE FORMATION OF DUPLEX AND TRIPLEX DNA STRUCTURES AND FLUORESCENT SILVER NANOCCLUSERS FOR THE DETECTION OF PYRIMIDINE-RICH DNA SEQUENCES

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In this work, strategies for the detection of pyrimidine-rich DNA target sequences based on the formation of duplex and antiparallel triplex structures are presented. The presence of the target is detected from the changes in fluorescence of silver nanoclusters stabilized by the corresponding complementary DNA probes.

It has been observed that the formation of antiparallel triplex structures produces changes in fluorescence properties that could be more useful for analytical purposes than those observed when only duplex structures are formed. In particular, the use of silver nanoclusters confined within a loop rich in cytosine-type bases in the antiparallel triplex structure resulting from the interaction of probe and analyte has been shown to produce an increase in red fluorescence. This latter probe has been shown to be selective against target sequences that have mismatches that could affect the formation of stable duplex structures, while it has been shown to tolerate a small number of purine mismatches that could affect the stability of the resulting antiparallel triplex structure. As a final remark, it should be noted that this methodology could also be used in the development of analytical procedures that allow the detection of antiparallel triplex structures, which are difficult to observe with other spectroscopic methods.

P1-053

ADVANCING NEUROPROTECTION THROUGH AVOCADO PEEL EXTRACTS: INTEGRATING BIOANALYTICAL TECHNIQUES AND CIRCULAR ECONOMY PRINCIPLES

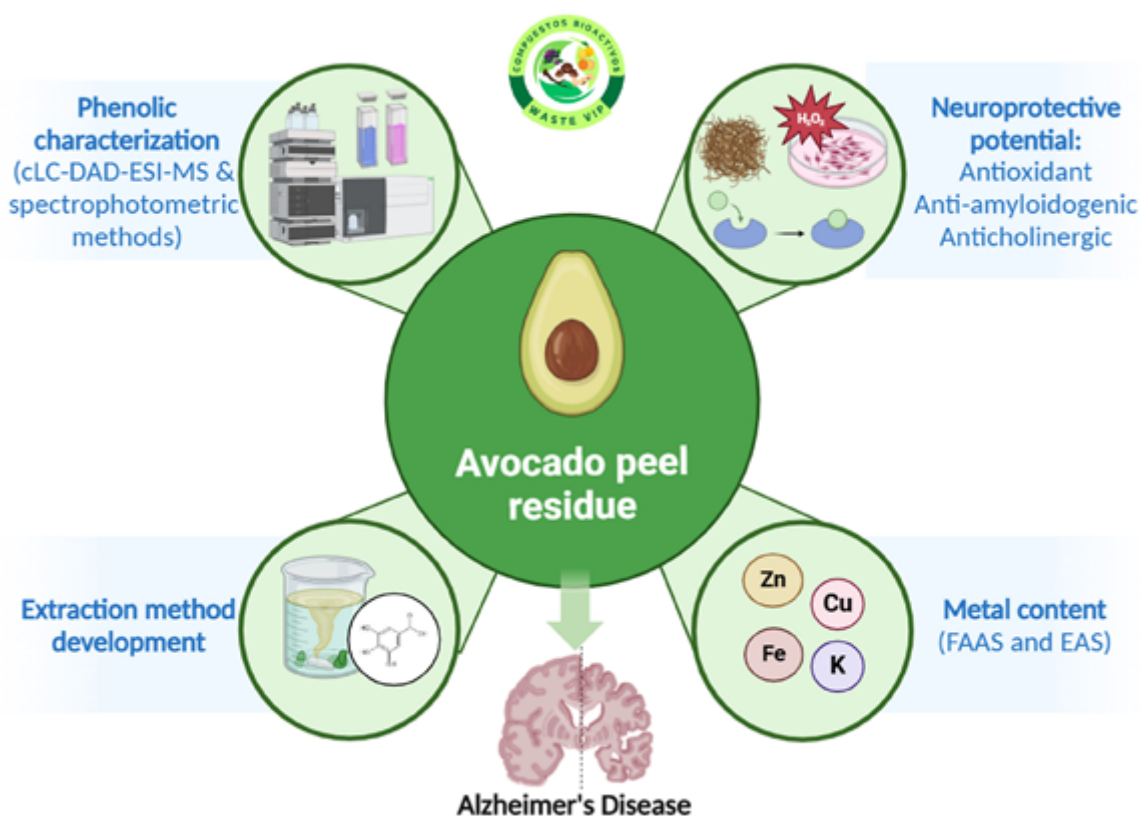
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Food waste biorefining is gaining attention for its potential to convert agri-food waste into valuable chemicals, energy and goods. One promising biorefining strategy is the valorization of biowaste, which enables the recovery of high value-added bioactive compounds from biowaste to meet the demand for natural and environmentally friendly products and to improve the safety and functionality of nutraceuticals (Esparza et al., 2020). Along these lines, one of the foremost and cutting-edge applications of bioactive compounds is their use for targeting neurodegenerative disorders, such as Alzheimer's disease (AD), the most common dementia, affecting 80% of cases worldwide. According to the "Amyloid Cascade Hypothesis", AD is largely attributed to the accumulation and aggregation of beta-amyloid ($A\beta_{42}$) protein, forming plaques that cause neuronal dysfunction. Metal ions and the reduced amount of acetylcholine (ACh) also exacerbate the disease by inducing oxidative stress and disrupting synapses (Chen et al., 2023; da Silva et al., 2022).

Within the extensive range of agri-food residues to be valorized, avocado consumption has surged in recent years, leading to significant waste (30% of the fruit), particularly seeds and peels rich in bioactive compounds like polyphenols (Figueroa et al., 2021). Despite extensive research, large-scale extraction methods are limited by complexity and toxic solvents. Thus, developing simple, rapid, green solvent-based extraction strategies for industrial use is crucial within a circular economy and biorefinery framework.

In light of the aforementioned, this study proposes the valorization of avocado by-products to obtain neuroprotective functional extracts rich in phenolic compounds. A green extraction method was developed and optimized using water as the solvent, evaluating various techniques (solid-liquid extraction (SLE), ultrasound, and microwave-assisted extraction). The phenolic composition was determined using spectrophotometric methods and liquid chromatography coupled to mass spectrometry (cLC-DAD-MS). Additionally, the method's sustainability was



assessed with the AGREEPrep tool. Subsequently, the neuroprotective potential of the optimal phenolic extracts from avocado peel was evaluated using various approaches. First, their antioxidant capacity was studied through the DPPH assay, lipid peroxidation inhibition assay (TBARS), and oxidative stress cytoprotection assay, employing the human neuroblastoma cell line (SH-SY5Y). Complementarily, the anti-amyloidogenic activity was evaluated against two metal ions, Fe(II) and Hg(II). Furthermore, the presence of Cu, Fe, Zn, Ca, and K was confirmed by atomic absorption and emission spectroscopy, respectively. Finally, the inhibition of acetylcholinesterase (AChE), due to its role as an ACh hydrolyzer, was assessed using Ellman's method.

The results demonstrated that avocado extracts obtained under optimal conditions (SLE, 15 min, 60 °C) were rich in gallic acid, ferulic acid, and kaempferol. Moreover, the promising *in vitro* neuroprotective activities suggest that avocado by-product extracts could be used as functional neuroprotective ingredients against AD.

ACKNOWLEDGEMENTS

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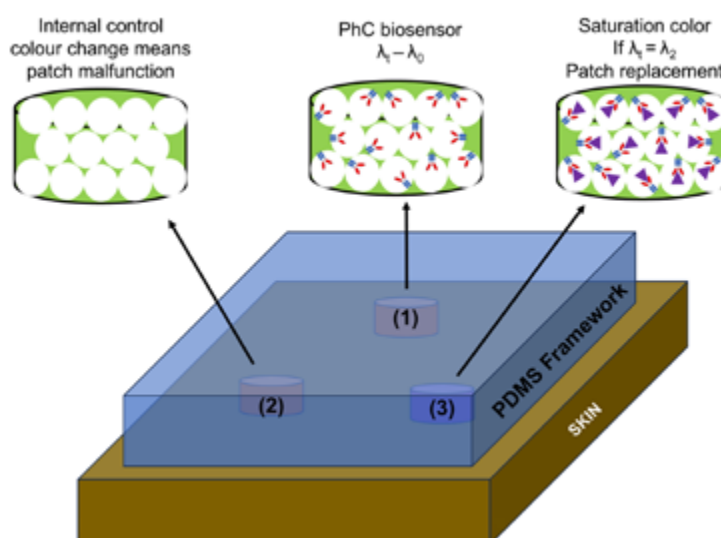
GELATIN PHOTONIC CRYSTALS AS WEARABLE BIOSENSORS FOR BIOMARKERS MONITORING IN SWEAT

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Chronic diseases have wide implications for society; they produce a significant economic burden on health systems because of the costs associated with ongoing health service use and productivity losses from morbidity and mortality. For this reason, patient self-monitoring is often thought to be cost saving when compared with standard or usual care. So, constant development of new wearable sensors and biosensors is promoted to monitor different biomarkers. Among them, sweat biomarkers are interesting owing to the minimal invasive sample collection. Moreover, new sensing strategies and materials have arisen from this research such as photonic crystals (PhCs). PhCs are materials with a periodic structure at nanoscale or microscale that confers unique optical properties. PhCs have periodic refraction index changes associated with their periodic structure, which affect light propagation across them at a range of frequencies called photonic bandgap (PBG). Photons with a frequency in the PBG cannot travel in all directions across the PhCs, which are translated into unique colorful owing only to the crystal nano/microstructure.^{1,2} Periodic nano/microstructures can be fabricated on materials in one-dimension (1D), two-dimensions (2D) or three-dimensions (3D) by cavities, holes, or porous. Moreover, PhCs can be achieved in almost all kinds of materials such as glass, polymers, conductive, semiconductor and dielectric films, and even flexible materials such as fibers, or hydrogels. PBG and their related color is highly sensible to small changes in the PhCs nano/microstructure, which can change easily by any physical or chemical stimuli from their environment. This characteristic made PhCs a highly attractive material for wearable sensors.^{3,4}

The work focuses on the manufacture of biosensor patches based on gelatin PhCs for monitoring of model target proteins in simulated sweat, as a first step to develop such patches towards sweat biomarkers of chronic diseases. PhCs have been developed using polystyrene (PS) monodisperse nanoparticles (NPs) of different sizes as templates of the PhCs nanostructures. After PS NPs arranged on a glass surface, gelatin was added to the template and crosslinked with glutaraldehyde to infiltrate the hydrogel in the PS NPs network. Finally, PS NPs have been dissolved by organic solvents to obtain the inverse-opal hydrogel PhCs based on gelatin. Such PhCs porous nanostructure and associated colour have been characterized and functionalized with anti-transferrin antibodies to demonstrate their capabilities as biosensors for transferrin determination in sweat samples as a dummy biomarker as such analyte is not expressed in sweat. Gelatin PhCs showed a suitable analytical performance for their used as wearable biosensors for biomarkers monitoring in sweat, promising for their used towards sweat biomarkers of chronic diseases.



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P1-055**IMPROVING THE PERFORMANCE OF ELECTROCHEMICAL IMMUNOSENSING BY EXPLOITING A NOVEL ELECTRODE MODIFIER THAT IMPARTS ANTIFOULING PROPERTIES AND ENABLES EFFICIENT IMMOBILIZATION OF BIOMOLECULES**

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Effective techniques for signal amplification and antibody immobilization are crucial for the development of immunosensors. To enhance the oriented covalent binding of antibodies and maximize the efficiency of immune recognition, this study introduces a novel nanostructured material to modify electrodes. Gold nanoparticles (AuNPs), which allow the controlled assembly of hydrated poly(3,4-ethylenedioxythiophene) (hPEDOT), are the backbone of the proposed methodology. As a result of the strong contact between the AuNPs surface and the thiophene groups, an ordered polymeric matrix doped with citrate is formed, providing a stable platform for antibody attachment. This modification significantly enhances the electrochemical properties of the modified electrode surface. In immunosensing applications, improved conductivity and electron transfer efficiency contribute to increased sensitivity. Additionally, the hydrated polymeric network plays a crucial role in minimising biofouling and non-specific adsorption - two major issues in electrochemical biosensing - ensuring accurate and reproducible measurements even in complex biological samples.

To evaluate the unique properties imparted by the nanostructured material, it was used in an electrochemical immunosensor with screen-printed electrodes and a sandwich format. Human endostatin, a protein associated with tumour angiogenesis in colorectal cancer (CRC), was selected as the target biomarker. Detection was performed by amperometry, using the hydroquinone/hydrogen peroxide redox system and a biotinylated detection antibody conjugated to streptavidin-horseradish peroxidase. The developed immunotool offered an attractive analytical performance with a detection limit of 0.04 ng mL⁻¹ and a linear range of 0.14–1.00 ng mL⁻¹.

Liquid biopsy samples from three groups (healthy individuals, patients with premalignant CRC lesions and patients with CRC) were used to further evaluate the immunoplatfrom beyond analytical validation. The results confirmed the promising potential of plasma endostatin levels determined with immunotechnology, after just a simple dilution step and without matrix effect, to accurately discriminate between these groups. All these results highlight the potential of the developed tool to contribute to the management of patients with CRC.

Acknowledgments

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P1-056

INNOVATING THERAPEUTIC DRUG MONITORING: APTAMER-BASED BIOSENSOR FOR RAPID AND EFFICIENT IRINOTECAN ANALYSIS

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Irinotecan (CPT-11) is an anticancer drug that had a significant impact on the treatment of patients with advanced colon rectal cancer¹, lung cancer^{2,3}, pancreatic, gastric⁴, ovarian⁵, and others.

Through its active metabolite, irinotecan primarily targets rapidly dividing cancer cells, but can also affect non-cancerous cells. As a result, irinotecan treatment is frequently associated with adverse effects, such as diarrhea, neutropenia, and fatigue.^{6,7}

For this reason, therapeutic drug monitoring (TDM) plays a critical role: it helps ensure that drug concentrations remain within the therapeutic window tailored to each individual patient. This approach minimizes the risk of toxic side effects while preserving the drug's efficacy.

Until now, TDM in cancer research is performed in core facilities by means of gold standard techniques, such as high performance liquid chromatography-mass spectrometry (HPLC-MS), which requires sample preparation and qualified personnel.⁸ As an alternative to HPLC, biosensors incorporating synthetic bioreceptors might offer a powerful tool for rapid, sensitive and specific drug detection.

This innovation supports real-time monitoring and precision medicine, ultimately enhancing patient outcomes.

In this presentation, we describe the use of aptamers that can selectively bind CPT-11. Aptamers are single-stranded oligonucleotides that can be designed to target a wide range of molecules, including small molecules. Their defined structure facilitates their binding to targets with high specificity and affinity. Moreover, aptamers exhibit remarkable stability and offer cost-effective synthesis, making them superior to proteins and antibodies, which are less stable and more expensive to produce.⁹

The biosensing platform features a gold surface functionalized with a thiolated oligocapture, which partially pairs to a specific aptamer. If the drug is present in the sample, it binds to the aptamer, causing it to detach from the oligocapture.

Functionalization steps are monitored by surface plasmon resonance (SPR). In addition, the decrease in SPR signal, caused by aptamer detachment from oligocapture, is correlated to CPT-11 concentration in the sample, thus providing a calibration curve.

This aptamer-based biosensor represents a promising proof of concept for real-time monitoring of irinotecan, which could support personalized treatment strategies and improve patient outcomes, once validated for clinical use.

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P1-057**STUDY OF THE OPERATING CONDITIONS FOR TAYLOR-ARIS DISPERSION ASSISTED MASS SPECTROMETRY****Szabo, Ruben¹; Felinger, Attila²; Horváth, Krisztián³; Gáspár, Attila¹.**

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Electrospray ionization mass spectrometry (ESI-MS) is a widely used technique for protein analysis. Although direct infusion is the fastest and most straightforward sample introduction method, numerous matrix components can cause significant interferences resulting in low-quality spectra or no information at all. The effectiveness of Taylor-Aris dispersion has recently been shown in the direct injection analysis of large molecules present in matrices incompatible with ESI-MS. This includes monoclonal antibodies in pharmaceutical formulations and native protein complexes in phosphate-buffered saline solution [1, 2]. This method offers a fast, straightforward, and cost-efficient technique for protein analysis.

Establishing the optimal conditions for these analyses can be challenging as numerous parameters affect the outcome of these measurements such as flow conditions, sample volume, analyte and matrix concentrations and characteristics. In Taylor Dispersion Analysis the diffusion coefficient of the analyte is determined by the observed peak variance based on a formula describing the theoretical concentration distribution. We found that by adjusting this formula to account for the non-negligible sample volume, it can be used to describe the expected taylograms under different flow conditions [3]. The signal-suppressing effects of the matrix components during ionization were described according to the work done by the Consortium for Top-Down Proteomics [4]. The proposed model enabled the in-silico investigation into the effects of operating conditions for Taylor-Aris Dispersion Assisted Mass Spectrometry.

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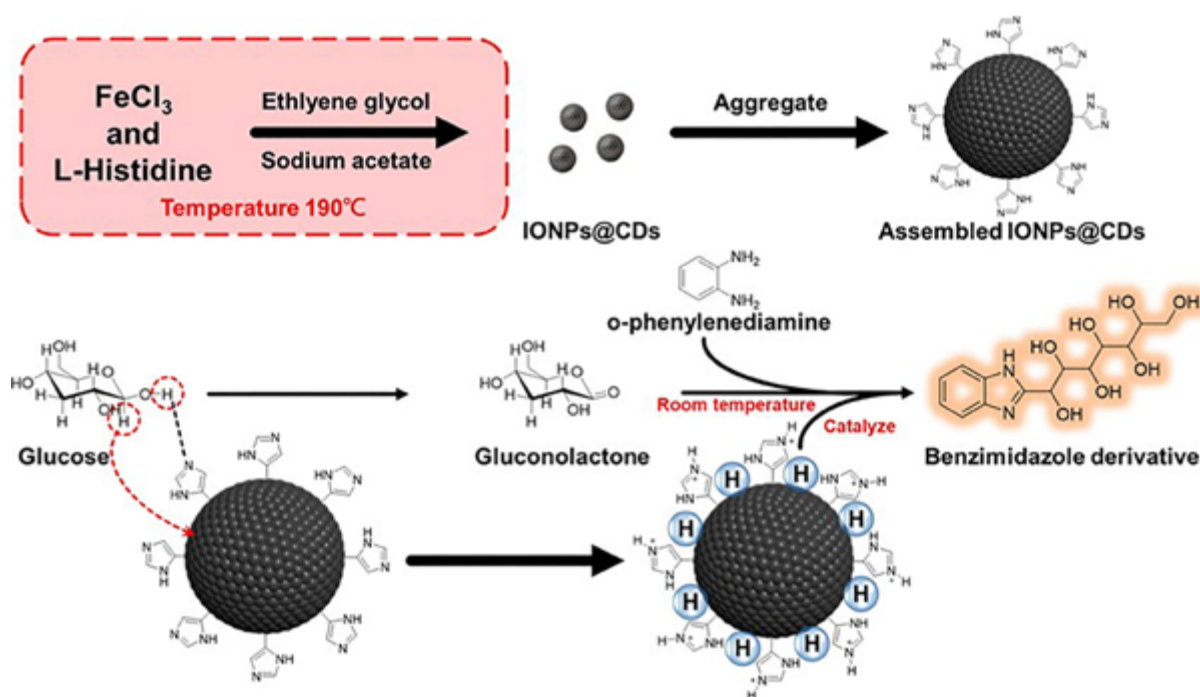
P1-058

A DUAL COLORIMETRIC AND FLUORESCENT SENSOR FOR NON-ENZYMATIC GLUCOSE DETECTION VIA CATALYTIC REACTION WITH O-PHENYLENEDIAMINE MEDIATED BY C-DOT-DECORATED Fe_3O_4 NANOPARTICLES

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We prepared assembled histidine-derived carbon dot-coated Fe_3O_4 nanoparticles (IONPs@CDs) by one-step solvothermal synthesis and apply them as a non-enzymatic catalyst for dual-mode glucose measurement in colorimetric and fluorescence modes. Histidine forms carbon dots at high solvothermal temperatures, initially attaching to small-sized iron oxide nanoparticles. Due to carbon-carbon interactions, the iron oxide nanoparticles with attached carbon dots aggregate into large spheres, thus forming the porous structure of IONPs@CDs. Since the imidazole moiety on the surface of the histidine-derived carbon dots mimics the activity of glucose oxidase, the assembled IONPs@CDs effectively facilitate the deprotonation of glucose and the transfer of hydride from glucose to the assembled IONPs@CDs. Hydride-loaded IONPs@CDs catalyze the reaction between OPD and the aldehyde form of glucose, producing benzimidazole derivatives with absorptive and fluorescent properties. ESI-MS confirms benzimidazole derivatives formation. The detection limits of the proposed sensing system for glucose in absorbance and fluorescence modes are 35 and 24 μM , respectively. Accuracy and reliability align well with commercial blood glucose meters used in the same sample. In addition, the system shows excellent stability and reusability. As a result of these advantages, the proposed sensing device is a practical, economical, and enzyme-free alternative for glucose monitoring.



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P1-059

DERIVATION OF HUMAN TOXICOKINETIC PARAMETERS AND CHEMICAL-SPECIFIC ADJUSTMENT FACTOR OF CITRININ THROUGH A HUMAN INTERVENTION TRIAL AND HIERARCHICAL BAYESIAN POPULATION MODELING

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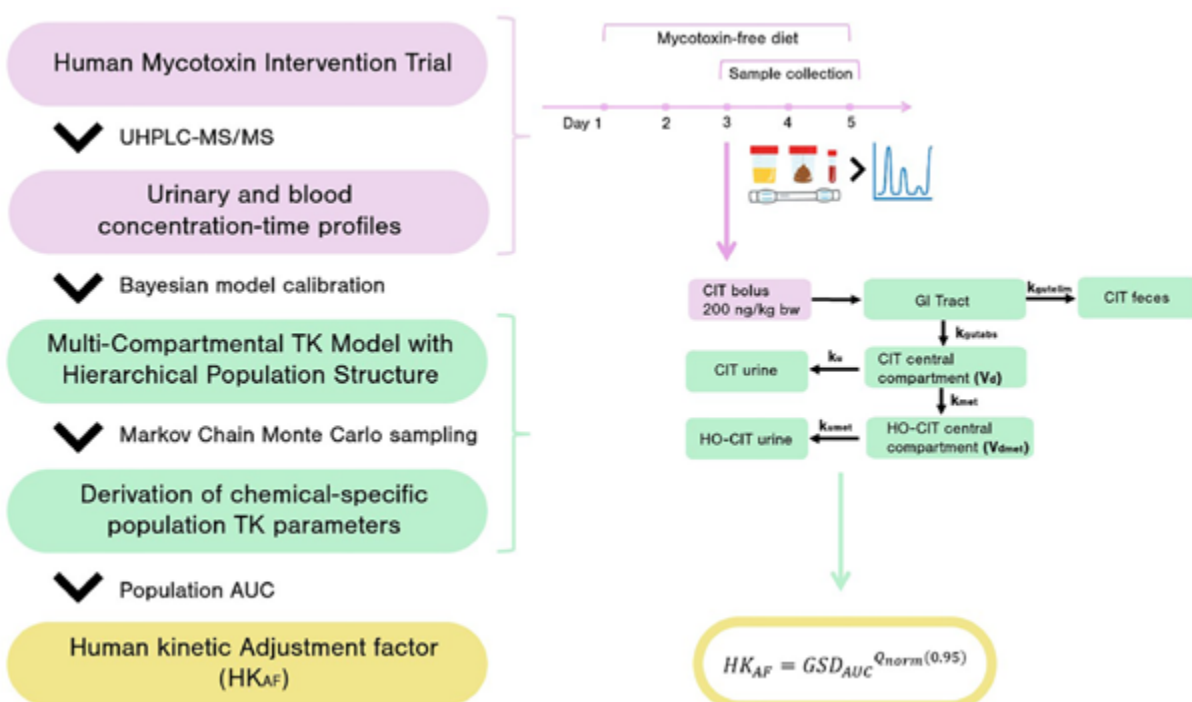
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Citrinin (CIT) is produced by various fungi that contaminate stored cereals and fruits. In humans, CIT is extensively metabolized into dihydrocitrinone (HO-CIT), for which no commercial analytical standards are available. Current data indicate that CIT exposure is widespread in the population^{1,2}, but its potential public health risks remain unclear due to the lack of occurrence and human exposure data. Specific toxicokinetic (TK) studies on CIT are incomplete, preventing a conclusive exposure assessment based on human biomonitoring (HBM) data^{3,4}.

To address this gap, extraction methods were developed for CIT in capillary blood collected via VAMS Mitra[®] tips, feces, and urine for subsequent UHPLC-MS/MS analysis. Chromatographic separation was achieved using an Acquity i-Class UHPLC system equipped with an Acquity HSS T3 (100 × 2.1 mm, 1.8 μm) UHPLC column and an Acquity Vanguard HSS T3 (10 × 2.1 mm, 1.8 μm) UHPLC pre-column (Waters[®], Manchester, UK). Analytes were detected using a Waters Xevo[®] TQ-XS tandem quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface operating in negative mode and using Multiple Reaction Monitoring (MRM) acquisition. The method was validated following ICH M10 guidelines for bioanalytical method validation and implementing the pipeline published by Desharnais et al. (2017)⁵. The lower limit of quantification (LLOQ) values obtained were < 0.05 ng/mL or ng/g of dried feces.

A human TK study was designed in accordance with the Declaration of Helsinki and ethical approval to investigate the toxicokinetics of CIT following a single bolus administration at a level of no concern for nephrotoxicity (200 ng/kg bw)³. Participants adhered to a controlled diet to minimize dietary CIT intake and collected autonomously individual urine, blood, and fecal samples for 48 hours post-administration. The urinary void volume was also collected at each sample collection point, allowing the back-calculation of the total mass of CIT excreted in urine. The validated methods were applied to 120 blood, 97 urine, and 18 fecal samples collected by 10 volunteers to characterize CIT's TK profile in humans.

Toxicokinetic modeling was performed using a multi-compartmental approach with a Hierarchical Bayesian population structure to determine CIT's population TK parameters^{6,7}. The model fitting was carried out using GNU MCSim v6.1.0 software⁸, an open-source tool, to perform MCMC simulation and to define posterior parameter values and uncertainty. All R codes and generated files are freely available in the GitHub repository https://github.com/liavisintin/TK_CIT. The model was structured based on the available experimental data and included gastrointestinal



(GI), fecal, urinary, and central compartments for CIT and HO-CIT. From the GI tract, CIT is either absorbed into systemic circulation (central compartment) at a rate of k_{gutabs} or eliminated via the GI tract at a rate of $k_{gutelim}$. Once in the central compartment, CIT is either excreted in urine (rate k_u) or metabolized (rate k_{met}). The metabolites are subsequently eliminated in urine (rate k_{umet}). The volume of distribution (V_d) and metabolite volume of distribution (V_{dmet}) define the relationship between the total amount in the body and blood concentration for CIT and HO-CIT, respectively.

The Bayesian approach accounts for the unavailability of input data for HO-CIT and total mass of CIT excreted in feces by incorporating prior distributions from the literature. The TK parameters derived for CIT align with preliminary human data and expand knowledge of human CIT toxicokinetics³. Based on the derived human area under the blood concentration-time curve (AUC), the calculated Human Kinetic Adjustment Factor (HK_{AF}) was 1.92, confirming that the level of no concern for nephrotoxicity in humans established by the European Food Safety Authority is a conservative threshold⁹.

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P1-060**APPLICATION OF GC-MS/MS METHOD FOR DETECTION OF METHANDIENONE AND ITS METABOLITES IN HUMAN URINE TO ANTI-DOPING CONTROL: ANALYSIS OF TRENDS OVER TIME AND POTENTIAL ORIGINES OF METHANDIENONE**

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Anabolic androgenic steroids (AASs) are the most widely abused substances in cases of unsportsmanlike conduct in sports. Over the past decade, their prevalence, among substances identified as the so-called adverse analytical findings (AAFs; also known as testing positives), has consistently remained at or above 40% each year [1].

AASs are synthetic analogues of the male sex hormone testosterone and have major anabolic physiological actions. This makes them attractive to athletes seeking to enhance performance and physical appearance by increasing muscle mass and strength. Their prevalence of being used in sports seems not to have declined despite health warnings related to side effects, which can lead to many comorbidities and potentially premature mortality [2,3].

Intense physical exertion in athletes' daily lives necessitates a relevant food regime and take specific dietary supplements, which are crucial for enhancing the body's recovery between training sessions and competitions. Unfortunately, the supplement use is not always entirely safe [4]. In addition to the black-market sale of the so-called "supplements" that openly list banned substances on their labels, many commercial supplements have been found to contain undeclared prohibited compounds [5,6]. Furthermore, the use of obscure chemical names on labels, which is a common practice, can conceal prohibited substances from consumers [5,6]. All these factors contribute to the fact that the abuse of prohibited substances, such as AASs, may be multidimensional. It is not only related to intentional doping but also to the widespread use of supplements among athletes with insufficient awareness of the risk of unintentional doping.

Methandienone is one of the most frequently detected AASs in the doping control by the laboratories accredited by the World Anti-Doping Agency (WADA) [1]. The metabolism of methandienone in humans has been extensively studied in the context of the anti-doping control, leading to the identification of numerous metabolites excreted in urine in free form or conjugated with glucuronic acid or sulfate [7-10]. Among these, the so-called long-term metabolites, e.g. 18-nor-17 β -hydroxymethyl-17 α -methyl-androst-1,4,13-trien-3-one (LTM1) and its C17 epimer – 18-nor-17 α -hydroxymethyl-17 β -methyl-androst-1,4,13-trien-3-one (LTM2), are particularly important as they enable the anti-doping laboratories to detect the use of methandienone for significantly longer period of time, with LTM1 detectable for up to several dozen days after the drug or a contaminated supplement has been administered, thereby enhancing the effectiveness of anti-doping controls. In addition to the metabolites excreted after oral administration of methandienone, there is evidence that the presence of methandienone and its characteristic metabolites, including 6 β ,17 β -dihydroxy-17 α -methylandrosta-1,4-dien-3-one, in urine, can be detected following topical administration of methandienone [11]. The research on analytical methods for the detection of methandienone and its metabolites in human urine has included GC-MS, LC-MS, GC-MS/MS, LC-MS/MS and GC-QTOF-MS [3,8-10,12].

In the present study, methandienone and its metabolites, mainly targeted in anti-doping control, such as LTM1, LTM2, 6 β ,17 β -Dihydroxy-17 α -methylandrosta-1,4-dien-3-one, 17 α -Hydroxy-17 β -methylandrosta-1,4-dien-3-one, 17 β -methyl-5 β -androst-1-ene-3 α ,17 α -diol, 17 α -methyl-5 β -androstane-3 α ,17 β -diol and 18-nor-17,17-dimethyl-5 β -androst-1,4,13-trien-3-one, have been investigated in terms of the anti-doping testings in Poland between 2014 and 2024. The qualitative method of methandienone and its metabolites (as a sum of free and glucuronic conjugated forms) in human urine has been applied using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). The analytical method enabled the Polish Anti-Doping Laboratory to identify 52 AAFs related to methandienone intake during the study period. The collected data has been analyzed over time considering factors, such as sport discipline, time of urine sample collection (in or out of competition) and athlete gender. Moreover, the identified metabolites and the presence or absence of other prohibited substances in urine samples have been examined to assess the possible time since drug administration and identify potential origins of methandienone intake.

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P1-061

QUANTIFICATION OF LABELED AND UNLABELED KETONE BODIES IN SERUM BY DEUTERATED β -HYDROXYBUTIRATE AND AcAc STABLE ISOTOPES AS INTERNAL STANDARDS

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Ketone bodies are lipid-derived metabolites involved in physiological processes such as fasting, exercise or acquisition of ketogenic diet. The primary ketone bodies in circulation are acetoacetate (AcAc) and β -hydroxybutyrate (β OHB)^[1], which serve as alternative energy sources during physiological states of glucose scarcity. Their production (ketogenesis) and utilization (ketolysis) are highly dynamic and studied using ¹³C-labeled tracers^[2]. Despite quick and reliable UPLC-MS/MS quantification method has been recently developed^[3], it relies on ¹³C-labeled internal standards, limiting its application in ¹³C-tracer studies. Additionally, AcAc synthesis from ethyl-AcAc yields multiple products, requiring rigorous controls to prevent confounding metabolic effects in *in vivo* and *in vitro* studies.

To optimize AcAc synthesis, temperature (60°C, 70°C, 80°C) and hydrolysis time (30 min to 3 h) were evaluated using ¹H NMR. The highest AcAc yield (99.2 $\pm$ 0.2% purity) was achieved at 60°C for 3 h. Optimized synthesis was further applied to produce deuterated AcAc (d_3 -AcAc) from d_3 -ethyl-acetoacetate in deuterated water, accounting for observed temperature-dependent tautomerization process of AcAc and thus dynamic deuterium loss. Quantitation of d_3 -AcAc was achieved through [¹³C₄]AcAc comparative measurement and the possible deuterium exchange was determined in extraction buffer and extracted mice serum at RT, 4°C, -20°C, over 72 h. The stability of d_3 -AcAc internal standard was consistent for up to 5 h across all conditions. The newly developed method demonstrated excellent linearity, sensitivity, and precision for quantifying both unlabeled (AcAc, β OHB) and labeled ([U-¹³C₄]AcAc, [U-¹³C₄] β OHB) ketone bodies. Compared to laborious conventional methods with limited sample capacity, this approach provides robust and reliable measurements with simplicity of sample preparation and analysis.

Our study establishes deuterated AcAc (d_3 -AcAc) as a novel internal standard, along with [3,4,4,4- d_4] β OHB enabling simultaneous quantification of labeled and unlabeled ketone bodies via UPLC-MS/MS. This advancement enhances the accuracy of metabolic tracing studies, improving our understanding of ketone body metabolism in physiological contexts.

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P1-062**EVALUATION OF THE DIGESTIBILITY AND BIOACCESSIBILITY OF FREE VS. ENCAPSULATED NEUROPROTECTIVE EXTRACTS USING THE INFOGEST PROTOCOL**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder without efficient treatment, however, different external factors such as the diet have crucial impact in this disease, highlighting the need for the study of nutraceuticals as neuroprotective agents. Natural neuroprotective extracts have shown promise results due to their antioxidant properties and cholinergic enzyme inhibition, but their low stability and poor bioavailability limit therapeutic potential. Encapsulation can enhance their effectiveness by improving stability, and gastrointestinal absorption and controlled release. This study evaluates three encapsulation techniques: hydrogels, liposomes, and Pickering emulsions to improve extract stability and bioaccessibility.

A blended extract was obtained through a sequential green extraction process combining Supercritical Fluid Extraction (SFE), Pressurized NaDES Extraction (P-NaDES-E), and antisolvent purification. The initial SFE step was carried out at 20 MPa and 60 °C for 2 hours with a CO₂ flow rate of 60 g/min, followed by P-NaDES-E using a ChCl:Gly (1:2) NaDES with 57% water at 64 °C for 27 minutes under 10 MPa. The resulting extract underwent antisolvent purification involving three extractions with 50% ethanol, two with 100% ethanol, and two with acetone. The final blended extract was composed in a 33:41:26 ratio (SFE:P-NaDES-E:antisolvent).

This enriched extract was then encapsulated using three different techniques aimed at enhancing its stability and bioavailability. (1) Optimized liposomal microencapsulation was performed at an extract concentration of 250 mg/mL, using a cryogenic and stirred method. (2) Pickering nanoencapsulation was developed by emulsifying the extract (250 mg/mL in sunflower oil) with water in a 1:1 ratio, stabilized by cellulose nanocrystals. (3) Alginate hydrogel encapsulation involved incorporating the extract (250 mg/mL in sunflower oil) into an alginate matrix. These encapsulation systems are intended to support further evaluation of the extract's functional properties, particularly in relation to neuroprotection and controlled release..

The simulated digestion of each sample was performed using the InfoGEST protocol, with bioaccessibility assessed through micellar fraction extraction. At each digestion phase, total terpenes, phenolics, and carotenoids were quantified, along with antioxidant activity (DPPH assay) and cholinergic enzyme inhibition (AChE assay).

This study supports the development of encapsulated nutraceuticals as viable candidates for Alzheimer's prevention and cognitive health. Enhancing the bioaccessibility of neuroprotective extracts through encapsulation offers a science-backed approach to improving neurodegenerative disease management.

P1-063**DEVELOPMENT AND OPTIMIZATION OF GREEN COLORIMETRIC METHOD FOR CHLORATE DETERMINATION IN AQUEOUS MEDIUM USING INDIGO CARMINE**

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The disinfection stage is important in diverse fields, from the food industry to the treatment of swimming pool water, as it aims to eliminate pathogenic microorganisms and improve product quality. However, the use of chlorinated disinfectants in processing water can lead to contamination of water with disinfection by-products (DBPs), such as chlorate, which can be absorbed by food and cause hormonal problems. . Halogenated disinfectants, particularly chlorine derivatives such as hypochlorous acid or chlorine dioxide, are commonly used due to their low cost and high efficiency in reducing the presence of microorganisms. Both give chlorate and many other disinfection byproducts, such as trihalomethanes.^{1, 2}

Analysis by ion chromatography and spectrophotometry, using reagents such as ortho-tolidine or indigo carmine, are the most common methods for chlorate analysis. Indigo carmine is not carcinogenic, so it seems more appropriate. The study successfully demonstrated the viability of an indigo carmine detection method based on its oxidative decolorization. Initially, the reaction was characterized using UV-Vis spectrophotometry to establish optimal analytical conditions, including reagent concentrations, pH, and reaction time. Indigo carmine reacts with both chlorate and chlorite, but it appears that modifying the medium conditions (pH or Fe(II) content) may change the selectivity. Thus, the idea is to use the procedure we know for the measurement of chlorate in a microplate with the aim of reducing reagent consumption while maintaining high sensitivity and reproducibility.

To further improve portability, the method was transferred to a paper-based analytical device. Homemade paper device allows the reaction to take place directly on the surface and it is expected to use reagent quantities equal to or less than 10 µL. The colorimetric response was performed using digital imaging analysis with a smartphone. Calibration curves obtained approaches demonstrated a linear response within a relevant concentration range, achieving detection limits suitable. Validation with real water samples confirmed the method's accuracy, showing strong correlation with reference technique.

In addition, we started working with reflectance measurements on paper-based supports to use them as a complementary technique to digital image analysis and UV-Vis spectrophotometric method in liquid samples. This approach allows for a possible multivariable analysis.

Future research will also investigate the potential of microfluidic methods to integrate these detection systems into ready-to-use commercial kits. Lastly, automation of image capture and result processing will be prioritized to enhance usability and reliability.

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P1-064**MICROWAVE-ASSISTED EXTRACTION OF PROTEINS FROM *ACHETA DOMESTICUS***

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The search for sustainable and affordable protein sources has become a global priority due to the growing population and the consequent increase in protein demand. Insects present high protein content with significant nutritional value and sustainable farming. House cricket (*Acheta domesticus*) has been recently authorized by the European Union to be used as an ingredient in foods formulations. Insect protein isolates from *Acheta domesticus* have been obtained by conventional alkaline extraction, with known limitations such as low sustainability, protein degradation, reduced solubility due to protein aggregation, low selectivity and yield, potential Maillard products formation under alkaline conditions, and co-extraction of chitin. Thus, the aim of this work was to develop a more sustainable methodology for extracting the protein fraction from house crickets.

In this work, microwave-assisted extraction (MAE) was employed with water as the extracting solvent. The temperature (40 – 170 °C), ethanol (EtOH) content (0 – 100 %), and extraction time (1 – 15 min) were optimized using a Box-Behnken experimental design. Additionally, the effect of the pH, the defatting of the sample, and the combination of MAE with enzyme-assisted extraction (EAE) was also studied. The responses analyzed were the protein and chitin content, and the presence of Maillard compounds. Results were compared with those obtained with a conventional alkaline extraction. Proteins extracted under optimal conditions were separated and characterized using gel electrophoresis (SDS-PAGE) and reverse-phase high-performance liquid chromatography (RP-HPLC). Extracted proteins were also hydrolyzed to study the potential bioactivity of released peptides.

Optimal conditions for maximizing the extraction of proteins while minimizing the co-extraction of chitin were: 3 % EtOH, 167 °C, and 15 min of extraction. Under these conditions, it was possible to extract 36.1 ± 0.8 g proteins / 100 g dried sample and 0.60 ± 0.09 g chitin / 100 g dried sample. Compared to a conventional alkaline extraction, MAE increased protein yield to 50 % without raising the chitin content. Maillard compounds were found in a lower concentration in MAE extracts, compared to the alkaline samples. The characterization of proteins revealed significant differences between extracts obtained by the two procedures employed and between insects at different maturity state.

P1-065**ELEMENTAL CONTAMINATION DURING THE MANUFACTURE OF SEMI-SOLID DERMOCOSMETIC FORMULATIONS****Bilandžija, Barbara¹; Margui, Eva²; Jug, Mario³; Jablan, Jasna⁴.**¹) Department of Analytical Chemistry, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia;²) Department of Chemistry, University of Girona, Girona, Spain; ³) Department of Pharmaceutical Technology, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia; ⁴) Department of Analytical Chemistry, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia.

High-quality and effective dermocosmetic products can significantly improve the general well-being and quality of life of individuals and thus indirectly contribute to health — in line with the World Health Organization's (WHO) definition of health as comprehensive physical, mental, and social well-being. Skin care products represent the largest segment of the global cosmetics market, with cosmeceuticals — topical formulations that combine cosmetic and pharmaceutical properties — being the fastest-growing category.

Dermocosmetic products are widely used in the daily lives of consumers of all age groups worldwide. Topical cosmetics in the form of creams, gels, lotions, and sprays are among the most commonly used consumer products. Daily consumption of cosmetics leads to consistent exposure to ingredients contained in cosmetics, some of which may have adverse health effects due to the repeated use and long-term retention of the cosmetic products at the site of application.

Potentially toxic elements (PTEs) are a group of hazardous substances in cosmetics that can accumulate in the skin and internal organs, where they can develop their toxic effects. PTEs in cosmetic products can originate from various sources, including raw materials (e.g., contaminated plants grown in industrial and polluted areas or contaminated mineral oils and derivatives like paraffin, silicones, and aliphatic hydrocarbons), production steps, packaging materials, and the intentional addition of metals in the form of pigments, UV filters, preservatives, and other cosmetic ingredients. Numerous negative effects of cosmetics on human health have been reported in the literature, e.g., cases of allergic reactions and negative systemic effects caused by the presence of PTEs in cosmetic products. Therefore, strict quality assurance throughout the production process — from raw materials to the final product — is crucial [1].

To meet the principles of environmentally friendly and sustainable analytical chemistry, recent efforts have focused on the incorporation of new, cost-effective screening and quantitative instruments such as total reflection X-ray spectrometry (TXRF) [2]. This study focuses on the development and optimization of analytical methods for multi-elemental analysis of topical cosmetic products using TXRF and inductively coupled plasma mass spectrometry (ICP-MS). Sample preparation strategies, including microwave-assisted digestions and green minimally treated approaches, were tailored to different formulations such as gels, emulsions, emulgels, and creams.

The results show considerable variability in the elemental composition of the products tested and highlight the need for routine elemental screening to ensure regulatory compliance, product consistency, and transparency for the consumer. Particular attention is paid to elements regulated by frameworks such as the EU Cosmetics Regulation 1223/2009.

Overall, this study highlights the critical role of elemental analysis in the development and quality control of dermocosmetic products and demonstrates the potential of TXRF as a rapid, cost-effective, and environmentally friendly tool for analyzing complex cosmetic matrices.

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P1-066**DISPERSIVE MINIATURIZED SOLID-PHASE EXTRACTION USING METAL-ORGANIC FRAMEWORKS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION TO DETERMINE PHYTOCHEMICAL COMPOUNDS IN ALOE VERA SAMPLES**

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Aloe barbadensis-Miller, commonly known as aloe vera, is well known for its bioactive gel, which contains a variety of phytochemicals such as anthraquinones, and chromones, among many others. These compounds have antioxidant, anti-inflammatory, and therapeutic properties. However, some anthraquinones, mainly those found in the outer leaf latex, are regulated given their potential genotoxic and laxative effects. The Commission Regulation (EU) 2021/468 of 18 March 2021 amending Annex III to Regulation (EC) No 1925/2006 of the European Parliament, for instance, restricts compounds like aloin A, aloin B, and aloe-emodin, among others. Consequently, reliable analytical methods are needed to monitor the presence of these compounds in aloe vera products.

Traditional extraction techniques for these phytochemicals are efficient but also characterized by the lack of sustainability, as they are time-consuming and require large amounts of organic solvents. To address these issues, sorbent-based microextraction techniques, such as miniaturized dispersive solid-phase extraction (μ -dSPE), have emerged as promising alternatives from a green perspective. The success of μ -dSPE relies on its simplicity, reduced solvent consumption, low generation of wastes, and improved efficiency given the adequate contact between the sample and the microextraction sorbent. Among the different sorbents available, metal-organic frameworks (MOFs) have gained attention because of their high porosity, impressive surface area, chemical tuneability, and adequate stability, even in complex samples.

This study evaluates the use of several MOFs as sorbents for μ -dSPE to extract key phytochemicals from aloe vera gel. Three MOFs were specifically evaluated, namely UiO-66(Zr), DUT-52(Zr), and CIM-100(Al). Their selection follows green features, as they all include low toxic-metals (Zr and Al). The microextraction performance was adequately optimized, ensuring in all cases a simple desorption step for the combination of the extracts with high-performance liquid chromatography with diode array detection (HPLC-DAD).

The MOF-based μ -dSPE-HPLC-DAD approach showed enabled the extraction, preconcentration and quantitation of key anthraquinones and chromones, among others, while reducing matrix interferences. Besides, the inherent green features associated to a microextraction approach are also present. These findings highlight the potential of MOFs as advanced sorbent materials for the determination of phytochemicals, offering a fast, selective, and environmental-friendly alternative for analytical sample preparation in natural product research.

P1-067**ANALYSIS OF TRICLOSAN AND THEIR PHOTOLYTIC TRANSFORMATION PRODUCTS IN WATER USING IN SITU IONIC LIQUID MICROEXTRACTION AND LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY**

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Triclosan (2,4,4'-trichloro-2'-hydroxydiphenylether, TCS) is an antimicrobial agent added to many household and personal care products. As a result of its extensive use, elevated concentrations of TCS are habitually found in wastewater, wastewater treatment plants (WWTP) and their receiving waters. TCS is considered a "contaminant of emerging concern" (CEC) due to the potential risks that its residual presence may constitute for human health and the environment.¹ On the other hand, degradation processes of CECs applied in the remediation of WWTP effluents generate transformation products (TPs) that may exhibit toxicological properties and aggravate environmental impacts.² Consequently, it must be a priority to understand the behaviour, persistence and degradation pathways of this emerging contaminant for developing effective water treatment methods as well as ensuring the protection of aquatic ecosystems and public health.

In the present study, the *in situ* ionic liquid dispersive liquid-liquid microextraction (IL-DLLME) technique was considered for the extraction of TCS and its main TPs formed during TCS's photo-oxidising process using ultraviolet A (UVA) irradiation with light emitting diode (LED). The *in situ* IL-DLLME is a green sample preparation technique characterised by the absence of dispersant solvent, so that the use of toxic organic solvents is reduced. For the *in situ* IL-DLLME, the cationic IL component 1-methyl-3-octylimidazolium and the anionic IL component bis(trifluoromethylsulfonyl)imide were employed for the extraction of 10 mL water sample containing 10% m/v sodium chloride. The *in situ* IL mixture resulted in a turbidity formed by very fine IL droplets dispersed through the aqueous phase. The final enriched IL extract was collected, diluted with acetonitrile and analysed by liquid chromatography combined with high resolution mass spectrometry (LC-HRMS) using a quadrupole-time-of-flight (QTOF) hybrid analyser for the identification of known and unknown TPs. In addition, verification of the identified TPs was carried out by LC with triple quadrupole. The identification of TPs by LC-QTOF was conducted through two untargeted strategies: a suspect screening based on the utilization of a home-made database with 46 compounds created from the TPs of the TCS described in the literature, and a non-target screening for novel TPs based on the spectral investigation of a deconvoluted and aligned list of peaks with suitable degradation trends.

The photodegradation of TCS reached a 59% efficacy during the illumination time range studied (0-240 min). All detected TCS TPs eluted earlier than TCS (retention time: 29.39 min). The experimental LC-QTOF parameters (precursor and product ions with their respective errors) of TCS and its TPs were obtained. In addition, LC-QqQ parameters such SRM transitions, fragmentation voltages, collision energies were optimized for the parent ion and each TP. Finally, 15 TPs of TCS were found through both untargeted strategies.

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P1-068**GREENNESS ASSESSMENT OF OFFICIAL ANALYTICAL METHODS AND EQUIVALENT METHODS UTILIZING SPME FOR SAMPLE PREPARATION****Lopez Garcia, Maria¹; Michel, Frank².**¹*Merck Life Science KGaA, Madrid, Spain;* ²*Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.*

Numerous official analytical methods established by organizations such as the US EPA, AOAC, ASTM, and ISO were formulated many years or even decades ago. Consequently, these methods often do not incorporate contemporary techniques for sample preparation and analysis. As a result, they typically necessitate substantial volumes of organic solvents for processes like liquid-liquid or Soxhlet extraction, which present environmental, health, and safety concerns [1]. Therefore, these methods fail to meet current standards for sustainable laboratory practices. In contrast, methodologies that utilize miniaturized and solvent-free sample preparation techniques offer a more environmentally friendly solution.

Solid Phase Microextraction (SPME), introduced in 1990, is a miniaturized and solvent-free sample preparation technique. SPME utilizes fibers coated with polymers or particles embedded in polymers to extract organic compounds from diverse matrices without the use of organic solvents. SPME is a robust and versatile sample preparation approach, recognized for its high sensitivity and selectivity, and has gained widespread acceptance in environmental, pharmaceutical, and food analysis. Its popularity was further bolstered by the automation of the entire sample preparation process using GC autosamplers starting in 1993. The adoption of SPME in official methods and standards further underscores its acceptance. Many attributes of SPME align closely with the 12 Principles of Green Analytical Chemistry [2], including its solvent-free nature, miniaturization, and ease of automation.

This study emphasizes the fundamental principles and advantages of SPME while examining various official methods utilized in environmental testing. A comparison will be made between these traditional methods and corresponding SPME approaches, showcasing the enhanced sustainability of SPME technique through the application of different metrics for Green Analytical Chemistry [3].

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P1-069**THE USE OF EUTECTOGEL AS A GREEN POLYMERIC SORBENT FOR EFFICIENT HEAVY METAL EXTRACTION FROM FOOD SAMPLES USING ELECTROCHEMICALLY CONTROLLED SOLID-PHASE MICROEXTRACTION****Marco Córdoba, Andrea¹; Díaz, Pedro¹; Aguirre, Miguel Ángel¹; Quijada, César²; Hidalgo, Montserrat¹.**

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Heavy metals occur naturally in the environment and, in appropriate concentrations, are essential for various physiological functions. For example, iron deficiency can impair oxygen transport to cells and weaken immune function. However, excessive exposure to these metals can lead to a range of health problems, including asthma, allergic reactions and serious diseases such as various types of cancer [1]. One of the main ways in which humans are exposed to these toxic elements is through the consumption of contaminated water or food. To mitigate potential health risks, it is essential to develop highly sensitive and accurate analytical methods for monitoring heavy metal concentrations in such samples.

In this context, the present study introduces a novel and efficacious approach that integrates solid-phase microextraction (SPME) with electrochemistry for the separation and preconcentration of selected analytes (i.e., As, Cu, Fe, and Pb), followed by detection using inductively coupled plasma optical emission spectroscopy (ICP-OES). Specifically, the study employs electrochemically controlled solid-phase microextraction (EC-SPME) to enhance metal extraction efficiency while minimizing reagent consumption, waste production, and energy usage.

In this work, a hydrophilic eutectogel composed of polymeric components (i.e., 2-hydroxyethyl methacrylate (HEMA) as monomer and polyethylene glycol dimethacrylate (PEGDMA) as cross-linker), hydrophilic DES (ChCl:EG in a 1:2 molar ratio) and 2-hydroxy-2-methylpropiophenone (HOMPP) as photoinitiator was employed to extract heavy metals from food samples. Following the UV curing of the eutectogel, the optimization of key parameters through multivariate analysis was carried out. The method with optimized parameters exhibited a notable enhancement in metal extraction. This innovative approach, which integrates microextraction with electrochemistry and utilizes a hydrophilic eutectogel as extractant phase, offers a promising alternative to conventional sample preparation methods.

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P1-070**BIODEGRADABLE ACTIVE FILMS WITH OLIVE LEAF NaDES EXTRACTS FOR SUSTAINABLE FOOD PACKAGING**

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The olive oil industry in Southern Europe generates over 21 million tonnes of waste annually, containing valuable polyphenolic compounds with strong antioxidant and bioactive properties. Recovering these compounds aligns with circular economy principles, offering a sustainable approach to agro-industrial waste management. Natural Deep Eutectic Solvents (NaDES) provide an eco-friendly and efficient alternative to conventional solvents for polyphenol extraction. These bioactive extracts can be incorporated into biodegradable polymeric matrices to develop active films with antioxidant and antibacterial properties, extending the shelf life of food products. This study explores the extraction of polyphenols from olive leaves using NaDES and their integration into biodegradable films, assessing their physicochemical, functional, and antimicrobial properties for food packaging applications.

Polyphenols were extracted using a NaDES system composed of choline chloride and glycerol (1:5 molar ratio, 30% water). The extracts were analyzed by HPLC-HRMS for identification, HPLC-LRMS for quantification of the main polyphenolic compounds, and FRAP assay for estimating their antioxidant capacity. The extracted polyphenols were then incorporated into an alginate-based biodegradable film crosslinked with calcium chloride. Nanocellulose fibers were also added to enhance mechanical properties. The NaDES extract was incorporated at 2.5–5 mL per gram of polymer, followed by ultrasound treatment for 2h. The mixture was cast onto Petri dishes, dried at 40°C, and immersed in 1% calcium chloride and redried. The films were characterized by FTIR and TGA for physicochemical properties, SEM for morphology, and additional tests were conducted to evaluate transparency, color, mechanical strength, swelling, permeability, polyphenol release, and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

The polyphenolic extracts were rich in oleuropein (0.37 mg/g), luteolin-7-O-glucoside (0.38 mg/g), and 3-hydroxytyrosol (0.11 mg/g), along with other bioactive compounds such as rutin, p-coumaric acid, hesperidin, diosmin, and quercetin. The antioxidant capacity was 19.6 mg Trolox equivalents per gram via FRAP, confirming their high potential. The resulting biodegradable films exhibited a uniform structure with favorable mechanical properties, while thermogravimetric analysis demonstrated their thermal stability, supporting their application in food packaging. The controlled release of polyphenols in aqueous media was evidenced by the gradual diffusion of oleuropein, luteolin-7-O-glucoside, and 3-hydroxytyrosol over time. Although no inhibition halos were observed in antimicrobial assays, bacterial colonization was significantly reduced in areas previously covered by the films, particularly at 24 and 48 hours post-removal. This suggests that the film antimicrobial activity is primarily contact-based rather than diffusion-driven.

These findings demonstrate that NaDES-extracted polyphenols from olive waste can be successfully incorporated into biodegradable alginate-based films, providing high antioxidant capacity, controlled release properties, and antimicrobial effects. This study highlights the potential of these bio-based materials as sustainable active packaging solutions for the food industry, contributing to waste valorization and the development of environmentally friendly alternatives.

P1-071**HIGH-RESOLUTION MASS SPECTROMETRY FOR POLYPHENOL PROFILING IN OLIVE LEAF NaDES EXTRACTS****Mir-Cerdà, Aina; Granados, Mercè; Saurina, Javier; Sentellas, Sonia.***Universitat de Barcelona, Barcelona, Spain.*

Waste generated by the olive industry is a significant source of antioxidant compounds that can be extracted and repurposed as raw materials for the chemical, cosmetic, and pharmaceutical industries. Among these bioactive compounds, flavonoids—such as luteolin and its numerous glycoside derivatives—are particularly noteworthy. Another prominent group of phenolic compounds is derived from dihydroxyphenyl ethanol and its esters with elenoic acid, leading to well-known compounds like oleuropein, which is highly valued for its health benefits. Additionally, hydroxybenzoic and hydroxycinnamic acids are present in these residues. Despite the extensive scientific literature on this subject, challenges remain in these compounds' structural elucidation, characterization, and quantification.

Olive leaves constitute one of the most abundant byproducts of the olive oil industry. In this study, the phenolic composition of olive leaf extracts was analyzed using liquid chromatography with ultraviolet detection coupled to high-resolution mass spectrometry (LC-UV-HRMS). Olive leaf residues were crushed, homogenized, and subjected to a solid-liquid extraction process involving mechanical shaking at 80°C for 2 hours. Natural Deep Eutectic Solvents (NaDES), specifically choline chloride:glycerol (1:5 m/m, 30% water), were selected as the extraction medium. Polyphenol identification in the resulting extracts was performed using HRMS in data-dependent acquisition mode in negative polarity using an Orbitrap HRMS instrument.

More than 65 phenolic compounds were tentatively annotated, with approximately 24 confirmed with reference standards. The most prominent identified compounds were oleuropein, along with its aglycone and glucoside derivatives, luteolin-7-glucoside, luteolin-4-glucoside, apigenin-7-O-rutinoside, 3-hydroxytyrosol, and verbascoside.

These findings highlight the potential of olive leaf residues as valuable sources of antioxidant compounds. Their recovery and reuse contribute to the sustainable recycling and valorization of agro-industrial waste, offering promising opportunities for various industrial applications.

P1-072**ENVIRONMENTALLY FRIENDLY AND HIGH-THROUGHPUT MULTI-MYCOTOXIN DETERMINATION IN HUMAN URINE BY UHPLC-MS/MS****Peris-Camarasa, Borja¹; Pardo, Olga²; Dualde, Pablo¹; Coscollà, Clara¹.**

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Mycotoxins, a group of naturally occurring secondary metabolites produced by fungi, are globally widespread and pose a serious risk to human health due to their toxicological effects, including carcinogenic, mutagenic, and neurotoxic properties. Their diverse biochemical properties lead to variations in metabolic pathways, with urine being a key excretion route. Given increasing concerns about environmental sustainability, the development of innovative analytical techniques aligned with Green Analytical Chemistry principles is essential. In response, a new methodology for environmentally friendly, rapid, efficient and simultaneous multi-mycotoxin determination has been developed and validated. The approach is based on a simple “dilute-and-shoot” sample preparation followed by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). This method targets twelve key mycotoxins: Aflatoxins B1, B2, G1, G2 and M1; Zearalenone and Zearalanone; Ochratoxin A; Alternariol; Alternariol monomethyl ether; Citrinin and Sterigmatocystin. Relative recoveries ranged between 79.2% and 113.5%, with all analytes demonstrating precision values below 20%. Limits of quantification (LOQs) varied from 0.005 to 0.5 ng/mL, matching or surpassing the sensitivity of previously published methods. Moreover, the measured concentration ranges encompass the levels typically found in human urine. The method’s environmental and practical performance was further assessed using AGREEprep and BAGI tools, positioning it as one of the most sustainable and user-friendly approaches currently available. Application of the method to 25 urine samples from adults in the Valencian Region (Spain) confirmed its effectiveness in real-world biomonitoring studies. In summary, the results indicate a measurable level of mycotoxin exposure in the studied population, underscoring the importance of continued surveillance and analytical innovation.

Acknowledgements

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P1-073**GREENING THE DERIVATIZATION PROCESS: ARYL AMINES AS STUDY CASE****Erendzenova, Danara; Gomara, Belen; Ramos, Lourdes.***IQOG-CSIC, Madrid, Spain.*

In the field of analytical chemistry, reactions of derivatization are frequently used to improve the chromatographic behavior or detectability of selected organic compounds. Numerous and diverse analytical derivatization methods have been described in the literature [1]. The derivatization reaction can be done before or after the instrumental chromatographic separation. For methods involving gas chromatography (GC), most derivatization approaches are carried out before the injection of the sample in the instrument. In general, these methodologies are time-consuming procedures involving hazardous reagents and, in some cases, an extensive manipulation of the extracts. They also contribute to increase of wastes associated to the sample preparation process and their yield can be variable.

Schiff base derivatives are obtained by condensation of a carbonyl group with a primary aliphatic or aromatic amine. This type of reaction has been used to give volatile derivatives for GC analysis [1]. This equimolecular reaction involves nucleophilic addition followed by dehydration to generate an imine derivate. The reaction typically involves aldehydes and ketones, is acid-catalyzed, and develops under relatively mild conditions but often requires long reaction times (2-4 h).

In an attempt to approach the principles of Green Analytical Chemistry [2], the feasibility of vanillin as a green reagent to form Schiff derivatives was evaluated using chloroanilines as model compounds. Different characterization techniques, including nuclear magnetic resonance, attenuated total reflectance-infrared spectroscopy and mass spectrometry, were used to follow the reaction and for the characterization of the formed derivatives.

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P1-074

ADVANCED EXTRACTION TECHNIQUES IN COMBINATION WITH NATURAL DEEP EUTECTIC SOLVENTS FOR THE IMPROVED EXTRACTION OF SAFFRON (*Crocus sativus*) BIOACTIVES

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There is increasing interest in obtaining plant-based bioactive compounds through sustainable methods to develop functional foods and dietary supplements. Saffron (*Crocus sativus*) contains important phytochemicals such as safranal, picrocrocin and crocins, which contribute to its distinctive aroma and color, while also providing various health benefits. Extensive research has shown that these compounds may help to support heart health, protect the nervous system, and possibly play a role in cancer prevention [1].

The high demand for saffron-based dietary supplements, the limited production of the plant and its high cost and the instability of bioactives require the optimization of the extraction process of these compounds in order to achieve the highest yields. Conventionally, solid-liquid extraction (SLE), using water or hydroalcoholic mixtures as extractants, has been applied for the recovery of saffron bioactives, while advanced extraction techniques such as microwave-assisted extraction (MAE) or ultrasound-assisted extraction (UAE) have scarcely been investigated for their faster and more effective extraction [2]. Recently, innovative solvents like biomass-derived biosolvents [for instance terpenoids and natural deep eutectic solvents (NADES)] have gained attention as cost-effective, sustainable, and biocompatible alternatives with excellent solvent capabilities [3]. In a previous study, choline chloride : ethylene glycol 1:2 (ClCh:EtG) showed to be an effective solvent for the simultaneous extraction of safranal and crocins from saffron [4] .

Then, the objective of this work was to explore the use of advanced extraction techniques (UAE and MAE) in combination with NADES in order to extract saffron's bioactive compounds, comparing these results with those obtained by conventional methods [5].

Firstly, different sample:solvent ratios (1:10, 1:20 and 1:40 w:v) were evaluated to obtain the highest yields with the lowest solvent consumption. A Box-Behnken experimental design was used to optimize UAE and MAE extraction conditions (temperature 40-80°C) and time (5-30 min). Samples were analysed by a reverse phase-liquid chromatography-mass spectrometry [(RP)LC-MS] method, previously optimized in the laboratory. Optimal MAE and UAE conditions were selected to maximize the recovery (in mg g⁻¹ dry sample) of safranal, picrocrocin and crocins. Finally, the potential cytotoxicity of the resulting NADES extracts was *in vitro* evaluated and AGREEprep software was used to determine the sustainability of the developed methods.

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P1-075**POLYOXYETHYLENE(10)TRIDECYL ETHER: EXPANDING THE POTENTIAL OF MICELLAR LIQUID CHROMATOGRAPHY WITHOUT ORGANIC SOLVENTS**

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The use of mobile phases containing a surfactant above its critical micelle concentration in reversed-phase liquid chromatography (RPLC) has attracted significant attention as an alternative chromatographic technique. In this separation mode, micelles form in the mobile phase, while surfactant monomers adsorb onto the stationary phase, altering its properties. Depending on the surfactant type (ionic or non-ionic), the coated stationary phase may exhibit a net positive charge or undergo changes in polarity and other characteristics while remaining neutral, which impacts chromatographic behavior [1,2].

Most studies on the use of surfactants as modifiers in RPLC focus on the anionic sodium dodecyl sulfate (SDS). SDS is effective in masking silanol groups (responsible for poor efficiency and peak tailing of basic compounds in hydro-organic RPLC with conventional columns), leading to improved peak profiles. However, SDS also has the drawback of yielding excessive retention of basic compounds (which are protonated at the working pH of the mobile phase), due to strong electrostatic interactions between the cationic species and the adsorbed anionic surfactant monomers. This often requires the addition of an organic solvent to reduce retention times to practical values.

The use of other surfactants, such as the non-ionic Brij-35, has been less explored. Brij-35 eliminates electrostatic interactions between cationic species and the column, enabling the separation of basic compounds and a wider range of analytes within suitable retention times, without the need of organic solvents in the mobile phase. However, its masking effect is less efficient, resulting in poorer peak shape. Therefore, the search for new non-ionic surfactants with properties similar to Brij-35, but capable of significantly improving peak shape, remains a key focus of interest in micellar liquid chromatography (MLC) [3].

In this work, we investigated the non-ionic surfactant polyoxyethylene(10)tridecyl ether in MLC, owing to its high solubility in water and lack of UV absorption. We used a group of β -adrenoceptor antagonists as probe compounds to evaluate its chromatographic performance. Polyoxyethylene(10)tridecyl ether was also combined with SDS to modify the chromatographic behavior and eliminate the need for organic solvents. The chromatographic performance was evaluated in terms of retention and peak shape. The column equilibration process, along with the reversible adsorption of the non-ionic surfactant on the alkyl-bonded stationary phase, was also assessed.

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P1-076**DETERMINATION OF LITHIUM BATTERY ELECTROLYTE COMPOSITION WITH POLYARC MICROREACTOR****Weber, Waldemar.***German, Duisburg, Germany.*

The production of organic electrolytes for batteries occurs at various locations worldwide. For quality assurance, it is essential to determine the precise composition of organic carbonates. Variations in their composition can significantly impact battery performance and safety. Accurate analysis enables manufacturers to maintain consistent quality and meet the stringent requirements of the industry, ultimately leading to more reliable and efficient energy storage solutions.

Typically, producers provide the weight percentages (%w) as the specified composition values. To determine this composition, different analytical technologies can be employed, including Gas Chromatography with Flame Ionization Detection (GC-FID), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography (LC), or Nuclear Magnetic Resonance (NMR). All these technologies, except for NMR, share a common characteristic: they provide molecule-dependent responses. Consequently, to ascertain the weight percentages (%w), an external calibration with all expected compound standards must be performed.

This calibration process is a common practice and usually does not pose a problem in routine operations. However, for semi-routine and non-routine laboratories, this requirement leads to a significant time investment. In this article, we present a new, highly accurate GC-based method to determine weight percentages from a single measurement without the need for external or internal calibrations.

The Polyarc system features a catalytic microreactor that enhances gas chromatographs equipped with flame ionization detectors (FIDs). It achieves this by converting all organic compounds into methane molecules before they are detected by the FID, effectively transforming the FID into a quantitative carbon detector (QCD). This innovative approach not only simplifies the analytical process but also significantly improves the accuracy of quantification by eliminating the need for calibrations.

By utilizing the Polyarc system, laboratories can streamline their analytical workflows, reduce time spent on calibration, and enhance the reliability of their results. This method provides a robust solution for quality assurance in the production of organic electrolytes, ensuring that manufacturers can consistently deliver high-quality products that meet industry standards.

Overall, the ability to accurately determine the composition of organic carbonates without the complexities of traditional calibration methods represents a significant advancement in analytical chemistry. This new approach facilitates better quality control and contributes to the development of safer and more efficient energy storage technologies.

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P1-077

SHRIMP SHELL-BASED BIOSORBENT FOR THIN-FILM MICROEXTRACTION OF RHODAMINE B AND RHODAMINE 6G FROM AQUEOUS SAMPLES

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A novel biosorbent derived from shrimp shells was evaluated for the determination of Rhodamine B (RhB) and Rhodamine 6G (R6G), two synthetic dyes widely banned due to their adverse effects on human health. Despite regulatory restrictions, these dyes continue to be used in poorly regulated markets to impart red and pink hues to various products [1,2]. Natural-origin materials have gained attention in recent years due to their biodegradability, biocompatibility, low cost, and eco-efficiency, positioning them as sustainable alternatives to commercial sorbents. Ideally, these materials do not require extensive treatments to enhance their sorbent properties, aligning with the principles of green analytical chemistry and circular economy. In our laboratory, we have previously studied materials such as cork, peanut shells, avocado seeds and grapefruit peel for the microextraction of emerging contaminants from environmental matrices [3-6].

The shrimp shell-based biosorbent was subjected to mild oxidation using 0.5 M hydrogen peroxide to improve its affinity toward the target analytes. Adsorption isotherms were conducted to determine the physicochemical parameters governing the sorption behavior of the dyes onto the modified material. Quantification of Rhodamine B (554 nm) and Rhodamine 6G (526 nm) was carried out using UV-Vis spectrophotometry. The biosorbent was further characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). It was then applied as the active phase in a Thin-Film Microextraction (TF-ME) system coupled with HPLC-FLD for the analysis of aqueous environmental samples. A TF-ME device with twelve stainless steel thin films was applied, enabling parallel extraction and desorption. The biosorbent was immobilized onto the films using double-sided adhesive tape, layered in three cycles for uniform distribution. Extractions were performed in 96-well ELISA-type plates with a volume of 2 mL per well.

The material exhibited an adsorption capacity ranging from 85–95%, with high selectivity for RhB and R6G over other dyes such as methylene blue and crystal violet (adsorption of approximately 50%). The optimal preoxidation condition was 0.5 M H₂O₂; higher concentrations led to reduced efficiency. In TF-ME, equilibrium was achieved within 15 minutes, and methanol was identified as an effective elution solvent. The medium pH significantly influenced the adsorption efficiency, with the optimal condition found at pH 2. Notably, the shrimp shell biosorbent demonstrated comparable analytical performance to the commercial Oasis® HLB phase and outperformed chitosan, despite both being derived from shrimp shells.

These results highlight the potential of this shrimp shell-based biosorbent as an eco-efficient, low-cost, and viable alternative for the analysis of synthetic contaminants in complex matrices, reinforcing the shift toward more sustainable analytical chemistry.

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P1-078**BIODEGRADABLE FILM-BASED EXTRACTION OF EMERGING CONTAMINANTS: A SUSTAINABLE STRATEGY WITHIN THE WHITE ANALYTICAL CHEMISTRY FRAMEWORK****Ceccardi, Erica¹; Di Carro, Marina²; Magi, Emanuele²; Benedetti, Barbara².**¹Università degli studi di Genova, Genova, Italy; ²Università degli Studi di Genova, Genova, Italy.

The increasing awareness of the environmental impact of analytical procedures has heightened the demand for more sustainable methodologies. In response, the concept of White Analytical Chemistry (WAC) has been introduced, aiming to balance analytical performance, environmental sustainability, and operational efficiency [1]. Emerging contaminants (ECs) are a broad group of substances whose occurrence in the environment has attracted the attention of the scientific community over the last two decades [2]. Given the extremely low concentrations of ECs and the presence of interfering substances in environmental matrices, sophisticated instrumentation, such as liquid chromatographic coupled to mass spectrometry, is often required to achieve the necessary levels of specificity, accuracy, sensitivity and precision [3]. However, in accordance with WAC principles, it is crucial to adopt environmentally friendly sample preparation techniques. The objective, in this context, is to achieve accurate analytical results while minimizing the use of toxic reagents and the waste production.

In this study, the potential of a biodegradable polymeric film (Mater-Bi) for the extraction of emerging contaminants from wastewater was initially investigated [4]. The optimized procedure allowed quantification of sixteen analytes from various classes, such as UV filters, pharmaceuticals, and additives, with satisfactory recoveries, precision, and minimal matrix effects. The film also demonstrated potential for extracting PerfluoroAlkyl Substances (PFAS), although different experimental conditions were needed for their extraction, prompting further targeted optimization.

A more comprehensive study was then conducted, focusing on the extraction of over 60 emerging compounds from water samples. Scanning Electron Microscopy (SEM) analysis revealed that the internal structure of the film was porous, prompting investigations into different configurations to better understand its interaction with the analytes. While three setups were explored to enhance cross-sectional area, recoveries showed no substantial differences. Following this, a complete mass balance of the extraction procedure was conducted to identify where analyte loss occurred. It was observed that some analytes, particularly the more polar ones, did not interact strongly with the film, while others, especially PFAS, were not fully desorbed during the back-extraction step. To address these challenges, two experimental designs will be employed. First, a Mixture Process Design will be used to assess the influence of various variables (solvent type, % of acid, time, and ultrasound power) on the "conditioning" step of the film. Subsequently, a quadratic model will be applied to investigate the optimal conditions for back-extraction, aiming to achieve complete desorption of the analytes.

In conclusion, this study presents a first approach with the green material Mater-Bi for the extraction of emerging contaminants. A multivariate optimization is currently underway to improve extraction efficiency and recovery rates, with the aim of expanding its applicability to a wider range of analytes, with particular attention to PFAS.

This work contributes to the development of greener methodologies in environmental monitoring, paving the way for more sustainable and efficient approaches to the determination of emerging contaminants.

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P1-079

SCREENING OF NATURAL DEEP EUTECTIC SOLVENTS TO EXTRACT BIOACTIVE COMPOUNDS FROM A WHEAT-BASED PHARMACEUTICAL WASTE

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Triticum vulgare is an annual herbaceous plant belonging to the genus *Triticum*, family Poaceae. It is one of the most widely cultivated crops around the world, with about 216 million hectares grown every year. The aqueous extract of *Triticum vulgare*, obtained from the germ, promotes tissue repair processes by stimulating chemotaxis and fibroblast maturation [1]. During the production process of this aqueous extract, a significant quantity of solid waste is produced, and to date, no alternative use has been identified.

Traditional techniques, such as organic solvent extraction, are widely used for their efficiency, but present disadvantages as long extraction times, risk of degradation of bioactive compounds and high consumption of organic solvents, which are often toxic, difficult to dispose of and harmful for the environment. To overcome these limitations and reduce environmental impact in line with the principles of green analytical chemistry, new-generation solvents such as natural deep eutectic solvents (NADES) have emerged in the last decade. These are formed by mixing two or more components to obtain a liquid and homogeneous mixture with a lower melting point than that of the separate individual components. NADES are easily biodegradable, have little or no toxicity, can behave as natural solvents and are also not expensive [2].

Therefore, the main aim of this study was to evaluate the extraction efficiency of NADES in order to improve the recovery of bioactive compounds of pharmaceutical or nutraceutical interest from wheat-based waste through a combination of targeted and non-targeted analyses.

In detail, we screened eight NADES based on different natural primary components. These systems were selected to cover a wide range of physicochemical properties, including pH, polarity, and viscosity, which were previously characterized. The composition of the NADES was as follows (molar ratio): lactic acid:glycerol:water (1:1:3), choline chloride:lactic acid:water (1:3:6), lactic acid:glucose:water (5:1:9), thymol:menthol (1:1), decanoic acid:thymol (1:2), betaine:1,3butanediol:water (1:3:10.6), choline chloride:2,3 butanediol (1:4), choline chloride:urea:water (1:2:3). The extraction process was performed as follows: 0.5 g of sample was suspended in 5 mL of NADES and the mixture was vortexed for 20 seconds. It was then subjected to mechanical agitation for 15 minutes at maximum speed and centrifuged at 5°C for 10 minutes at 9000 rpm. The supernatant obtained was collected, diluted with MeOH containing 0.1% formic acid, filtered and finally subjected to analysis by LC-Q-ToF-HRMS. The results showed that the best NADES, in terms of higher recovery of ferulic acid, was betaine:1,3butanediol:water (molar ratio, 1:3:10.6). The higher extraction efficiency may be attributed to the solvent's pH (5.9), low viscosity (5.56 mPa·s at 20 °C), and the presence of water, which together enhance solvation and mass transfer. The key variables affecting the extraction, such as NADES volume, extraction time, temperature and centrifugation time, were optimized using a multivariate optimization strategy. Response surface methodology (RSM), based on a central composite design (CCD), was employed to reduce the number of experiments and improving extraction efficiency.

This extraction approach was compared with conventional hydroalcoholic extraction (EtOH/H₂O, 80:20 v/v), yielding comparable results and demonstrating that NADES-based extraction represents a promising alternative to traditional organic solvent methods. Non-targeted analysis revealed that both extraction approaches enabled the recovery of a comparable number of chemical features, including various compound classes such as amino acids and sugars.

This study highlights the potential of utilizing this waste as a source of ferulic acid and as a possible raw material in other production processes, considering that NADES allow for the direct preparation of ready-to-use extracts with numerous applications in the food and pharmaceutical industries.

Keywords: wheat, waste management, NADES extraction, bioactive compounds, green sample preparation, circular economy

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P1-080**ECO-FRIENDLY EXTRACTION AND SEPARATION OF ORGANIC CONTAMINANTS IN COMPLEX FOOD SAMPLES USING NATURAL DEEP EUTECTIC SOLVENTS AND nano-LC**

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White analytical chemistry has evolved from green analytical chemistry by integrating its foundational principles—such as reducing reagent consumption, waste generation, and energy use—with a strong emphasis on analytical functionality, including both performance and operational simplicity. This integrated approach has led to the development of analytical methods that are environmentally sustainable without compromising reliability or ease of implementation [1]. Within this context, the use of alternative solvents plays a crucial role, particularly natural deep eutectic solvents (NADES). These solvents, formed by combining two or more naturally occurring components, represent a green alternative to conventional organic solvents due to their low toxicity and minimal associated health risks. Moreover, their low volatility and high solvating capacity for a broad spectrum of compounds—attributed to the diverse intermolecular interactions they can establish—make NADES highly versatile and promising as extraction media in analytical procedures [2,3].

In response to the growing demand for sustainable analytical methodologies, this study explores two complementary approaches based on NADES and nano-liquid chromatography (nano-LC) for the determination of organic contaminants in complex food matrices. Both strategies aim to reduce environmental impact while maintaining analytical efficiency, selectivity, and practicality. The first approach focuses on the analysis of alkylphenols and bisphenol A in olive and sunflower oils. Liquid-liquid microextraction using various NADES based on natural compounds such as vitamins, organic acids, alcohols, and amino acids was employed to determine their suitability for analyte recovery. Nano-LC separation was optimized through the evaluation of different stationary phases, achieving an efficient separation in less than an 11-minute run. In parallel, a second approach applied the same analytical principles to herbal aqueous samples, using similar model analytes. NADES specifically formulated from menthol combined with either vanillin or cinnamic acid were selected for their low toxicity, natural origin, and favourable physicochemical properties, which support effective extraction. Both approaches were systematically evaluated using analytical metric tools to assess their environmental impact, robustness, and applicability, confirming the potential of NADES-based microextraction combined with nano-LC as a green and effective solution for monitoring organic contaminants in food products.

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P1-081**ADDED-VALUE OF AGRONOMIC WASTES: GREEN EXTRACTION AND UHPLC-MS/MS ANALYSIS OF PHTHALIC ACID ESTERS IN BANANA LEAVES USING NATURAL DEEP EUTECTIC SOLVENTS****Conde-Díaz, Adrián; Santana-Mayor, Álvaro; Herrera-Herrera, Antonio V.; Socas-Rodríguez, Bárbara; Rodríguez-Delgado, Miguel Ángel.***Departamento de Química, Unidad Departamental de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL), San Cristóbal de La Laguna, Spain.*

Banana is one of the most widely produced tropical fruits worldwide. In Spain, its cultivation is concentrated in the Canary Islands, where optimal agroclimatic conditions yield high-quality fruits distinguished by their aroma, flavor, high sugar, and low starch content—features that set them apart from bananas produced in Asia, America, and Africa [1]. In this context, the valorization of banana by-products for use in food and nutraceutical applications emerges as a promising and sustainable strategy to address two pressing challenges: the growing problem of food waste—which reaches 88 million tons annually in Europe, with an associated economic impact of 143 billion euros [2]—and the increasing difficulty faced by Canarian producers in competing with external markets. Reducing food loss and waste represents a key opportunity to lower production costs, increase food system efficiency, and promote environmental sustainability [3]. However, to ensure the safety and quality of revalorized banana-derived products, it is essential to assess the potential presence of organic pollutants. This is the case of phthalic acid esters (PAEs), widely used as plasticizers and well-known endocrine disruptors that pose health risks and can contaminate crops, such as bananas, due to their ubiquitous presence [4].

This study developed an ultrasound-assisted extraction (UAE) method using Natural Deep Eutectic Solvents (NADES)—a novel class of green solvents—combined with ultra-high performance-liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for the determination of 13 PAEs in banana leaves intended for valorization. NADES were synthesized from natural metabolites, including vitamins, sugars, and alcohols. Extraction conditions were optimized following a one-variable-at-a-time approach. The analytical validation demonstrated excellent recovery, precision, linearity, and sensitivity, enabling trace-level detection of all target plasticizers. Finally, the method was applied to analyze 22 banana leaf samples from various islands in the Archipelago. This approach aligns with White Analytical Chemistry principles, balancing sustainability from Green Analytical Chemistry with practical and robust analytical performance, thus offering a reliable and eco-friendly tool for assessing the safety of agronomic waste-derived products [5].

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P1-082**SIMULTANEOUS ENANTIOMERIC DETERMINATION OF TRYPTOPHAN-RELATED METABOLITES BY ELECTROKINETIC CHROMATOGRAPHY. APPLICATION TO THE ANALYSIS OF URINE SAMPLES****Adámez-Rodríguez, Sandra; Marina, María Luisa; Castro-Puyana, María.***Universidad de Alcalá. Dpto. de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares, Madrid, Spain.*

The identification and quantification of biomarkers that allow the understanding or prediction of pathologies are crucial for establishing specific clinical therapies. Biomarkers are metabolites that differ in their presence or concentration between healthy and pathological states. In certain instances, biomarkers are not a given metabolite but one of its enantiomeric forms. This fact has increased the interest in the development of innovative analytical strategies to obtain the enantiomeric separation of chiral molecules in biological samples^[1,2].

Tryptophan (Trp) is a chiral amino acid obtained through diet which is involved in different and vital biological processes such as intestine homeostasis, cancer, mood disorders, anxiety or depression. The principal metabolic pathways related to Trp are protein synthesis, kynurenine production, and serotonin obtention^[3]. This protein amino acid is mainly metabolized into kynurenine (Kyn), whose levels are linked to inflammatory processes, hormonal disruption, immune system regulation by cellular energy generation and neuroplasticity^[4]. On the other hand, 5-hydroxytryptophan (5-HTP) is produced as a result of Trp hydroxylation while 5-HTP decarboxylation leads to serotonin, a neurotransmitter that participates in several biological processes such as cognition, learning, sleeping, satiety or memory^[5]. Considering the biological relevance of Trp and its related metabolites, and taking into account that except serotonin all of them are chiral compounds; the development of methodologies capable of providing the individual determination of each enantiomer is required.

In this work, an innovative approach based on the use of electrokinetic chromatography-(EKC) was developed to achieve the simultaneous enantioseparation of Trp, Kyn, 5-HTP, and serotonin. The most appropriate conditions to obtain the chiral separation were optimized in terms of the type and concentration of chiral selector, temperature, and voltage applied. Thus, under the most favorable experimental conditions, the simultaneous enantiomeric separation of all the metabolites analyzed was achieved with resolutions values higher than 3.8 in less than 20 min. Subsequently, the analytical characteristics of the developed methodology were evaluated, showing good performance for the determination of Try-related metabolites. Before applying the developed EKC method to the analysis of urine samples, a sample treatment consisting of salt precipitation and solid-phase extraction to clean-up and preconcentration was performed. In this way, L-Kyn and L-Trp were determined at physiological levels in the urine of a healthy volunteer.

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P1-083**A COMBINATION OF VORTEX-ASSISTED LIQUID-LIQUID MICROEXTRACTION WITH FLUORESCENCE DETECTION (VALLME-FLD): AN INNOVATIVE APPROACH FOR A GREEN AND HIGHLY SENSITIVE ANALYSIS****Bazel, Yaroslav; Kakalejčíková, Sofia.***Department of Analytical Chemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovakia.*

A novel design for vortex-assisted liquid-liquid microextraction, combined with fluorimetric detection (VALLME-FLD), was proposed and successfully tested for determining picric acid (PA), sodium dodecyl sulfate (SDS), and rhodamine B (RhB) in real samples. The determination of PA and SDS is based on the formation and extraction of an ion associates (IA) in the presence of the basic polymethine dyes, which serve as the analytical species for fluorescence measurement. Various factors influencing the relative fluorescence intensity of microextracts were studied. Optimization primarily focused on investigating the influence of chemical characteristics (such as pH, dye concentration, solvents choice, interference studies) as well as other factors like mixing and vortexing time, speed and effects of centrifugation. The VALLME-FLD approach represents a significant advancement, characterized by high sensitivity and selectivity, minimising the use of organic solvents, facilitating direct sample preparation, and reducing analysis time which meets the requirements of green analytical chemistry. The methods have been successfully applied to real samples, confirming their practical applicability in environmental and analytical chemistry [1].

Additionally, a new green and highly sensitive method for RhB determining based on deeply eutectic solvents (DES-VALLME-FLD), was developed. The extraction efficiency of conventional solvents and various DES systems composed of tetrabutylammonium bromide and alcohol (hexanol, octanol, decanol) in different ratios was compared. DFT calculations of the intermolecular electrostatic and non-covalent interactions of the most stable forms of RhB with DES and water explain the experimental DESs' extraction efficiency. The analytical characteristics of the developed DES-VALLME-FLD method were also investigated. Based on these results, it can be concluded that the LOD values when using the microextraction method with fluorescent detection are more than an order of magnitude lower than in the case of a similar method with UV-Vis detection of microextracts. The developed method was successfully applied to real samples (tap water, energy drink, and lipstick) [2].

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P1-084**DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD BY MICELLAR LIQUID CHROMATOGRAPHY FOR DEGRADATION STUDIES OF CHEMOTHERAPEUTIC DRUG IRINOTECAN****Carda Broch, Samuel¹; Bose, Devasish²; Peris Vicente, Juan³; Durgbanshi, Abhilasha².**¹Universitat Jaume I, Castellón, Spain; ²Doctor Harisingh Gour Vishwavidyalaya (A Central University), Sagar, India;³Universitat de València, Burjassot, Spain.

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin) is a widely prescribed chemotherapeutic drug. Approved by the U.S. Food and Drug Administration (FDA) in 2015 under the trade name Onivyde for advanced pancreatic cancer, it has since been used to treat various tumors, including colorectal, pancreatic, ovarian, and lung cancers, either alone or in combination with other antitumor agents.

In order to ensure therapeutic efficacy, the finished product must pass through rigorous and strict control quality tests, including the quantification of the active components, stability assessments under stress conditions, and impurity profiling. Indeed, environmental factors, such as light, temperature, pH, oxygen, moistness, additives, excipients, and other ones can significantly impact the stability of pharmaceutical substances. Stress testing is a valuable tool for identifying degradation products and gaining crucial insights into the stability of active components. As per the International Council for Harmonisation (ICH) guidelines, drug stability testing aims to determine shelf life and establish appropriate storage conditions.

Liquid chromatography is the gold standard technique for pharmaceutical analysis due to its automatation, versatility, selectivity and reliability. Therefore, some methods based on LC-MS and hydroorganic HPLC-UV have been developed to quantify irinotecan. However, these techniques exhibit important drawbacks: LC-MS is a costly technique that needs sophisticated maintenance, while hydroorganic HPLC uses large volumes of toxic and pollutant solvents. What's more, both require long, cumbersome and sometimes non-robust purifications steps. Therefore, we aim to develop greener and more easy-to-conduct alternative procedure.

We have developed a micellar liquid chromatography (MLC) method for irinotecan quantification and applied it to forced degradation studies, including thermal degradation, exposure to domestic light sources, acid and alkali hydrolysis and oxidation. Chromatographic analyses were performed using a mobile phase of 0.15 M SDS - 6 % pentanol, buffered at pH 7 with phosphate salt, running through a C18 column under isocratic mode at 1 mL/min. Detection was at 268 nm. Irinotecan was eluted at 6 min. Mobile phase optimization was performed using Response Surface Methodology (RSM) with a Central Composite Design approach, considering SDS concentration, organic modifier content, and pH as factors, while retention time served as the dependent variable. This approach minimized the number of required experiments (20 runs) while providing valuable insights into the effects of these factors on retention time.

The method was successfully validated by the guidelines of International Conference on Harmonization demonstrating excellent selectivity, linearity ($r^2 > 0.999$), calibration range (0.25 – 30 mg/L), trueness (100.1 - 101.4%), precision (<1.7%) and robustness. The developed procedure is easy to perform, rapid, cost-effective, safe, environmentally-friendly and sensitive enough for forced degradation studies. Stability tests revealed that irinotecan remains thermally stable as a solid up to 80°C but undergoes significant degradation in its marketed formulation under oxidative (46.7% degradation with hydrogen peroxide), thermal, and photolytic conditions (83.5% degradation under a 100 W tungsten filament, 30.3% under daylight for 24 h). Hydrolysis studies showed complete degradation in acidic conditions (0.1 M HCl, 4 h reflux), 18.7% degradation in neutral conditions (24 h), and 46.7% degradation under alkaline conditions (0.1 N NaOH, 4 h reflux).

P1-085**VALIDATION STUDIES FOR DETERMINATION OF LAMIVUDINE AND ITS DERIVATIVES BY DIRECT INJECTION - MICELLAR LIQUID CHROMATOGRAPHY****Carda Broch, Samuel¹; Peris Vicente, Juan²; Kassuha, Diego³.***¹Universitat Jaume I, Castellón, Spain; ²Universitat de València, Burjassot, Spain; ³Universidad Católica de Cuyo, San Juan, Argentina.*

Lamivudine is an antiretroviral drug included in a large number of HAART regimens in the treatment against HIV infection. The study of its metabolic pathways may bring us useful information to understand and found the personal (genetic, habits, diet, gender, age, social and natural environment, etc.) and medical (dose, timeline, etc.) factors potentially affecting the pharmacokinetics, and to relate them to the therapeutic and adverse effect. This may be used to ameliorate the treatment. This must be conducted by determining the plasmatic concentration of lamivudine and its metabolites at several times after tablet ingestion.

In the present study, a simple and sensitive method by micellar liquid chromatography (MLC) was optimized and validated for the simultaneous analysis of lamivudine and its main metabolites, including lamivudine (3TC), 3TC-methanol, 3TC-ethanol, 3TC-n-propanol, 3TC-n-butanol, 3TC-n-pentanol, 3TC-n-hexanol and 3TC-n-octanol. All of them, demonstrated to exhibit important biological activity against HIV and HBV. The developed chromatographic method uses an HPLC system equipped with a C-18 column (250 x 4.6 mm). The detection was carried out by a UV detector at a wavelength of 272 nm. The mobile phase (0.15 M SDS/4% 1-butanol buffered at pH 7.4) flows at 1 mL/min under isocratic mode, at 30°C. Zidovudine was used as internal standard for a major statistical analysis. Under these conditions, analytical separation was performed in less than 12.5 min, with retention times (t_R , min): 3TC: 2.4; 3TC-Metha: 3.1; 3TC-Etha: 4.0; 3TC-nPro: 4.9; 3TC-nButa: 5.8; 3TC-nPenta: 6.9; 3TCnHexa: 8.1 and 3TC-nOcta: 10.4. The MLC method was validated according to the FDA guideline, showing and excellent sensitivity, selectivity, adequate LODs and LOQs, precision, recoveries and robustness.

P1-086

PERFORMANCE COMPARISON OF DIFFERENT GC-HRMS ANALYTICAL METHODS FOR QUANTITATION OF FRAGRANCE ALLERGENS IN PERFUME CONCENTRATES

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Introduction

Following the extension to 81 fragrance allergens regulated in Annex III of regulation EC 1223/2009 in 2023, including 57 chemically defined molecules, the quantitative analysis of these regulated compounds has become more complex, particularly in multi-compound perfume matrices. The standardized AFNOR analytical method EN 16274:2021 in 2021 makes it possible to meet the regulations but generates long analysis and reprocessing times. The objective of this project is to develop an alternative analytical method to save analyses and reprocessing time and to improve resolution and sensitivity. Two different GC-HRMS technologies performances were selected and evaluated in this study: GC-Orbitrap HRMS and GCxGC-ToF.

Methods

Two different GC-HRMS technologies performances were evaluated and compare with the GC-MS standardized AFNOR analytical method. For each approach, two different methods were developed to quantify the entire list of 57 allergens and associate isomers (65 analytes in total). Quantitation was performed for each method by determining one quantitative ion and two qualitative ions for each analyte of interest.

GC-Orbitrap (Thermo) analyses were carried out on two different methods: one using an apolar column and another one using a polar column. GCxGC analyses were carried out on two different instrumentations. The first method was developed on a GCxGC-ToF HRT+ (Leco); the second method was developed on a GCxGC-ToF BT4D (Leco) with a similar set of columns for both dimensions.

Preliminary data

Several methodology criteria were used to interpret the determined values:

- Correct quantitation: The allergen has been correctly identified and the quantitative result is in the quantitative range of acceptability (30% deviation accepted)
- Correct identification outside quantitative range acceptability: The allergen is truly identified but has been quantified outside the 30% deviation of acceptance.
- False Positive Identification: The allergen is not present in the sample but has been mistakenly identified.
- False Negative Identification: The allergen is expected in the sample but has been mistakenly not detected.

Conclusions on the Orbitrap methodology: The use of a single method and a single column makes it possible to almost identify systematically 100% of the allergens present in a perfume concentrate without generating false positives and/or negatives. The apolar column is to be preferred in the first instance in view of the identification results observed. However, the application of a second method with a polar column makes it possible to significantly improve the quantitative results by reducing the differences in values by more than 30%. The impacted values are mainly allergens quantified below 200 ppm and above 20,000 ppm for which the uncertainties are higher.

Conclusions on the GCxGC-ToF methodology: The comprehensive GC analysis allows in a single analysis to obtain identifications close to 100%, as for the Orbitrap methodology. On the other hand, the precision of the quantitative values is today lower than what is obtained in Orbitrap. In the case of HRT, the sensitivity of the device and the restricted dynamic range do not make it possible to achieve performance equivalent to an Orbitrap for allergen quantitative trace analyzes. Concerning BT4D, method development should be considered to significantly improve the quantitative results currently obtained. As was observed with the Orbitrap method, matrix specificities can cause identification difficulties depending on the samples.

Novel aspect

To our knowledge, this is the first-time different analytical methodologies are compared for the analysis of fragrance allergens.

P1-087**ACCURATE AND ROBUST METHOD FOR PLANT VOLATILOME ANALYSIS BY GC-MS****Ferretti, Marysol¹; Spiezia, Giuseppe²; Lo Scalzo, Mariachiara¹; Todeschini, Valeria²; Lingua, Guido²; Conterosito, Eleonora¹; Guerrieri, Emilio³; Gianotti, Valentina¹.**¹Università del Piemonte Orientale, Vercelli, Italy; ²Università del Piemonte Orientale, Alessandria, Italy; ³Centro Nazionale delle Ricerche, URT IPSP CNR-DISIT UNIUPO, Alessandria, Italy.

The volatilome encompasses all volatile organic compounds (VOCs) emitted by an organism. Plants typically produce VOCs belonging to distinct chemical classes, including aldehydes, ketones, esters, and terpenes, which are involved in various biological and ecological functions such as intra- and interspecific communication, defense against herbivores, and pollination. Furthermore, the VOC profile contributes to the organoleptic properties of plants, influencing their commercial value in the food and agricultural markets¹. VOCs composition can also vary in response to different stimuli to which the plant is subjected, leading to the formation of new evolved molecules or very subtle concentration variations, resulting in a great variability in the collected samples². Consequently, the sample collection method and analysis must be robust and reliable, to minimize the uncertainty that affects the results. Gas-chromatography tandem mass spectrometry (GC-MS) is currently regarded as the gold standard for identifying and quantifying such molecules in this type of samples, due to their high sensitivity and selectivity³; for this reason, this work proposes a quantitative GC-MS method, optimized and validated following the AOAC Guidelines⁴ for fifteen VOCs typically present in *Solanum lycopersicum* (tomato plant). Different figures of merit have been evaluated, including linearity and alternative fitting models by means of ANOVA and F-test, limits of detection and quantification, inter- and intra-day precision. The validated method has been then applied to real tomato plant volatilome samples collected with a tailored setup with an airtight entrainment system, forced to pass through an adsorption cartridge. The analytes were then eluted through a solvent desorption before GC-MS analysis; this step has been also evaluated in terms of recovery, in order to assess the trustworthiness of the data obtained.

The method developed in this study serves as a highly reliable analytical tool for characterizing plant volatilome. By integrating optimized protocols for gas chromatography-mass spectrometry (GC-MS) with meticulous validation processes, this approach enhances our ability to investigate plant secondary metabolism and its ecological significance. These insights lay the foundation for enhancing the quality and resilience of agricultural products while deepening our understanding of plant-environment interactions.

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P1-088**ENANTIOMERIC SEPARATION OF DUVELISIB BY ELECTROKINETIC CHROMATOGRAPHY. APPLICATION TO THE QUALITY CONTROL OF PHARMACEUTICAL FORMULATIONS AND TO THE ANALYSIS OF SERUM AND WASTEWATER SAMPLES****García-Cansino, Laura; García, María Ángeles; Marina, María Luisa.***Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares (Madrid), Spain.*

A significant percentage of commercially available drugs are chiral. Chirality plays a crucial role in the pharmaceutical field, as the enantiomers of a drug can exhibit distinct biological activities [1]. The International Council on Harmonization (ICH) guidelines states that enantiomeric impurities in pharmaceuticals should be detected at or below 0.1% with respect to the active enantiomer, which highlights the need to develop highly sensitive enantioselective analytical strategies for the quality control of chiral drugs [2]. In this context, Electrokinetic Chromatography (EKC) has positioned itself as a key tool in the separation of enantiomers being cyclodextrins (CDs) the most employed chiral selectors, given their recognized stereospecific recognition capability. Duvelisib (active substance of Copiktra®), marketed as pure enantiomer (S-duvelisib), is prescribed for the treatment of chronic lymphocytic leukemia (CLL), a cancer affecting white blood cells, or small lymphocytic lymphoma (SLL), a similar cancer primarily affecting the lymph nodes. It is used in cases where the disease has returned or has not responded to at least two prior treatments [3].

The aim of this work was to develop an analytical methodology by EKC allowing the separation of duvelisib enantiomers and to apply it to: i) the quality control of a simulated pharmaceutical formulation; ii) the study of the stability of the pharmaceutical formulation submitted to a simulated gastrointestinal digestion; and iii) the determination of duvelisib enantiomers in biological (serum) and environmental (wastewater) samples after preconcentration by a semi-automated micro-solid phase extraction (μ SPEed). Under optimized conditions (sulfobutylated- β -CD (SB- β -CD) in formate buffer (pH 3.0)), the chiral separation took place in 5.1 min with a resolution of 3.0. Analytical characteristics of the developed method were evaluated in terms of linearity, trueness, and limits of detection (LODs) and quantification (LOQs). The enantiomeric impurity was detected below 0.1% with respect to the active enantiomer demonstrating the validity of the developed analytical approach to the quality control of pharmaceutical formulations. The study of the stability of the pharmaceutical formulation proved to be stable for 4 h. Finally, the method was applied to the analysis of duvelisib enantiomers in serum and wastewater samples after μ SPEed microextraction. For this purpose, different microextraction cartridges were tested (octyl silica (C8), octadecyl silica (C18-RPS), octadecyl silica hydrophilic ODS (C18-ODS), porous PS/DVB normal phase (PS/DVB), porous PS/DVB reversed phase (PS/DVB-RP)), with PS/DVB-RP offering the highest recovery percentages (86 $\pm$ 8% for R-duvelisib and 85 $\pm$ 7% for S-duvelisib). The method was then optimized considering the volume of MeOH as elution solvent (100, 150, 200 or 250 μ L), and the preconcentration ratio between sample volume and volume of a solution of SB- β -CD in formate buffer (pH 3.0) used as reconstitution solvent of the extract (5:1, 10:1 and 15:1) for maximum extraction efficiency (100 $\pm$ 10% for R-duvelisib and 99 $\pm$ 10% for S-duvelisib) and applied to the determination of duvelisib enantiomers in serum and wastewater samples.

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P1-089

COMPARISON OF DEGRADATION KINETICS AND PRODUCTS OF NOVEL DRUGS: ETRASIMOD AND RITLECITINIB

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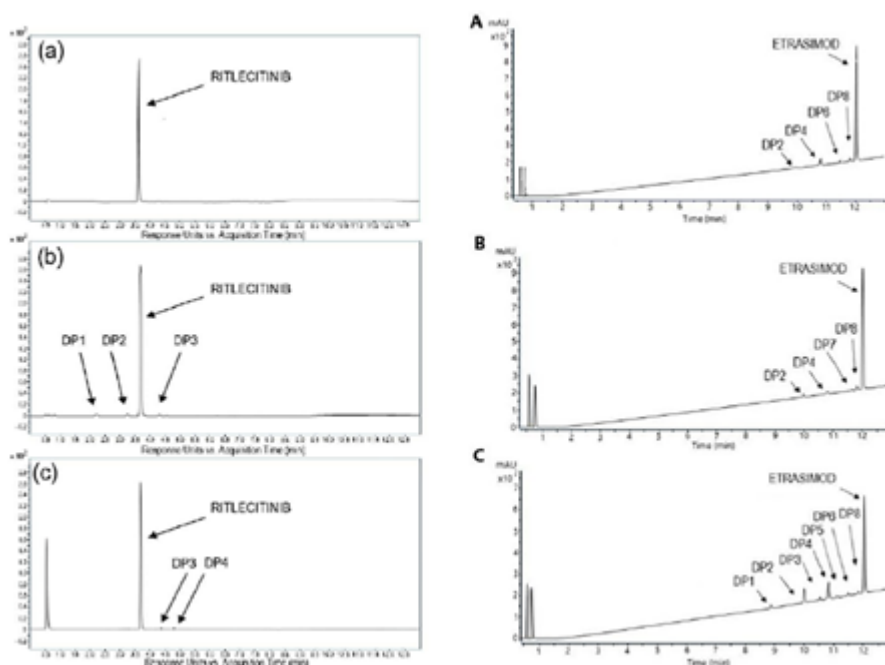
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Etrasimod and ritlecitinib are novel therapeutic agents with distinct mechanisms of action used to treat inflammatory and autoimmune diseases. Etrasimod, a selective sphingosine-1-phosphate receptor modulator, is effective for ulcerative colitis, while ritlecitinib, a Janus kinase 3 inhibitor, is utilized for conditions such as alopecia areata and inflammatory bowel diseases.

Both studies highlighted the application of UHPLC-DAD-MS/MS techniques for precise quantification and identification of degradation products and stability analysis. Etrasimod's stability was evaluated using green analytical chemistry principles, ensuring eco-friendly methods. Similarly, ritlecitinib's analysis incorporated green and blue metrics, aligning with sustainable practices.

Etrasimod degradation followed first-order kinetics under acidic, photolytic, and oxidative stress, showing the fastest degradation in acidic environments (rate constant: $k=0.022\text{ h}^{-1}$). Conversely, ritlecitinib exhibited second-order kinetics in basic conditions (rate constant: $k=0.000016\text{ h}^{-1}$) and zero-order kinetics under oxidative stress (rate constant: $k=0.181\text{ h}^{-1}$), indicating dependence on reactant concentration and stressor type. Ritlecitinib was stable in acidic, thermal, and photolytic conditions, while etrasimod displayed moderate stability in basic and thermal stress environments. Etrasimod yielded eight degradation products under acidic, oxidative, and photolytic stress, with DP1, DP3, and DP5 forming uniquely under photolytic conditions (Figure 1.). Key products shared across stressors included DP2, DP4, and DP8. In contrast, ritlecitinib generated four degradation products under basic (DP1, DP2, DP3) and oxidative (DP3, DP4) conditions (Figure 2.), with no degradation under thermal, acidic, or photolytic stress. Both drugs demonstrated distinct degradation pathways, underscoring their unique chemical stability profiles.

The comparative analysis of etrasimod and ritlecitinib revealed unique stability profiles and degradation pathways under varying conditions. These insights are essential for optimizing formulations and storage protocols and ensuring drug quality. These findings are pivotal for advancing the therapeutic application of these agents while maintaining environmental sustainability.



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P1-090**CHROMATOGRAPHIC RETENTION AND ENANTIORESOLUTION OF CHIRAL COMPOUNDS USING CELLULOSE-1 AND CELLULOSE-5 CHIRAL STATIONARY PHASES AND AQUEOUS ACETONITRILE MOBILE PHASES****Martín-Biosca, Yolanda¹; Llácer-Martí, Isabel¹; Pardo-Cortina, Carlos¹; Medina-Hernández, María José¹; Escuder-Gilabert, Laura¹; Sagrado, Salvador².**¹*Dep. Química Analítica, Universitat de València, Valencia, Spain;* ²*Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico, Valencia, Spain.*

Molecular chirality has had a growing impact on science over time, with pivotal implications for life sciences, agriculture, food and environmental sciences, and several other fields. In this respect, chiral separation techniques are paramount, with high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) being the preferred choice for many researchers. The rationale behind enantioseparations in HPLC using CSPs lies in the formation of diastereomeric transient complexes between enantiomers of the chiral analyte and a chiral organic molecule (so-called chiral selector, CS) present in the CSP, through several types of enantioselective noncovalent interactions.

A large number of CSPs with a wide variety of CSs have been developed, the most widely used (~90% of reported applications) being polysaccharide-based CSPs containing amylose or cellulose benzoate or phenylcarbamate derivatives as CS, primarily those with -CH₃ and/or -Cl substituents at different positions of the phenyl group. The nature and position of these substituents, as well as the polysaccharide backbone, are key factors in the enantioselectivity of these type of CSPs.

The extensive use of polysaccharide-based CSPs is attributed to their exceptional enantiorecognition capacity, enabling broad applicability to structurally diverse organic molecules. This is due to their ability to establish multiple enantioselective noncovalent interactions (i.e., steric and van der Waals forces, hydrogen bonding with the carbamate group, π - π stacking with phenyl groups, and electronic interactions with halogen atoms). Another key advantage of these CSPs is their compatibility with all partition HPLC modes (i.e., normal-phase, reversed-phase (RPLC), hydrophilic interaction liquid chromatography (HILIC) and polar organic liquid chromatography). The nature and composition of the mobile phase modulates the chiral recognition process in these CSPs and must be carefully selected to achieve a given enantioseparation, in conjunction with the nature of the CSP.

In this communication, the effect of the nature of substituents in phenylcarbamate-type cellulose-based CSPs on the retention and enantioresolution of chiral compounds is investigated. The CSPs studied are Lux Cellulose-1 (Cell1, cellulose tris(3,5-dimethylphenylcarbamate) and immobilised Lux Cellulose-5 (Cell5, cellulose tris(3,5-dichlorophenylcarbamate), with two -CH₃ and -Cl substituents in the 3,5-position of the phenyl moiety, respectively. Both columns have identical dimensions (3 μ m, 150 $\times$ 4.6 mm i.d.) and are from the same supplier (Phenomenex, Torrance, CA, USA). Nine mobile phases consisting of 5 mM NH₄HCO₃ (pH=8.0) and acetonitrile (ACN) in varying proportions (30-98% (v/v) ACN, covering RPLC and HILIC modes) are used. Chromatographic parameters obtained for 77 neutral and basic structurally unrelated chiral compounds (with only a single asymmetric centre) belonging to 19 families of drugs and pesticides are compared.

Acknowledgments

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P1-091**EXPLORING CHROMATOGRAPHIC RETENTION AND ENANTIORESOLUTION OF CHIRAL COMPOUNDS USING CELLULOSE-2 AND CELLULOSE-4 STATIONARY PHASES IN LIQUID CHROMATOGRAPHY****Martín-Biosca, Yolanda¹; Alcaraz-Sáez, Simón¹; Pardo-Cortina, Carlos¹; Medina-Hernández, María José¹; Escuder-Gilabert, Laura¹; Sagrado, Salvador².**¹Dep. Química Analítica, Universitat de València, Valencia, Spain; ²Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Valencia, Spain.

High performance liquid chromatography (HPLC) is the most widely used chromatographic technique for the separation of enantiomers. In this methodology, a chiral selector can be either adsorbed or immobilized on the stationary phase, forming what are known as chiral stationary phases (CSPs). CSPs can be roughly classified into chiral small organic molecule-based CSPs and macromolecule-based CSPs. Nowadays, approximately 90 % of the chiral separations are performed by HPLC using polymer-based CSPs, with those based on natural polysaccharide derivatives (phenylcarbamate or benzoate derivatives of cellulose and amylose) being the most widely used.

Cellulose and amylose are linear helical polymers consisting of D-glucose molecules with β -1,4 or α -1,4 glycosidic linkages, respectively. Their hydroxy groups are usually derivatised with benzoate or phenylcarbamate moieties with $-\text{CH}_3$ and/or $-\text{Cl}$ substituents on the aromatic ring. These CSPs contain chiral helical grooves and cavities, whose shape and size depend on the polysaccharide backbone and the nature of the substituents, fostering a large number of interactions (hydrophobic, electrostatic, hydrogen bonding, van der Waals forces and steric factors) involved in chiral separation. All these possibilities yield a large variety of derivatives with different selectivities and applications to many structurally diverse compounds. Among these derivatives, some CSPs differ only in the position of substituents on the aromatic ring. For instance, tris(3-chloro-4-methylphenylcarbamate) cellulose (Cell2) and tris(4-chloro-3-methylphenylcarbamate) cellulose (Cell4) differ solely in the arrangement of the $-\text{CH}_3$ and $-\text{Cl}$ substituents on the phenylcarbamate groups. This variation, though seemingly minor, could have a substantial impact on enantioselectivity.

Additionally, polysaccharide-based CSPs are highly versatile, compatible with various liquid chromatography modes: normal phase, reversed-phase (RPLC), hydrophilic interaction (HILIC), and polar organic chromatography. The mobile phase's nature and composition influence enantiorecognition by altering the polysaccharide's intramolecular hydrogen bonds. When using hydroorganic mobile phases with aprotic solvents like acetonitrile (ACN), CSPs can exhibit dual HILIC and RPLC behavior based on the water content. High water content enhances hydrophobic interactions, increasing retention (RPLC behavior), while low water content promotes hydrophilic interactions, decreasing retention (HILIC behavior). In protic solvents like methanol (MeOH), only RPLC behavior is observed, as MeOH competes for hydrophilic interactions, enhancing hydrophobic analyte-CSP interactions.

In this communication, a comparative study on the retention and enantiorecognition ability of chromatographic systems comprising Cell2 and Cell4 CSPs and aqueous acetonitrile (ACN) mobile phases is performed. CSPs used are from Phenomenex (Madrid, Spain) and have identical dimensions (3 μm , 150 $\times$ 4.6 mm i.d.). Mobile phases consist of mixtures of 5 mM NH_4HCO_3 (pH = 8.0) and ACN in varying proportions (30-98 % ACN, v/v). Chromatographic parameters obtained for 77 structurally unrelated chiral compounds (with only a single stereogenic centre) belonging to 19 families of drugs and pesticides are compared. The primary objectives were to investigate the potential RPLC/HILIC behavior of the chromatographic systems under study and to evaluate the impact of the position of the $-\text{CH}_3$ and $-\text{Cl}$ substituents on the phenylcarbamate groups on both retention and enantioresolution.

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P1-092**EXPLORATION OF RETENTION MECHANISMS OF POROUS GRAPHITIC CARBON AS A STATIONARY PHASE IN HPLC**

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For many years, high-performance liquid chromatography (HPLC) has primarily utilized silica-based particles for the separation of both small and large molecules. Since the inception of HPLC, these silica materials have offered the necessary mechanical stability, batch-to-batch reproducibility, and the versatility to modify the properties of the stationary phase using various silylation reagents. While alternative HPLC packing materials, including carbon, alumina, zirconia, and titanium dioxide, are available, they have not achieved the same level of popularity as silica-based materials. This lack of adoption is likely due to the different and often poorly understood retention mechanisms associated with these alternatives and the challenges in controlling them.

Porous graphitic carbon (PGC) has been recognized as a possible stationary phase in HPLC for many years [1,2]. The benefits of using PGC over traditional silica-based stationary phases include its stability across the entire pH range (1-14) and its ability to withstand temperatures of up to 250 °C. Initially, it was believed that this fully carbonaceous stationary phase functioned solely as a highly hydrophobic material; however, experiments with polar compounds have challenged this assumption. PGC often shows significantly better retention of polar compounds compared to C18 materials, leading to the introduction of the term PREG (Polar Retention Effect on Graphite). Knox and Ross, who were instrumental in the development and chromatographic characterization of PGC, sought to elucidate this unexpected polar retention behavior [3].

This study will focus on a novel PGC material with a smaller particle size (2.7 µm), a narrower particle size distribution, and enhanced mechanical stability. The research will investigate how the stationary phase interacts with various nonpolar and polar analytes of differing sizes and geometries. In particular, polar compounds known to exhibit weak retention on silica-based reversed-phase columns will be selected for analysis. Insights drawn from the resulting chromatograms will help clarify the fundamental retention mechanisms at play on this carbon stationary phase. A comparison with silica-based columns will further illuminate these mechanisms. Additionally, this work will include application examples that demonstrate how PGC columns provide additional selectivity options, facilitating easier HPLC method development alongside classical silica-based columns.

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P1-093**HPLC PERFORMANCE OF WIDE-BORE MONOLITHIC COLUMNS BASED ON PENTAERYTHRITOL TETRAACRYLATE AND POLYHEDRAL OLIGOMERIC SILSESQUIOXANE-METHACRYLATE****Moravcová, Dana; Planeta, Josef; Karásek, Pavel; Roth, Michal.***Institute of Analytical Chemistry of the CAS, Brno, Czech Republic.*

Highly crosslinked monolithic capillary columns with an inner diameter of 450 μm have been developed through radical polymerization of pentaerythritol tetraacrylate, polyhedral oligomeric silsesquioxane-methacrylate, and either n-octadecyl methacrylate or sulfobetaine methacrylate. The polymerization was conducted in the presence of methanol, dodecyl alcohol, and polyethylene glycol lauryl ether. The performance of these monolithic columns was evaluated using scanning electron microscopy, inverse-size exclusion chromatography, and isocratic high-performance liquid chromatography separation of various model compounds.

The prepared columns exhibit efficiency surpassing 100,000 theoretical plates per meter and demonstrate high selectivity, irrespective of whether hydrophobic (n-octadecyl methacrylate) or hydrophilic (sulfobetaine methacrylate) monomers were utilized in their fabrication. Their versatility makes them ideal for multimodal chromatography, effectively separating a diverse array of compounds across a wide range of polarities, including alkylbenzenes, phenolics, aromatic carboxylic acids, as well as mixtures of purine and pyrimidine bases, nucleosides, and 2-deoxynucleosides. The developed preparation protocol results in columns of remarkable stability, achieving a minimum theoretical plate height of just 6–10 μm . These columns maintain their peak performance with efficiency diminishing by less than 12% over a rigorous two-month period of daily operation.

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P1-094**FAST REACTION YIELD MONITORING BY CE ANALYSIS OF DICARBOXYLIC ACIDS IN KEROGEN VALORISATION PROCESS****Muldma, Kati¹; Umernkova, Violetta¹; Närep, Angelica¹; Kontson, Tiina²; Kaldas, Kristiina¹.**¹Tallinn University of Technology, Tallinn, Estonia; ²Estonian Business and Innovation Agency, Tallinn, Estonia.

Valorisation of kukersite - mineral resource that is currently mainly used for oil and electricity production, is a highly attractive field which has been investigated for several years [1], [2], [3]. In this work kerogen – the organic part of kukersite, is oxidized into different chain-length dicarboxylic acids (DCA) and organic acids with nitric acid. Dicarboxylic acids applications are wide and can be used in the production of plasticizers, adhesives, lubricants and even acne treatment medication. A continuous flow reactor equipped with a built-in sampling option for the oxidation of kukersite was used. This kilogram-scale laboratory pilot unit was the winner of TalTech's development project of the year 2024. It is important to acquire information about the on-going reaction to ensure that a steady state of the reaction has been achieved. The samples were analysed using an Agilent 7100 capillary electrophoresis (CE) system (Germany) equipped with a UV diode array detector to determine the yields of DCA-s. The analytes of interest were dicarboxylic acids with a chain length of 4 to 10 carbons. CE is an excellent tool for this work as it allows the quantification of DCA-s in less than 10 minutes directly from the sample matrix. Furthermore, with a slightly modified background electrolyte (BGE) it was possible to monitor the remaining nitrate content which allowed to calculate the nitric acid used in the reaction.

An optimised CE method from previous work was used [4]. Simultaneous analysis of formic acid, acetic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid and sebacic acid as their anions was achieved. The separation of formic acid and acetic acid was important as they were present in the samples and their migration times were similar to succinic and suberic acid. The optimal background electrolyte for the analysis of DCA-s consisted of 15mM maleic acid as the UV absorbing agent, 0.002% cetyltrimethylammonium bromide (CTAB) for the capillary wall modifier at pH of 11.8. For the determination of nitrate and sulfate (degradation product of kukersite) the maleic acid concentration was changed to 20mM to ensure baseline separation. The analytes were detected with indirect UV detection at 380nm (reference 210nm), and the analysis was carried out using fused-silica capillary with an internal diameter of 50 µm and outer diameter of 350 µm with a total length of 70cm (effective length 61.5cm). The sample was injected hydrodynamically by applying a pressure of 50mbar for 8 seconds and the separations were performed at 25°C at a voltage of -25kV. The sample preparation consisted only of neutralization to ensure that all the dicarboxylic acids were solubilized and centrifugation if needed to separate the insoluble part.

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P1-095**FROM RPLC TO SURFACTANT-MODIFIED LIQUID CHROMATOGRAPHY: A UNIFIED MODEL FOR RETENTION ACROSS SEPARATION MODES****Ruiz Angel, Maria Jose; Tereba Mamani, Carlos Josue; Baeza Baeza, Juan Jose; Garcia Alvarez-Coque, Maria Celia.***Universitat de València, Burjassot, Spain.*

Reversed-phase liquid chromatography (RPLC) is the most widely used liquid chromatographic technique due to its high performance, broad applicability to various analytes, and straightforward retention mechanism, which is based on the interaction between solutes and the apolar stationary phase, primarily governed by solute polarity [1]. However, solutes with extreme polarities can exhibit excessively high or low retention, leading to poor selectivity or reduced efficiency. To overcome these limitations, additives are incorporated into the mobile phase to modify the interaction between solutes and the stationary phase, effectively modulating retention and selectivity. Surfactants are particularly useful in this regard, as they introduce diverse retention mechanisms that can enhance resolution [2,3].

In alkyl-bonded columns, surfactant monomers strongly adsorb onto the stationary phase via their hydrocarbon chains, orienting their polar headgroups toward the mobile phase. With ionic surfactants, this results in the formation of an asymmetric bilayer that behaves as an ion-exchanger for oppositely charged ionic solutes, thereby increasing their retention. In contrast, solutes with the same charge as the surfactant are repelled, eluting at the void volume.

Chromatographic behavior is influenced by surfactant concentration. Below the critical micelle concentration (CMC), the system operates under submicellar conditions, where micelles are absent, and the column's maximum adsorption capacity is not fully utilized. These conditions are typically associated with ion-pair chromatography. When the surfactant concentration exceeds the CMC, micelles form, and the stationary phase becomes saturated, leading to another chromatographic mode, known as micellar liquid chromatography (MLC). In MLC, solutes interact with both micelles and the modified stationary phase, significantly impacting selectivity, analysis time, and efficiency. However, micellar solutions tend to have weak elution strength and broad chromatographic peaks, requiring the addition of a small amount of organic solvent. The presence of organic solvent alters the CMC, reduces surfactant adsorption, and requires careful concentration control, as exceeding a critical threshold can disrupt micelles. When micelles break down, ion-pair interactions between solutes and surfactant monomers dominate, resulting in high submicellar liquid chromatography. This mode offers improved analysis time, selectivity, and peak shape [4].

The primary goal of modifying both the stationary and mobile phases with surfactants is to enhance selectivity. However, surfactant-mediated chromatographic modes involve multiple variables, including organic solvent content, surfactant concentration, and pH, making the identification of optimal separation conditions more challenging. Therefore, reliable mathematical models are essential to predict retention behavior and optimize chromatographic performance. Developing a unified retention model for submicellar and micellar conditions is particularly challenging, especially when considering the gradual transitions between different separation regions: conventional RPLC, low submicellar, micellar and high submicellar. This study aims to develop a mathematical model based on Langmuir and Sips isotherms to predict retention times across a wide range of concentrations of the anionic surfactant sodium dodecyl sulfate (SDS), in the presence of acetonitrile. The model is designed to predict retention in conventional RPLC, low submicellar, micellar and high submicellar modes, as well as the transitions between these modes. A set of compounds with diverse chemical properties, including basic, weakly acidic, and amphoteric substances, is used to train and assess the applicability of the models.

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P1-096**VERSATILE APPLICATION OF A NOVEL SUPRAMOLECULAR DEEP EUTECTIC SOLVENT AS A CHIRAL SELECTOR IN CAPILLARY ELECTROPHORESIS FOR THE ENANTIOSEPARATION OF PSYCHOACTIVE SUBSTANCES****Stavrou, Ioannis J.¹; Ioannou, Katerina A.²; Kapnissi-Christodoulou, Constantina².**¹European University Cyprus, Nicosia, Cyprus; ²University of Cyprus, Nicosia, Cyprus.

The development of efficient, sustainable, and selective chiral selectors remains a central challenge in analytical separation science. In this work, we report the preparation and application of a novel supramolecular deep eutectic solvent (SUPRADES) composed of sulfated- β -cyclodextrin (S- β -CD) and citric acid (CA), evaluated as a sole chiral selector (CS) in capillary electrophoresis (CE). This innovative CS was systematically studied for its ability to enantioseparate fluorine-substituted amphetamine analogs and synthetic cathinone derivatives.

The SUPRADES system demonstrated remarkable chiral recognition capabilities across all tested classes of compounds. In the enantioseparation of six fluorinated amphetamine analogs, baseline separation was achieved for all analytes in less than 21 minutes. Optimization studies revealed that a 0.050% v/v concentration of S- β -CD-CA in the background electrolyte (BGE) was sufficient to achieve baseline separation in five out of six compounds, while 4-fluoroamphetamine (4-FA) required a slightly higher concentration of 0.15% v/v.

Similarly, for the separation of the five cathinone derivatives (3-MMC, 4-MMC, 3,4-DMMC, 4-MEC, and MDMC), the SUPRADES system enabled baseline separation of all enantiomeric pairs. The optimal concentration of S- β -CD-CA varied depending on the analyte, ranging from 0.050% to 0.10% v/v, with resolution values (R_s) between 1.5 and 2.4, demonstrating the tunable nature of the chiral environment created by the SUPRADES.

These results demonstrate the efficiency of the SUPRADES in separating chiral compounds and particular amphetamine and cathinone derivatives. Its simple preparation procedure, enantioselectivity, and environmentally friendly composition position it as a promising alternative to conventional chiral selectors in CE-based analyses.

P1-097

MODELLING RETENTION IN LIQUID CHROMATOGRAPHY: EVALUATING SOLUTE-SOLVENT INTERACTIONS IN REVERSED-PHASE AND HILIC BY MEANS OF ABRAHAM'S SOLVATION PARAMETER MODEL

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Manufacturers of liquid chromatography columns offer a diverse range of supports, including fully or superficially porous silica particles, monolithic, and polymeric options, as well as various tube and particle sizes and bonded phases. This variety significantly enhances separation opportunities but also poses challenges for rapid and efficient method development. In this context, tools for characterizing chromatographic systems, combined with user expertise, are crucial.

In this study, we propose a rapid method based on Abraham's solvation parameter model to characterize analyte behavior in chromatographic systems, with a particular focus on HILIC due to the extensive catalog of available bonded phases. Abraham's model elucidates the contributions of different solute-solvent interactions to chromatographic retention, as described by the following general equation for neutral (unionized) compounds [1]:

$$\log k_i = c + e \cdot E_i + s \cdot S_i + a \cdot A_i + b \cdot B_i + v \cdot V_i$$

where k is the chromatographic retention factor, $e \cdot E_i$ and $s \cdot S_i$ account for polarizability and dipolarity interactions, $a \cdot A_i$ and $b \cdot B_i$ for hydrogen bonding, and $v \cdot V_i$ for the effect of the solute volume. The uppercase letters of these terms represent the molecular descriptors of the i -th solute, while the lowercase letters are the coefficients characterizing the selectivity of the chromatographic system. c is a constant term, independent of the solute properties, primarily related to the stationary/mobile phase ratio and normalization of descriptors.

In the proposed methodology, the hold-up volume and the v coefficient are determined from the injection of a few n -alkyl ketone homologues, while e , s , a and b are rapidly measured from the chromatographic selectivity factors of carefully selected pairs of non-ionized (neutral) compounds [2]. Using this approach, we characterized the solute-solvent interactions governing retention in HPLC systems (cavity formation, hydrogen bonding and dipolarity/polarizability interactions) for a reversed-phase (XBridge C18) and several HILIC columns (ZIC-HILIC, ZIC-cHILIC, Chromolith Performance Si, Chromolith Diol, LiChrospher 100 Diol, Acentis Express OH5, and Purospher STAR NH2), in mobile phases containing acetonitrile and methanol as organic modifiers. Ethanol, due to their lower toxicity to human health and the environment, was also considered as a potential substitute for acetonitrile.

The obtained results demonstrate the complementarity between reversed-phase and HILIC. In the latter separation mode, a decrease in chromatographic retention has been observed with an increase in the molecular volume of the solute, attributed to the greater cohesion of the aqueous layers acting as the stationary phase relative to the mobile phase with a high content of organic solvent. Conversely, hydrogen bonding interactions favor partitioning with the stationary phase, particularly the hydrogen bond basicity of the solute when the eluent contains acetonitrile.

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P1-098

AN ENHANCED HIGH-SENSITIVITY AND LARGE-SCALE METABOLOMICS PLATFORM FOR CHARACTERIZATION OF HUMAN EXPOSOME

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Background and objectives: The exposome, defined as the cumulative measure of environmental or external exposures and their biological responses across the lifespan, has become a crucial research focus in biomedical sciences. Metabolomics plays a fundamental role in characterizing the intricate interplay between genetics, exogenous exposures, and endogenous metabolism. However, the complexity of the exposome demands continuous advancements in analytical methodologies.

Materials and methods: In this study, we present a significantly improved high-throughput metabolomics platform for exposome research, validated for the simultaneous quantification of 1100 metabolites in plasma and urine. Plasma sample preparation includes protein precipitation in a Sirocco Protein Precipitation plate while for urine, a simple dilution provided optimal performance in terms of metabolite coverage. This enhanced method integrates an upgraded mass spectrometry system (transitioning from the 6500 QTRAP to the 7500 QTRAP), with separate positive and negative ionization runs. It employs scheduled multiple reaction monitoring (sMRM) mode in combination with isotopically labelled internal standards.

Results: The changes in the mass spectrometry system result in enhanced sensitivity, improved linearity (0.01–10,000 ng/L), and increased metabolite coverage. The expanded metabolite panel includes new metabolite classes, such as tryptophan pathway metabolites, isothiocyanates, kynurenines, glucosinolates, flavonoids, indole derivatives, and resorcinol derivatives, broadening the scope of exposome characterization. Key improvements include a substantial improvement in sensitivity, with 86% of metabolites now detectable below 50 ng/mL, compared to 75% in the previous method. Furthermore, limits of quantification (LOQs) for critical bioactive compounds, such as valerolactones and flavonoids, have been significantly lowered, facilitating more precise biomonitoring of dietary and environmental exposures. The platform employs green analytical strategies, minimizing solvent consumption and ensuring sustainable high throughput metabolomic fingerprinting. Finally, this metabolomics approach was satisfactorily validated in terms of linearity, recovery, matrix effects, specificity, limits of quantification, intraday and interday precision, and carryover

Conclusions: This updated methodology and the inclusion of these key metabolites allows a deeper understanding of the interactions between diet, environmental exposures, and human health. It also offers an advanced tool for large-scale exposome investigations, epidemiological studies, and clinical research, reinforcing the role of metabolomics in understanding the impact of environmental factors on human health with better

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P1-099**IN-TRAP HEADSPACE LIQUID-PHASE MICROEXTRACTION FOR HIGHLY SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF BROMIDE IN WATER SAMPLES****Vishnikin, Andriy; Tamen, Aimad-Eddine; Bazel, Yaroslav.***Department of Analytical Chemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovakia.*

A new dynamic variant of headspace liquid-phase microextraction (HS-LPME) termed in-trap HS-LPME (IT-HS-LPME) has been proposed. The donor phase and microvolume of the extraction phase are placed in two different hermetically sealed vessels connected by a tube. To intensify the mass transfer of volatile compounds, air or an inert gas is passed through both phases in series. Intensification of the extraction process of volatile substances by gas flow allows to significantly, up to 1-2 orders of magnitude, increase the efficiency of HS-LPME and simultaneously reduce the extraction time compared to the passive variant of extraction. This method does not have problems typical of the existing HS-LPME approaches, such as instability of microdrop holding and too small volume of the extraction phase. The proposed methodology allows to improve the sensitivity of the analysis, as a high ratio of donor and acceptor phases can be achieved. Purging the analyte by gas flow allows to significantly increase the extraction efficiency even from large sample volumes, which was previously difficult. The potential of the method was evaluated by determining the bromide content of water samples. Bromide in the donor phase was converted to volatile Br_2 by oxidation with $25 \text{ mmol L}^{-1} \text{ KBrO}_3$ in $0.3 \text{ M H}_2\text{SO}_4$. Bromine was displaced from the sample solution by air flow and absorbed by phenol red solution. After conversion of phenol red to bromphenol blue, the absorbance of the acceptor phase was measured at 590 nm . For the case of extraction from 80 mL of donor solution into $250 \mu\text{L}$ of acceptor phase and an extraction time of 15 min , the calibration graph was linear in the range of 10 to $1000 \mu\text{g L}^{-1}$ (as Br^-) with a detection limit of $3 \mu\text{g L}^{-1}$. The developed method was successfully applied to the determination of bromide content in natural waters.

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P1-100**INTACT PROTEIN ANALYSIS OF SNAKE VENOMS WITH CZE-MS**

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Venoms consist of several biologically active components, primarily peptides and proteins. these toxin components have the potential to cause lethal effects [1]. Top-down mass spectrometric technique when combined with capillary zone electrophoresis, becomes highly effective in studying the structural and dynamic characteristics of intact proteins. This methodology can be utilized for the analysis of complex protein molecules such as snake venom [2].

In this research work we demonstrated the potential analytical performance of capillary zone electrophoresis coupled with mass spectrometry (CZE-MS) for the intact protein analysis of similar venom samples. Using 1 M formic acid (pH=1.9) as BGE, minimal adsorption and narrow peaks shapes - thus good separation efficiencies - were obtained for the protein components of the venom samples. The precision of migration times and peak areas were 1.9-2.8 RSD% and 0.8-7.2 RSD%, respectively and the theoretical plate numbers were 32000-238000 for peaks having signal-to-noise ratio (S/N) larger than 50.

More than 250 different toxin components (7-10 kDa) were detected in the venoms obtained from snakes of 9 different subspecies (belonging either to *Naja* or *Dendroaspis* species). The protein contents of the venoms of the same subspecies collected from different geographical regions are similar and differ only in a few (less than 10%) components. However, the venoms collected from different organism (within the same species) exhibit very different protein patterns.

Notably, our findings revealed discrete protein patterns among venoms from different subspecies, highlighting the unique fingerprinting potential of venom in various snake populations.

Key words: snake venom, intact protein analysis, capillary electrophoresis-mass spectrometry.

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P1-101**COUPLING SUPERCRITICAL FLUID CHROMATOGRAPHY WITH ION MOBILITY SPECTROMETRY: A MINIATURIZED CHIP-BASED PLATFORM FOR RAPID ANALYSIS****Welters, Klaus¹; Schwieger, Julius¹; Thoben, Christian²; Nitschke, Alexander²; Zimmermann, Stefan²; Belder, Detlev¹.**¹Leipzig University, Leipzig, Germany; ²Hannover University, Hannover, Germany.

The recent widespread adoption of artificial intelligence, machine learning, and data science methods has made a significant impact on analytical chemistry and its typical workflows, thereby rendering the measurement step and the subsequent data generation the rate-determining steps. Aiming to match the speeds afforded by modern data processing, numerous efforts at reducing analysis times by chromatography as the most-common separation method have been made.

In this endeavor, supercritical fluid chromatography (SFC) offers the potential for higher separation speeds at lower pressures in comparison to HPLC, owing to its use of supercritical carbon dioxide (scCO₂) and the associated high diffusion coefficient and low viscosity.[1] The addition of polar modifiers affords compatibility with both normal-phase and reverse-phase methods, enabling access to a broad range of analytes. However, in addition to speed, other aspects such as affordability, robustness, environmental friendliness and portability have also gained in importance, not only for the separation system but also for the detection technique.[2] Ion mobility spectrometry (IMS) represents a cost-effective and robust alternative to established techniques, offering a second separation dimension through characteristic ion mobilities that enhance compound identification.

While early efforts to couple SFC with IMS in the 1980s and 1990s faced limitations due to underdeveloped instrumentation, recent advancements in SFC technology, particularly miniaturization,[3] have sparked a renewed interest in this pairing. Building on prior work with chip-based SFC platforms, we present a novel miniaturized chipSFC-IMS system. Leveraging eluent decompression and a custom high-resolution IMS with fully shiftable electric potentials, the integration of chipSFC with IMS enables high-speed separations of complex samples, drastically reducing analysis time while utilizing a detector capable of delivering structural information at a rapid acquisition rate and low cost. The chipSFC-IMS platform demonstrated exceptional reproducibility and sensitivity. Short columns and high flow rates resulted in record-speed SFC-IMS analysis of enantiomers in just six seconds as well as the successful analysis of a mixture containing five structural isomers. This innovative platform demonstrates the potential to advance SFC-IMS hyphenations and broaden their applicability in modern analytical chemistry.[4]

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P1-102**RECENT ADVANCES IN THE ADSORPTIVE RECOVERY OF RARE EARTH ELEMENTS****Zaitsev, Volodymyr; Artiushenko, Olena.***Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro, Brazil.*

The demand for Rare Earth elements (REEs) has rapidly grown since they are used in high-tech and sustainable technologies such as high-performance magnets, rechargeable batteries, and low-energy fluorescent lamps. Due to the latest political tensions and low substitutability of REEs mining technology in China, which is the leading supplier of REEs, the REEs world market is at risk. Most REEs occur together in mineral deposits, often widely dispersed and at exceptionally low concentrations. Their ore mining requires high energy, generally derived from conventional sources, and generates massive quantities of waste. For example, mining 1 ton of rare earth oxide mixture in China generates 60,000 m³ of waste gases, 200 m³ of acidified water, and 1.4 tons of radioactive waste since most REEs deposits contain uranium or thorium [1]. One of the crucial concepts of industrial strategy to reduce supply risks related to critical raw materials is to increase their recycling [2]. Only about 1% of REEs used in different applications are recycled today. However, it was demonstrated that the production of Nd by recycling computer hard drives is 80% less toxic than mining and consumes 60% less energy [3]. It is suggested that an environmentally sustainable technology of REEs recycling will substitute or at least supplement the ore treatment [4]. Among others, end-of-life electronic equipment such as mobile phones, fluorescent lamps, and computer hard drives (e-waste) can be a promising source for REE recovery. A critical parameter for adsorptive recovery of REEs from e-waste is selectivity, which can be provided by functionalization of the adsorbent surface. Due to the different chemical compositions, technologies of ore treatment are unsuitable for the recuperation of REEs from e-waste. Instead, methods of extraction and adsorption can be more appropriate. The adsorption technique offers several significant advantages, such as less waste, reduced organic solvent usage, high enrichment factor, and rapid phase separation. Because of the new demand for better recycling of REEs using less harmful technology, a significant increase in the number of publications dedicated to REEs adsorption is observed. The scope of this presentation is to provide insight into current achievements in the adsorptive recovery of REEs from solutions with a high content of transition and heavy metals. The presentation covers recent advances in the synthesis and application of metal-chelating, ion-imprinted, and porous polymers; silica-based adsorbents with covalently and non-covalently attached ligands; magnetic and green adsorbents; metal-organic frameworks; and carbon-based nanomaterials for REEs adsorption. Special attention was paid to emerging materials, such as green and hybrid adsorbents containing carbon nanoparticles. The adsorbents' efficiency, selectivity, and peculiarities of their application are revealed by critical analysis of the essential conditions of the rare earth elements recovery experiment. The advances and drawbacks of each class of the adsorbent presented were highlighted in the review, as well as modern tendencies in developing adsorbents

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P1-103**ANALYSIS AND CHARACTERIZATION OF ALGAL PEPTIDES: A CHROMATOGRAPHY- AND MASS SPECTROMETRY-BASED APPROACH**

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In Chile, chronic non-communicable diseases (NCDs) are the leading cause of mortality, representing a significant public health challenge. These diseases include hypertension, type 2 diabetes, and cardiovascular diseases. Despite the development of pharmaceutical treatments, the search for new therapeutic solutions remains a top priority. In this context, microalgae have emerged as a promising source of bioactive compounds that could offer innovative approaches for treating or preventing these chronic conditions.

Scenedesmus obliquus is a microalga rich in proteins, fatty acids, carotenoids, etc. However, despite its promising composition, research into its therapeutic applications is still limited. The objective of this study was to isolate and characterize bioactive peptides from *S. obliquus* that can inhibit two key enzymes involved in the development of risk factors for NCDs: the Angiotensin-Converting Enzyme (ACE), which is associated with hypertension, and Dipeptidyl Peptidase-IV (DPP-IV), which plays a role in prevention of hyperglycemia.

Protein extraction was conducted using phosphate buffers at pH levels from 2 to 12, and the protein content was determined using the Bradford method. To generate peptides pepsin, trypsin, papain, and alcalase, with digestion times ranging from 2 to 8 hours and enzyme-to-protein ratios of 1:10, 1:20, and 1:50 being tested. The efficiency of digestion was evaluated through TNBS assay, SDS-PAGE, and high-resolution planar chromatography (HPTLC).

The study utilized a bioactivity-guided fractionation approach, where successive fractionations of *S. obliquus* extracts were performed to isolate fractions that exhibited inhibitory activity against the selected enzymes. After each fractionation step, enzymatic assays were conducted to evaluate the inhibitory activity, which allowed the selection of the most promising fractions. To quantify the inhibitory capacity of the peptides on ACE, High-Performance Liquid Chromatography (HPLC) was employed. This advanced technique allowed for precise measurement of enzyme inhibition. Additionally, DPP-IV inhibition was assessed through spectrophotometric assays at 405 nm. Once the active fractions were identified, the amino acid sequences of the peptides in the smallest fractions were determined using HPLC coupled with a Time-of-Flight Mass Spectrometer (TOF-MS). The HPLC analysis was conducted using a Kinetex C18 column (100 mm × 4.6 mm, 4.5 μm) at 35°C, with ultrapure water (A) and acetonitrile (B), both containing 0.1% formic acid, as the mobile phase.. MS analysis was conducted in positive mode with an electrospray ionization (ESI) voltage of 4.5 kV.

The results demonstrated that the optimal extraction buffer for *S. obliquus* was phosphate at pH 10. In terms of digestion time, trypsin and alcalase were most effective at 4 hours, while pepsin and papain showed optimal results at 2 hours. Regarding the enzyme-to-protein ratio, the best results for trypsin and pepsin were achieved at a 1:100 ratio, while alcalase performed best at a 1:50 ratio, and papain at a 1:20 ratio. Bioactivity-guided fractionation successfully isolated peptide fractions between 3 and 10 kDa from each enzyme, capable of inhibiting ACE and DPP-IV. The highest inhibition percentages were observed for the <3 kDa peptide fraction from alcalase, which achieved up to 80% inhibition of ACE and 65% inhibition of DPP-IV.

This study highlights the potential of *S. obliquus* as a source of bioactive peptides with inhibitory effects on key enzymes related to NCDs. The identification of peptides such as KATRT (*m/z* 347.0227) and KQRS (*m/z* 274.1873) opens up avenues for their potential use as therapeutic agents for treating hypertension and type 2 diabetes. The integrated approach of fractionation, enzymatic bioassays, and advanced analytical techniques like HPLC and TOF-MS provides a solid foundation for further research into microalgae as natural sources of bioactive compounds with therapeutic potential for chronic non-communicable diseases.

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P1-104**EXTREME PRESSURE COLLOIDAL NanoLC-MS/MS FOR ULTRA-FAST IN-DEPTH PROTEOMICS AND LOW-INPUT SAMPLE****Wen, Hanrong¹; Zhang, Bo²; Broeckhoven, Ken¹; Eeltink, Sebastiaan¹.**¹Vrije Universiteit Brussel, Brussels, Belgium; ²Xiamen University, Xiamen, China.

Proteomics is increasingly contributing to the development of quantitative biology, precision medicine and public health. It now faces tremendous number of samples from different species, human population, and single cells from tissues and organs. This demands a throughput on the scale of thousands, even millions of samples per day to provide sufficient data for answering specific biological questions. However, current throughput of fast in-depth proteomics is limited to 50 – 120 samples per day on single platform, constrained by the separation speed of nanoflow liquid chromatography (nanoLC) and acquisition speed of MS instrument. To increasing the analysis throughput, faster chromatography and mass spectrometry are needed, along with automated sample preparation and data analysis.

As the rate-determining step, nanoLC separation must be completed within a few minutes, or even below one minute, to enable over 1000 samples per day on single nanoLC-MS/MS platform, which presents a significant challenge. One promising approach is applying nanoparticle material in a chromatography column. According to kinetic performance limit (KPL) calculation, we found that, by using a 1500 bar nanoLC instrument, the analysis time could be reduced to 1/6 with same separation efficiency by changing the particle size from 1.5 μm to 500 nm. With an extreme pressure instrument (with maximum pressure of 3000 bar), the analysis time can reduce to 1/10, potentially support 600-1000 samples per day on single platform. When self-assembly of nanoparticle is considered, ordered column bed can further reduce the analysis time compared to a random packed column, even when accounting for the drastic increase of flow resistance. In addition to fast analysis, the reduced surface area and stationary phase diffusion makes the non-porous nanoparticle-based column suitable for high-recovery, high resolution, low-input analysis of rare and small amount sample, such as phosphoproteomics, glycomics and single-cell studies.

Using the single particle frit and convection-driven assemble of stabilized silica colloid, we successfully assembled silica nanoparticle materials inside fused silica capillary. The flexibility of single particle frit and stability of silica colloid (stable for over 12 h) support robust assemble of various nanoparticle sizes (200 – 750 nm) into capillaries of different inner diameter (25 – 200 μm) and length up to 300 mm. This fabrication capability can cover all column formats possibly required in ultra-fast nanoLC. During the assembling, we assumed that nanoparticle will be guided to the favored position inside capillary by convection through the formed column bed channel and weak interaction between particles. We studied the impact of convection speed, nanoparticle concentration and aspect ratio, showing the potential to fabricate 'perfectly' packed chromatography columns via colloidal assembly. Preliminary peptide mapping and DDA/DIA proteomics by C18-grafted nanoparticle column was performed on 1500 bar Vanquish-timsTOF platform and compared with conventional sub-2 μm nanocolumn under fast and low-input proteomics, demonstrating promising future of nanoparticle column for ultra-fast proteomics on a 3000 bar extreme pressure platform.

P1-105

QUANTIFYING COLIBACTIN: AN ONLINE SOLID-PHASE EXTRACTION-LC-MS/MS METHOD FOR ASSESSING GENOTOXIN PRODUCTION IN DIVERSE MICROBIAL SPECIES

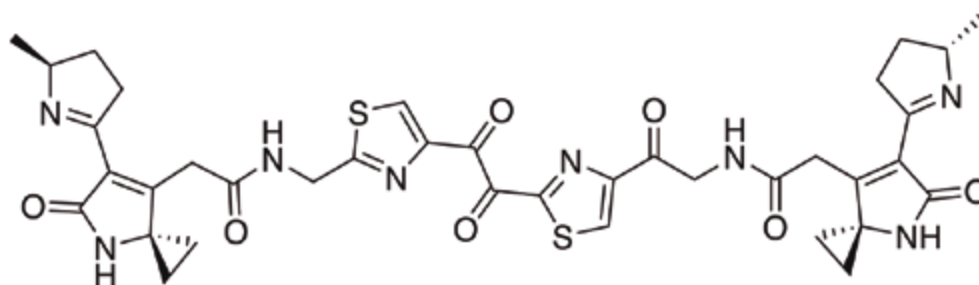
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Several microbial species in the human gut are increasingly associated with colorectal cancer, although their direct role in oncogenic mutations remains to be fully elucidated. Certain bacterial strains, such as *Escherichia coli*, which are frequently but not exclusively isolated from colorectal cancer patients, harbor the *pks* pathogenicity island. This genetic cluster directs the synthesis of Colibactin, a small-molecule genotoxin that causes DNA double-strand breaks in eukaryotic cells. Such damage could lead to cell cycle arrest and inflammatory conditions that can foster the development of colorectal cancer.

Due to its highly reactive molecular structure, the isolation of Colibactin has not yet been achieved. Consequently, quantitative analytical efforts concentrate on *N*-myristoyl-D-asparagine, a more stable cleavage product formed at the end of the Colibactin biosynthesis pathway. However, this indirect quantification approach presupposes myristic acid to be the dominant fatty acid within the cleavage product spectrum. Therefore, especially when analyzing diverse microbial species, it is vital to confirm that *N*-myristoyl-D-asparagine is indeed the most abundant byproduct.

For analysis, reversed-phase high-performance liquid chromatography (RP-HPLC) was employed. To streamline sample preparation and avoid labor-intensive extraction methods, turbulent flow chromatography (TFC) was incorporated for online sample cleanup prior to the LC analysis. For accurate quantification, a novel, custom-synthesized ¹³C-labelled internal standard was used. Hyphenation with electrospray ionization tandem mass spectrometry (ESI-MS/MS) enabled sensitive and selective detection. The established online TFC-RPLC-ESI-MS/MS method offers a reproducible analysis of diluted culture supernatants and was applied to a range of microbial species and their strains to assess Colibactin production.



Colibactin

P1-106**UNRAVELING HUMAN LYMPHOMA CELL PHOSPHOLIPID COMPOSITIONS BY MEANS OF LC-MS/MS UTILIZING MOBILITY RESOLVED FRAGMENTATION****Wieland, Dominik¹; Schmitt, Anja²; Hailfinger, Stephan²; Hayen, Heiko¹.**

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Many pathological conditions can be associated with lipid alterations, spanning from shifts in double bond positions to significant remodeling of the lipidome. Human lymphoma cell lines react differently to induction of ferroptosis, an iron mediated cell death, based on lipid per-oxidation. However, the precise biological processes and lipid species involved still remain unclear. Phospholipids, as major building blocks of the cellular membrane, are the main lipid category contained in human cells. Especially ether-linked phospholipids with highly unsaturated fatty acyl moieties are suspected to play a key role in ferroptotic processes. The molecular diversity and subtle changes between lipid species necessitate the utilization of powerful hyphenated mass spectrometric techniques for their analysis.

Human lymphoma cells were cultivated and provided by collaboration partners. For lipid extraction, an established protocol according to MATYASH *et al.* was conducted.[1] Human lymphoma cell lipid extracts were analyzed utilizing reversed phase liquid chromatography coupled to trapped ion mobility spectrometry (TIMS), enabling the separation of selected constitutional isomers. Mass spectrometric measurements were conducted on a quadrupole time of flight mass spectrometer (Q-TOF-MS) utilizing mobility resolved fragmentation. The resulting diagnostic MS/MS spectra, along with ion mobility values, facilitated confident lipid assignment. Combined with retention time and accurate mass, this four-dimensional data set was processed with MetaboScape software, enabling unequivocal lipid annotation. Manual validation of the data was conducted via lipid class specific four-dimensional Kendrick mass plots as well as confidence scores generated by MetaboScape software.

This methodology was implemented across eleven different human lymphoma cell lines with varying susceptibility towards ferroptosis. For each cell line, six biological replicates were included to mitigate potential deviations from cell cultivation. In total, more than 150 individual lipid species, spanning over ten lipid classes, were identified. Observed variations in ferroptosis susceptibility may be linked to changes in the phospholipidome. Therefore, multivariate data analysis strategies like hierarchical clustering heatmaps, principal component analysis, or volcano plots were applied to explore conspicuous changes in species level lipid composition.

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ANALYTICAL METHOD BASED ON UHPLC-MS/MS FOR THE TARGETED ANALYSIS OF MULTI-CLASS PFASs IN ATMOSPHERIC PARTICULATE MATTER

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Per- and polyfluoroalkyl substances (PFASs), a large group of synthetic chemicals widely used in a variety of industries and consumer goods due to their thermal stability and oil- and water-repellent properties, have received more attention in recent years. These same properties make PFASs highly persistent pollutants, and numerous adverse health effects have been reported, including cancer, impacts on fertility and fetal development, and disruptions to the endocrine and immune systems. Due to their toxicity and persistence, the continuous release of PFASs into the environment has raised significant global concerns. These substances have been found in every environmental compartment, but research and regulations have primarily focused on groundwater and foodstuffs to this point (1, 2). Inhalation of atmospheric particulate matter (PM) might be a significant human exposure pathway to several PFASs, as recent studies have demonstrated the presence of several of them in PM (3,4). Our research focuses on assessing the occurrence of PFASs in PM collected from various locations in Spain and Portugal, as well as exploring their *in vitro* inhalation bioaccessibility (i.e., the fraction dissolved in lung fluids) and bioavailability (i.e., the fraction dissolved in human plasma after crossing a membrane), using physiologically based extraction tests with simulated fluids. To support this, a methodology for the analysis of target PFASs is required, being ultra high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) among the most suitable techniques, providing high sensitivity and selectivity (5).

The present work comprehends the development of a UHPLC-MS/MS method for the separation and quantification of 53 multi-class PFASs, comprising 27 target compounds and 26 isotopically labelled-analogues used as surrogates or internal standards, including those regulated under EU legislation (e.g., Directive 2020/2184 and Regulation 2023/915) and emerging PFASs of growing concern. Chromatographic separation was performed using an ACQUITY UPLC BEH C18 (1.7 μ m, 2.1 x 100 mm) column, with gradient elution consisting of two mobile phase components (20 mM ammonium acetate in both methanol and water), using also an ACQUITY UPLC (2.1 x 50 mm) isolator column to delay contamination from the mobile phase and instrument. Additionally, a secondary pump with a diverter valve was used to direct non-relevant portions of the chromatographic run to waste. The MS/MS detector operated in negative mode (ESI⁻), and data were acquired in selected reaction monitoring (SRM). For this purpose, optimal MS/MS transitions were studied for each compound, as well as the conditions yielding more efficient separation, including the solvent composition (proportion of methanol: water) of injected extracts and linearity assessment.

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P1-108**DEVELOPMENT OF A UHPLC-QTOF-MS METHOD FOR THE COMPREHENSIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN ALOE VERA LEAVES****Santana-Mayor, Álvaro; Conde-Díaz, Adrián; Rodríguez-Ramos, Ruth; Socas-Rodríguez, Bárbara; Herrera-Herrera, Antonio V.***Departamento de Química, Unidad Departamental de Química Analítica, Facultad de Ciencias, Universidad de La Laguna (ULL), San Cristóbal de La Laguna, Spain.*

Aloe vera (*Aloe barbadensis* Mill.) is a widely cultivated succulent plant from the Asphodelaceae family, highly valued for its medicinal properties and rich chemical composition. Its chemical profile, very rich in bioactive compounds, has found applications in functional foods, cosmetics, and pharmaceuticals [1]. The global aloe vera market has seen significant growth, rising from 2.65 billion USD in 2023 to 2.86 billion USD in 2024, with Europe leading at 33.92% market share. Projections estimate the market to reach 5.34 billion USD by 2035 [2]. This growth drives the need for robust analytical methods to accurately characterize the chemical profile of aloe vera leaves, crucial for quality control in value-added product development. With this purpose, analytical techniques including LC-MS, GC-MS, and NMR are usually used, alongside emerging 2D screening methods [3].

In this study, a UHPLC-QTOF-MS method was developed for the separation and quantification of a wide variety of bioactive compounds from different chemical families in fresh aloe vera leaves. These include organic acids, fatty acids, sterols, vitamins, and other antioxidants linked to aloe vera's health benefits. Chromatographic separation was studied using different experimental conditions, achieving a final run time of less than 15 minutes. High-resolution mass spectrometry conditions were optimized in MSE mode, selecting the most intense ion for quantification and additional ions for compound confirmation. The method enabled baseline separation of all analytes, allowing for unequivocal identification and sensitive quantification at low concentration levels.

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P1-109**ACCURATE SIMULATION OF BENCHTOP NMR SPECTRA WITH HQSpectrum**

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Low-field Nuclear Magnetic Resonance (NMR) spectroscopy has become increasingly popular in recent years, as benchtop spectrometers are economical, compact and easy to operate. However, with typical resonance frequencies of 60-100 MHz, the recorded spectra often feature overlapping peaks and broad multiplets, which make them more difficult to analyze.

Our work employs HQSpectrum, a software solution to simulate NMR spectra and to compare them with experimental data. To prepare the input for HQSpectrum, chemical shifts and J-coupling constants were calculated via density functional theory using an in-house workflow that automatically explores the conformational flexibility of the compounds of interest. Alternative approaches for NMR parameter computation are currently under development. Based on a full set of chemical shifts and J-couplings, the NMR spectrum is calculated using the solver implemented in HQSpectrum. With suitable approximations, the solver simulates spectra for tens of spins in a matter of minutes, which are accurate and detailed for high fields and low fields alike. Individual spin contributions to the overall simulated spectrum are available as part of the solution, aiding the assignment of overlapping peaks. Parameters can be interactively hand-tuned within the HQSpectrum web interface to match experimental results.

Interesting use cases emerge from the combination of experimental with computed NMR parameters. For example, realistic benchtop NMR spectra can be simulated if chemical shifts that were assigned from a high-field NMR spectrum of the same molecule are combined with calculated J-couplings that would be otherwise unavailable. This demonstrates how simulation technology can improve data re-usability between different NMR spectrometers in quality control and other application areas.

P1-110**SPERMINE AS A STABILIZER OF HEPES-BASED GOLD NANOPARTICLES: UV/Vis AND SERS STUDY****Kendel, Adriana; Gudan, Nikolina.***University of Zagreb Faculty of Science, Zagreb, Croatia.*

Surface-enhanced Raman scattering spectroscopy is a technique in which the inelastic scattering of molecules adsorbed on metal nanostructures, called SERS substrates, is enhanced by up to 10^{10} times compared to classical Raman scattering.[1] The main characteristics of SERS substrates include stability and scattering enhancement efficiency. Among the most commonly used metal substrates are colloidal nanoparticles, usually prepared by reducing metal salts with analytes, which can act as both reducing agents and stabilizers of the metal nanoparticles. Colloidal suspensions of nanoparticles are subject to aggregation over time, and to increase the stability of the resulting nanoparticles, various stabilizing agents such as polymers, surfactants, and proteins are added during synthesis.[2]

In this study, gold nanoparticles were prepared by reduction of tetrachloroauric(III) acid with 4-(2-hydroxyethyl)-1-piperazineethylenesulfonic acid (HEPES buffer) [3]. The SERS efficiency of the prepared colloid was investigated using rhodamine 6G (R6G) and (E)-1,2-di(pyridin-4-yl)ethene (BPE) as model molecules. SERS and UV/Vis spectroscopy were used to investigate how the addition of *N,N*-di-(3-aminopropyl)butane-1,4-diamine (spermine) to the colloidal suspension affects the stability of metal nanoparticles and the enhancement of light scattering.

In colloids prepared with and without spermine, the strongest SERS effect was observed for BPE molecules, most likely due to the lack of charge and optimal structural features. In contrast to BPE, the positively charged rhodamine 6G molecules were electrostatically repelled from the gold nanoparticles because the HEPES molecules were located on the metal surface in the form of zwitterions. When spermine molecules are introduced into the system, they change the surface charge of the nanoparticles and at the same time create a steric barrier between the nanoparticles and the test molecules, so the obtained spectra of the model molecules showed less intense bands compared to the unmodified colloid. However, such modified colloidal nanoparticles were more stable over time compared to gold colloids prepared without stabilizer molecules.

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P1-111**DEVELOPMENT OF A SIMULTANEOUS ANALYTICAL METHOD FOR PESTICIDE RESIDUES IN HERBAL MEDICINES USING MODIFIED QuEChERS AND UPLC-MS/MS**

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A reliable and efficient analytical method was developed for the simultaneous determination of eight pesticide residues in herbal medicines using a modified QuEChERS extraction procedure coupled with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). Target analytes were selected based on their frequent usage in Peony Root, Astragalus Root, Angelica Gigas Root, and Cornus Fruit. Extraction was performed using a modified QuEChERS protocol, followed by analysis in multiple reaction monitoring (MRM) mode. Quantification was conducted via matrix-matched calibration. Method validation was carried out following SANTE/2020/12830 guidelines across the four herbal matrices. Calibration curves exhibited excellent linearity ($R^2 > 0.98$) over the concentration range of 0.001–0.05 mg/kg. The method showed good sensitivity with a limit of quantification (LOQ) of 10 ng/mL. Recovery rates ranged from 81.3% to 112.6%, with relative standard deviations (RSDs) below 11.7%. The results demonstrate that the modified QuEChERS approach not only reduces sample preparation time but also ensures high accuracy and reproducibility. This method is suitable for routine monitoring and regulatory assessment of pesticide residues in herbal medicine matrices.

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P1-112**A MULTI-TECHNIQUE APPROACH BASED ON MICRO-RAMAN SPECTROSCOPY AND ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY FOR THE DETERMINATION OF SMALL MICROPLASTICS IN MILK POWDER: A FOCUS ON ANALYTICAL CHALLENGES AND METROLOGICAL ISSUES****Barbaresi, Marta; Piergiovanni, Maurizio; Masino, Matteo; Bianchi, Federica; Mattarozzi, Monica; Careri, Maria.***Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy.*

Microplastics (MPs) are ubiquitous anthropogenic pollutants that can be found in the environment and food samples. The European Commission is driving the development of legislation needed to tackle their potential threat to human health and the environment. Currently, interpretation and comparison of analytical data are hampered by the unavailability of standardized and harmonized methods for MP sampling, characterization and counting as well as by the lack of reference materials for metrological traceability.¹ In particular, small-microplastics (SMPs, 10-100 µm) are difficult to isolate, enrich and detect in matrices containing high concentration of organic compounds, without compromising the integrity of the particles and altering polymer composition.¹ Furthermore, another analytical issue is cross-contamination throughout the analytical workflow: implementing a quality control procedure is essential to assess environmental contamination, define realistic reporting limits (ISO/NP 16094-2) and ensure reliable results.

In this context, the present study focuses on the development and validation of an analytical approach for chemical identification, physical characterisation and counting of SMPs in milk powder (infant formula) based on the use of micro-Raman spectroscopy and Environmental Scanning Electron Microscopy.^{2,3} To maximize the impact of the study, both primary and secondary particles of polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) were considered as representative test materials. As part of a collaboration within the European project PlasticTrace, BAM (German Federal Institute for Materials Research and Testing) produced and provided PET tablets to be tested as SMPs reference materials.

At first, blank filtration experiments were performed to establish good practices to be implemented into a clean room and develop a multi-step cleaning procedure to minimize cross-contamination. After selecting the appropriate filtration system and filters (Si, 5 µm), the acquisition parameters for micro-Raman measurements were properly selected to obtain high-quality spectra and representative coverage of filter area while avoiding laser-induced SMP degradation and fluorescence phenomena; in addition, the use of ParticleFinderTM and IDFinderTM (Horiba) software for Raman analysis permitted to automate particle analysis. Automatic particle counting on ESEM micrographs was performed using ImageJ-Fiji software.

Concerning the isolation of SMPs by selective removal of matrix organic/inorganic matter that inhibits microplastic detection, different approaches for the digestion of organic matrices were tested, obtaining satisfactory results by combining multi-enzymatic digestion with alkaline hydrolysis.⁴ Excellent recovery values were obtained from SMP fortified milk powder, which were in the 80-120% range for all polymers investigated. Ongoing activities involve the assessment of reporting limits per polymer type within defined size classes. Further activities such as real sample analysis and participation in inter-laboratory testing schemes are planned as a final part of the funded projects.

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P1-113**A COMPARATIVE STUDY OF ROBUSTNESS TO NOISE AND INTERPRETABILITY IN RAMAN SPECTRAL DENOISING WITH U-Net ARCHITECTURES**

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Raman spectroscopy is a powerful analytical tool for molecular characterization, but its utility is often hindered by low signal-to-noise ratios (SNR), particularly at short integration times. To overcome this challenge, we developed U-Net-based deep learning models [1] to denoise Raman spectra, leveraging their encoder-decoder architecture to suppress noise while preserving key spectral features. While U-Net has been previously applied to signal denoising tasks, this study introduces a novel comparative framework that investigates how training under varying noise conditions affects model generalization and interpretability in Raman spectroscopy.

We evaluated two models: a Multi-Condition (MC) model trained on spectra with varying noise levels, and a Single-Condition (SC) model trained on a single noise condition. Both models were assessed using metrics such as Mean Squared Error (MSE) and Pearson correlation coefficients (PCC) to quantify denoising accuracy and robustness. To gain deeper insight into model behavior, we employed interpretability techniques such as saliency maps and Jacobian matrix analysis to visualize which spectral regions the models prioritized during denoising.

Our results reveal that the MC model not only achieves superior denoising performance across a broader range of noise conditions but also demonstrates more stable and meaningful attention to key spectral features. These findings highlight the importance of training diversity and provide the first detailed interpretability analysis of U-Net-based denoising in Raman spectroscopy. This work establishes a foundation for more transparent and generalizable deep learning approaches in time-constrained spectral acquisition scenarios, with implications for clinical diagnostics, environmental monitoring, and industrial applications.

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P1-114**COMPARATIVE STUDY ON THE INFLUENCE OF SAMPLE INTRODUCTION SYSTEM ON THE SIZE UNCERTAINTY IN THE CASE OF Ag AND Pt NANOPARTICLE CHARACTERIZATION BY SINGLE PARTICLE ICP-MS (spICP-MS)**

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Single particle inductively coupled plasma mass spectrometry (spICP-MS) is a powerful analytical technique that is increasingly used for the determination and characterization of metal-based nanoparticles (NPs) in various types of samples and complex matrices. In recent years, considerable efforts have been devoted to the development of novel approaches/systems for more efficient introduction of NPs suspension in order to improve the analytical characteristics of the method, such as higher transport efficiency, lower LOD size, and other key parameters in nanoparticle analysis.

In accordance with the findings of our preceding study, it has been determined that the sample introduction system can exert a substantial influence on the size resolution of the spICP-MS method. The pulsation of the peristaltic pump emerges as a predominant factor in calculating the uncertainty associated with the diameter of individual nanoparticle, especially for NPs with smaller size [1].

In order to investigate the possibility of reducing the noise of the measured signal and improving the size resolution of the method, four different configurations of the sample introduction system were tested. The configurations tested were as follows: (i) peristaltic pump and pneumatic MicroMist® nebulizer; (ii) peristaltic pump and a two-channel, flow-focusing MultiNeb® nebulizer; (iii) syringe pump and MicroMist® nebulizer; and (iv) syringe pump with MultiNeb® nebulizer.

In the investigation of the MultiNeb® nebulizer, two options were examined. The first option involved the simultaneous aspiration of the sample through both capillaries. The second option entailed the utilization of one capillary for sample introduction, with the second capillary employed for water aspiration.

In order to evaluate the effect of the four sample introduction designs over the size uncertainty, a set of ionic standards for Ag and Pt were gravimetrically prepared and measured. The concentrations of the standard solutions were selected to provide a delivery of analyte mass for a given dwell time, corresponding to nanoparticles with diameters ranging from 20 to 100 nm. The sample flow rate for the two pumps was determined through gravimetric analysis before and after the instrumental measurement. The transport efficiency was determined by applying the size method using a reference material with certified sizes of Ag and Pt nanoparticles, respectively.

The combined uncertainties of the analyte masses were initially calculated and subsequently transferred to the uncertainties of the diameters of the spherical nanoparticles, which were estimated as confidence intervals. These confidence intervals were used to experimentally determine the size resolution of the spICP-MS method.

A comparison of the results indicates that, in all cases, superior size resolution is achieved when utilizing a syringe pump. This phenomenon can be attributed to the distinct operational mechanism of the syringe pump, which enables a substantial reduction in pulsation, as observed with peristaltic pumps. The syringe pump, when employed in conjunction with the MultiNeb® nebulizer and with the sample introduced through both channels, attains optimal outcomes. In this configuration, the confidence interval in the diameter of the individual nanoparticles, estimated to be approximately ± 1.5 nm across the entire investigated size range, was determined.

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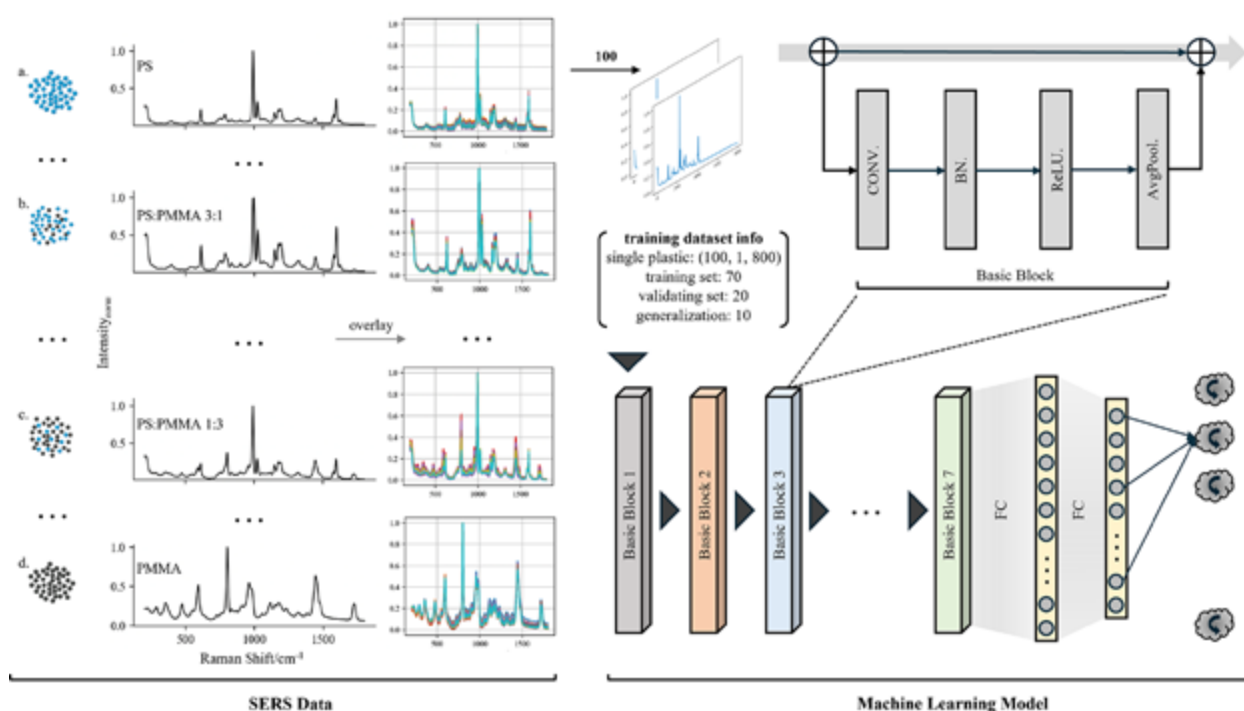
MACHINE LEARNING-ASSISTED SERS ANALYSIS OF NANOPLASTIC PARTICLES

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The widespread use of plastic has led to micro-nano plastics pollution, which has emerged as a major environmental concern over the last decade. Plastic fragments have been found in soil, oceans, air, and organisms, meaning humans are continually exposed to micro-nano plastics. Micro-nano plastics have been linked to many serious health problems, including heart disease and stroke¹. In particular, nanoplastics are considered to pose greater health risks due to their small size, which allows them to penetrate human skin and mucosal barriers and accumulate in tissues more easily. Some studies have been conducted to analyze nanoplastics from the single particle perspective, however, for multivariate mixed and aggregated samples of nanoplastics, a significant analytical knowledge gap persists, primarily due to the limited availability of reliable analytical methods². In this study, we developed a data-driven analytical framework combining surface-enhanced Raman spectroscopy (SERS) with machine learning to quantitatively determine the composition of nanoplastic particle mixtures^{3,4}.

To establish a simplified representative model system, polystyrene (PS) and polymethyl methacrylate (PMMA) nanoparticles (100 nm) are selected as representative nanoplastics. We systematically prepare a series of binary mixtures with PS and PMMA concentrations varying from 0% to 100%. The corresponding SERS spectra of these mixtures are normalized and used as input for the machine learning model. To improve training efficiency, we construct a CNN-based machine learning model using the ResNet framework to identify and classify SERS spectral characteristics of binary mixtures with different ratios⁵. For each type of compound with different PS and PMMA concentrations, we use spectral data from 100 different samples as training data. The data undergoes multiple feature extraction layers and fully connected layers, with the output being the corresponding predicted classification. Under 30 epochs of training, we validate the trained model with a test dataset, achieving an accuracy of approximately 99.7%. Additionally, the model demonstrated strong generalization capabilities in proportion estimation, accurately predicting PS content even for intermediate concentrations not included in the training data. For instance, a test sample containing 15% PS was correctly classified as falling between the 10% and 20% levels, closely matching the true value, which indicates that the model can interpolate between trained concentrations and capture subtle spectral variations associated with compositional changes. Since the intensity of Raman spectra at different frequency domains reflects the concentration of corresponding molecules or molecular groups. We aim to develop an enhanced feature extraction-based machine learning model for the prediction and classification of multicomponent mixtures



based on this indication. So, matrix filters were employed to amplify local spectral differences in mixtures with varying concentrations and use these difference combinations as input for the multicomponent machine learning model to detect the proportion of individual plastic components in complex mixtures. The proposed approach enables non-destructive, label-free, and rapid quantification of nanoplastics in complex mixtures.

Keywords: Nanoplastics, Quantification, Machine Learning, Surface-Enhanced Raman Spectroscopy

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P1-116**GC/QqQ METHOD FOR QUALITATIVE ANALYSIS OF Androst-3,5-diene-7 β -ol-17-one IN HUMAN URINE: VALIDATION AND APPLICATION TO ANTI-DOPING CONTROL**

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The Polish Anti-Doping Laboratory, Warsaw, Poland.

According to the Anti-Doping Testing Figures covering the last several years, the Hormone and Metabolic Modulators substance class, which is prohibited in sport, has consistently ranked fourth among the most frequently identified substances by the laboratories accredited by the World Anti-Doping Agency (WADA) [1]. This class includes several subclasses, the first of which comprises aromatase inhibitors. These substances limit the conversion of androgens into estrogens by binding to the aromatase enzyme and rendering it inactive [2]. Consequently, aromatase inhibitors are often misused by athletes to counteract the unwanted side effects of anabolic androgenic steroid administration, such as gynecomastia and excessive water retention. By reducing estrogen levels, aromatase inhibitors can potentially enhance the anabolic effects of testosterone, leading to increased muscle mass and strength. The most recent aromatase inhibitor explicitly included in the WADA Prohibited List is androsta-3,5-diene-7,17-dione, also known as arimistane.

The development of analytical methods for detecting prohibited substances and their metabolites is crucial for anti-doping efforts, as their use can provide athletes with an unfair advantage in competitive sports. It is particularly important to develop and improve detection methods for metabolites, which are often excreted over a longer period than the parent compound. In recent years, several studies have been published on the metabolism of arimistane in the context of anti-doping analysis [3,4,5]. All authors reported that the main metabolite of arimistane in urine is androst-3,5-diene-7 β -ol-17-one. Its presence in urine has been confirmed using GC/MS [3], GC/qTOF-MS [4], LC/qTOF, and LC/QqQ [5].

In the present study, the detection of androst-3,5-diene-7 β -ol-17-one in human urine by gas chromatography coupled with tandem mass spectrometry (GC/QqQ) has been validated and applied to anti-doping control in sports. This analytical method enabled the Polish Anti-Doping Laboratory to confirm the presence of androst-3,5-diene-7 β -ol-17-one in urine samples multiple times in recent years (once in 2022 and twice in 2023).

The GC/QqQ qualitative method for androst-3,5-diene-7 β -ol-17-one in human urine was validated, and acceptable validation parameters were obtained. Qualification was based on multiple reaction monitoring (MRM) ion transitions: 325.0 $\rightarrow$ 235.0, 430.0 $\rightarrow$ 325.0, 430.0 $\rightarrow$ 235.0, 430.0 $\rightarrow$ 415.0, 325.0 $\rightarrow$ 169.0, and 430.0 $\rightarrow$ 247.0. The internal standards used were d5-etiocholanolone, d3-testosterone, and methyltestosterone, monitored at MRM ion transitions 424.0 $\rightarrow$ 334.0, 435.0 $\rightarrow$ 209.0, and 446.3 $\rightarrow$ 301.2, respectively. The validation of the qualitative method for androst-3,5-diene-7 β -ol-17-one was performed to assess characteristics, such as specificity, matrix effect, limit of detection, limit of identification (LOI) [6], precision and carryover. The LOI, defined as the lowest concentration of an analyte in a sample at which the analyte can be confirmed with a 95% identification rate, was 2.21 ng/mL, which is significantly lower than the corresponding Minimum Required Performance Level of 20 ng/mL [7]. Another validation parameter, such as linearity, was also investigated, yielding an acceptable result.

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P1-117**IDENTIFICATION OF MICROPLASTIC PARTICLES EXPOSED TO ENVIRONMENTAL AND BIOLOGICAL MEDIA BASED ON THEIR NANOSECOND AND FEMTOSECOND LIBS SPECTRA**

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Laser-induced breakdown spectroscopy (LIBS) is a laser analytical spectroscopy method that can be efficiently used for the qualitative discrimination of various samples, among them plastics or plastic microparticles. Our project focuses on improving the accuracy of the LIBS identification of small microplastic particles (< 500 micrometers) dispersed in various liquid media. Prolonged exposure to various environmental (e.g. seawater, sewage sludge) and biological matrices (e.g. gastric juice, saliva) as well as to heat or UV radiation can potentially cause a contamination or change in the surface functionalization of microplastic particles, which can influence their laser spectroscopy spectra, including LIBS spectra. This in turn can influence the performance of LIBS as an analytical method used for the identification of microplastic particles for medical diagnostic or environmental monitoring purposes [1-3].

In our project, we extensively assessed the changes observable in the LIBS spectra of five common plastic types induced by such exposures. We also studied in detail, how the measurement conditions, such as the laser pulse duration (nanosecond or femtosecond) as well as the quality and pressure of the ambient gas (e.g. air and argon), can influence the spectra. We also investigated the accuracy of the discrimination provided by various supervised machine learning algorithms. Our goal was to optimize the experimental conditions and find the spectral features that can be reliably used to identify microplastic particles even if they are aged or contaminated.

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P1-118**POLARIZATION MODULATION INFRARED REFLECTION ABSORPTION SPECTROSCOPY (PM-IRRAS) AS A TOOL FOR INVESTIGATING LOW-DIMENSIONAL MATERIALS****Wöhler, Sebastian; Chauhan, Jatin; Cherevan, Alexey; Lendl, Bernhard; Ramer, Georg.***Technische Universität Wien, Vienna, Austria.*

In this work, polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) was employed to investigate surface bound organic and inorganic thin layers with high surface selectivity. The technique is based on the differential response of s- and p-polarized light at grazing incidence ($\sim 80^\circ$) on reflective surfaces, where only the p-polarized component generates a significant electric field. By rapidly modulating polarization states, PM-IRRAS selectively detects vibrational modes involving dipole changes normal to the interface, whilst minimizing background interferences caused by the substrate.¹

Thin films of 3,4-Ethylenedioxythiophene (EDOT) and its azide-functionalized analogue EDOT-N₃ were electropolymerized on gold substrates and analyzed by PM-IRRAS to probe molecular vibrations of surface-bound species in the mid-IR range. Distinct spectral features corresponding to the EDOT polymer (PEDOT) backbone were observed in both samples, while the azide-functionalized polymer showed a clear absorption band near 2100 cm⁻¹. Furthermore, the presence of the terminal azide groups in PEDOT-N₃ provides a reactive moiety for further modification of the polymer, e.g. using on surface azide-alkyne copper-catalyzed click reactions.²

To demonstrate the applicability of PM-IRRAS to inorganic systems, cobalt-molybdenum polyoxometalate (POM) clusters were adsorbed onto a few-nanometer thin ALD layer of TiO₂ on a silicon substrate. Despite the lower reflectivity of silicon compared to metals, the spectra revealed characteristic terminal Mo=O stretching bands corresponding to the inorganic {CoMo₃} core of the POM, indicating successful surface attachment.

These results highlight the versatility of PM-IRRAS for probing a range of interfacial systems of organic and inorganic materials on both, metallic and dielectric supports. Future studies will target vibrational characterization of 2D materials, where the strong surface sensitivity of PM-IRRAS is expected to be especially advantageous. Additionally, PM-IRRAS will serve as a complementary reference method to scattering-type scanning near-field optical microscopy (s-SNOM), given that both techniques share the same surface selection rules.³

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P1-119**SERS ANALYSIS OF NARINGENIN USING GOLD AND SILVER NANOPARTICLES****Petrović, Petra; Baričević, Antonija; Miljanić, Snežana.***University of Zagreb Faculty of Science, Zagreb, Croatia.*

Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical technique that enhances Raman signal of molecules adsorbed on metallic nanostructures, such as gold or silver nanoparticles. The enhancement of the Raman scattering enables the detection of analytes in sub-micromolar concentration range as well as structural analysis of biomolecules [1]. Naringenin, a natural flavonoid found in citrus fruits, is of particular interest due to its antioxidant, anti-inflammatory, and anticancer properties, making it a relevant target in pharmacological and biochemical studies [2].

In this study, Raman spectrum of solid naringenin was acquired to assign characteristic vibrational bands of the flavonoid. In contrast, the Raman spectrum of naringenin in ethanol was not observed, indicating that naringenin is a weak Raman scatterer in solution, when its vibrational signals are not measurable using conventional Raman spectroscopy. Consequently, the SERS was employed to enhance the signal. Citrate-stabilized gold and silver nanoparticles were prepared, characterized, and used as SERS active substrates. An aggregating agent, MgSO_4 (0.1 M), was added into the colloidal suspensions to induce controlled aggregation of the metallic nanoparticles, resulting in the observed Raman scattering of naringenin in the low concentration range. Concentration dependent SERS spectra pointed to the limit of detection at 1×10^{-5} M, while the most intense and well-resolved spectra were observed at a concentration of 1×10^{-4} M. Differences in the SERS spectra of naringenin on silver and gold nanoparticles implied metal-dependent adsorption mechanism of naringenin: via the fused six-membered rings and the carbonyl group on silver, and via the isolated hydroxylated benzene ring on gold.

In conclusion, SERS enabled the detection and vibrational characterization of naringenin in solution, not possible by conventional Raman spectroscopy due to the naringenin weak scattering properties. The characteristic vibrational bands were successfully assigned in the SERS spectra, allowing detection of naringenin at low concentrations. These results demonstrate the effectiveness of SERS as a sensitive and reliable technique for the analysis of natural compounds with low Raman activity.

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P1-120

METALS AND METALLOIDS IN DIFFERENT SIZE FRACTIONS OF PRINTED CIRCUIT BOARDS BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

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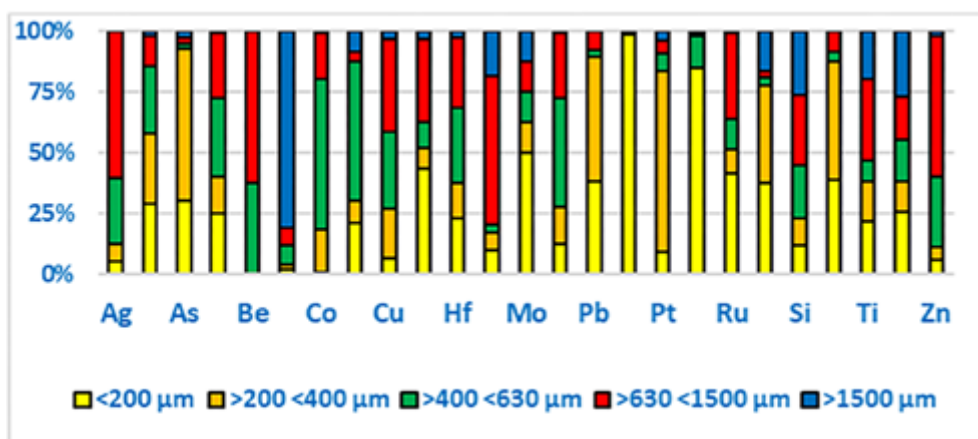
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Over the last half century, the increase in the world population and the well-being of those living in the most advanced nations, together to the escalation in acquisition of electrical and electronic devices, have led to a growth in electronic waste (WEEE). In this study five granulometric fractions obtained from computers mother boards were investigated to quantify 25 elements.

Inductively coupled plasma emission spectrometry (ICP-OES) and Inductively coupled plasma mass spectrometry (ICP-MS) are the most employed multi-elemental analytical techniques to quantify several elements in a large concentration range in complex environmental and food matrices [1, 2] as in the solution obtained from the treatment of electronic waste or from others [3, 4]. In our case, after mineralization by microwave, using a mixture of strong acids and hydrogen peroxide, the ICP-OES technique was used. One of the benefits of this method is the low detection and quantification limits which can be particularly relevant for elements present at trace levels. In fact, the LOQ values referred to the solid sample, ranged from $4 \cdot 10^{-5}$ to $5 \cdot 10^{-3}$ %.

Copper is the main element of printed circuit boards (8.5 to 26%), aluminium is the second (2.5 and 6.1%), while tin ($0.1 \div 1.6\%$), lead ($0.4 \div 1.2\%$) and antimony ($0.1 \div 0.4\%$) probably derive from the alloys used in soldering. Ni ($0.01 \div 0.7\%$) probably comes from the plating of the pins that connect the various devices. Au ($0.01 \div 0.03\%$) and Ag ($0.02 \div 0.2\%$) may also have had the same origin since they are used in the most expensive and efficient motherboards. Zinc ($0.4 \div 15.0\%$) is the fourth most abundant metal. Figure shows the Distribution of single elements in the different granulometric fractions.

Taking into account the percentages and the value of the main precious elements in the electronic waste, as those analysed by us, the rational and organized recovery of the aforementioned material could lead to a non-negligible economic profit and the protection of the environment and the health of workers.



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P1-121**NANOSCALE IMAGING AND IDENTIFICATION OF CONSTITUENT PHASES WITHIN Ca-BASED NON-METALLIC INCLUSIONS IN ULTRA-HIGH STRENGTH STEELS**

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The performance and mechanical properties of ultra-high-strength steel can be significantly influenced by non-metallic inclusions such as sulfides, oxides, and nitrides because they can enable sites for crack initiation. In this paper, simultaneous synchrotron-based X-ray fluorescence (XRF) and wide-angle X-ray scattering (WAXS) imaging were performed on non-metallic inclusions (NMI) in ultra-high strength steel samples at the NanoMAX beamline of the fourth-generation synchrotron radiation facility MAX IV Laboratory. To analyze the data, principal component analysis (PCA) was used to reveal in detail the nanoscale structure of these Ca containing NMIs with elemental and phase specificity. The nanoscale spatial resolution and simultaneous elemental and phase identification provide valuable insights into how the NMI composition and structure affect the physical properties of the steel. The observed phases and their locations within the NMI can contribute significantly to hydrogen embrittlement in ultra-high strength steels.

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P1-122**PREPARATION OF STANDARDIZED PLASTIC MICROPARTICLES BY VAT PHOTOPOLYMERIZATION 3D PRINTING FOR STUDIES AIMING AT THEIR DETECTION BY LASER SPECTROSCOPY**

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Research on micro- and nanoplastic particles is becoming more and more intense every year. These projects are concerned with the environmental, biological and analytical aspects of the problem of plastic particle pollution. Such research requires micro- or nanoparticle standards with a known composition, size and shape. Many polymer particle standards can be commercially obtained, but they often only come in a spherical shape and the available size range is also very limited [1,2].

In our present project, we have explored the possibility to use high resolution vat photopolymerization 3D printing technology to produce plastic microparticles with size, shape and compositional control. By establishing a tight control over the LCD illumination conditions (pixel pattern, exposition time, light intensity, etc.) and optimizing the deposition and harvesting conditions, we successfully achieved the production of particles with very good repeatability and good productivity. Rod, square, disk and triangle shaped particles were fabricated in various sizes, ranging from tens of micrometers to a few hundreds of micrometers. The morphology and topology of the particles were studied by light microscopy and contact profilometry. The composition of the particles can be controlled by the resin type – we have already worked out the methodology for several resins [3,4].

In our presentation, we will provide an illustrated description of the preparation procedure as well as imaging and tabulated numerical statistical data describing the properties of the plastic microparticles fabricated. We will also demonstrate the use of these particles in our ongoing research aiming at the detection of plastic microparticles by laser spectroscopy methods.

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OTKA K 146733 and 2024-1.2.3-HU-RIZONT-2024-00032 as well by the Austrian Research Promotion Agency (FFG) within the frameworks of the Important Project of Common European Interest (IPCEI) on Microelectronics and Communication Technologies.

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P1-123**THE PERFORMANCE OF CCD SPECTROMETER-BASED UV-VIS DETECTOR FOR PORTABLE MICROCOLUMN LIQUID CHROMATOGRAPHY DEVICE****Gogařová, Zuzana; Moravcová, Dana; Šesták, Jozef.***Institute of Analytical Chemistry of the CAS, Brno, Czech Republic.*

Regular HPLC systems are large and designed for laboratory use. However, smaller HPLC systems are becoming increasingly popular because they offer benefits such as portability, in-situ analysis, and space-saving. The development of portable liquid chromatography (LC) systems for analysis at the point of sampling requires new approaches to miniaturization of individual components. One key part of any LC system is detector, which convert the result of chromatography separation into a chromatogram, a record containing qualitative and quantitative information. In optical detectors, the design of the flow cell is very important for the overall performance of the LC system. In this study, we present initial results of testing a compact UV-VIS absorbance detector which includes a miniature deuterium lamp, a fused-silica capillary flow cell developed in our laboratory and a compact CCD spectrometer [1,2]. The detection cell is connected to the light source and CCD spectrometer using optical fibres integrated into the cell structure. For gradient chromatography separation of the test analytes, we compared the performance of our detector with a commercial DAD detector, which has an 80 nL rectangular flow cell. The compact design makes the setup appropriate for portable HPLC systems that use 0.2-0.3 mm I.D. microcolumns.

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P1-124**COMPARISON OF VARIOUS METHODS FOR DETERMINING NSAIDS IN EQUINE BLOOD AND URINE SAMPLES**

Jarek, Anna; Wójtowicz-Zawadka, Marzena; Wicka, Mariola; Wojtkowiak, Katarzyna; Tarka, Monika; Chajewska, Katarzyna; Olas, Magdalena; Konarski, Patryk; Kwiatkowska, Dorota.

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Due to their character, body structure, and physical capabilities, horses play a unique role in people's lives. Horses are kept as companion animals and for other purposes, such as breeding, production, and sports competitions. In the latter case, animals are subject to anti-doping control in many aspects analogous to those applicable to athletes.

Requirements regarding the scope of analysis are contained in documents of organizations such as the International Federation of Equestrians (FEI – Fédération Équestre Internationale) or the International Federation of Horseracing Authorities (IHMA). The first of them publishes yearly the 'Equine Prohibited Substances List' containing a list of the so-called 'Banned Substances' and 'Controlled Medications'. The substances included in the FEI List contain various classes of doping compounds, such as androgenic-anabolic steroids, stimulants, beta-agonists, and a wide range of nonsteroidal anti-inflammatory drugs (NSAIDs), among others. In turn, the second one published guidelines and recommendations regarding issues such as residue limits or international screening limits.

According to the FEI List, the use of NSAIDs in horses should be strictly controlled, and any amount of compounds that are detected in a urine or blood sample taken from a horse during competition should be reported. In order to adapt to changing FEI requirements, the Polish Anti-Doping Laboratory had to develop and improve the analytical methods used. In the case of the GC-MS technique, the change from GC-MS to GC-MS/MS allowed the LOD to be reduced from 25 ng/ml to <1 ng/ml. In turn, for the LC-MS/MS technique, the use of different measurement equipment allowed a significant change in the LOD of the tested compounds and a shortening of the analysis time. Additionally, using the dilute and shoot procedure for some of the tested compounds significantly improved the throughput of the method at the sample preparation stage while ensuring low LOD for the tested compounds.

P1-125**COMPARISON AMONG INCANDESCENT, SUPERCONTINUUM LASER, AND SUPER LUMINESCENT DIODE EXCITATION SOURCES IN PORTABLE NIR-SWIR HYPERSPECTRAL SCAN IMAGING**

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Hyperspectral scan imaging (HSI) in various spectral regions has come up in the last years as a reliable and versatile technique towards the chemical characterization of materials. Indeed, it has been extensively used for *in situ* diagnostic applications in biology, geology, cultural heritage, and other since it represents an effective and expeditious providing the unique potential of non-destructive and non-invasive chemical mapping. Focusing on the NIR-SWIR HSI, during the selection of a proper excitation source several factors must be considered, especially the availability of sufficiently high spectral power density in the region of interest to speed up the data collection and high focusability for achieving satisfactory spatial resolution.

In this work, incandescent source, supercontinuum laser (SCL), and superluminescent diode (SLD) have been compared for vibrational excitation in NIR-SWIR HSI characterization of Asbestos Containing Materials (ACM) and a set of samples of interest in conservation of cultural heritage including paint layers and corroded copper alloys conservation. The measurements were carried out using a homemade portable NIR-SWIR hyperspectral scan imaging tool [1]. This was equipped with high resolution spectrometer, compact XYZ translation stages, and dedicated own-built software. Following the optimization of the excitation setups, point scan NIR-SWIR reflectance images of ACM allowed successfully mapping and quantifying asbestos inclusions in proximity of the sample surface and identify alteration materials in paint layers and corroded copper alloys. The possibility to exploit the high nominal intensity provided by SLD and SCL resulted to be critically dependent on the specific experimental setups that allowed to mitigate their intrinsic fluctuations.

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P1-126

100-FOLD REDUCTION IN THE QUANTIFICATION LIMIT OF NATURAL GAS ODORANT BY GC BY COOLING A NANO-GRAVIMETRIC DETECTOR

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Natural gas, a fossil energy source formed naturally in underground deposits from the decomposition of organic matter, is nowadays widely used for transport, industry, domestic use etc. Natural gas is a mixture mainly composed of methane (75-100%) and contain minor constituents (hydrocarbons up to hexane, permanent gases) and sometimes pollutants.

Since natural gas is odorless, an odorant compound (usually containing sulfur) is added at the entry points of distribution network, enabling leaks detection without causing health issues associated to short-term exposure. There are two key challenges related to the monitoring of odorant concentration in natural gas. The first one is ensuring that the odorant concentration complies with regulatory standards, which vary from country to country. For instance, in France where tetrahydrothiophene (THT) is used, its concentration must be in the range from 4,2 to 11,1 ppm (15 and 40 mg/m³) [1]. The second challenge arises during the de-odorization process at international borders, where it is necessary to quantify ultra-trace levels of odorant. In such case, detection limits must reach sub-ppm level, requiring highly sensitive instruments. While thermal conductivity detector (TCD) meets the requirements of the first challenge, its sensitivity is not sufficient for the second one. An alternative detection process has to be designed to address this issue.

The nano-gravimetric detector (NGD) based on nano-electromechanical systems (NEMS) technology [2] has been introduced for miniaturized chromatographic systems linked to on-line and in-situ analysis. NGD is a non-destructive compact detector, suitable to run in ATEX environments. NGD is able to ensure detection and quantification of a wide range of compounds. Its response increases with the adsorbed mass of analyte, a consequence of an adsorption/desorption mechanism within the detector. This makes it an asset for on-line analysis of heavy compounds [3]. The partition equilibrium between the gaseous phase and the adsorbent layer of the detector is temperature dependent [4] ; indeed, at lower temperatures, adsorption is favored. Thus, a prototype for cooling an NGD has been developed so that sensitivity should be increased enough to reach detection limit in sub-ppm range.

In this context, a study was conducted on the response of THT using a cooled NGD to enable trace-level detection. Over the studied range of temperatures, peak height increases exponentially as temperature decreases, and noise does not vary significantly. With the limit of quantification (LOQ) being modulated by temperature, it has been demonstrated that the LOQ of THT was reduced by a factor of 100 between 50°C and -65°C going from 18 ppm to 0,17 ppm, thus largely meeting regulatory requirements regarding THT content in natural gas in France. The extrapolation of the obtained results even suggests that an LOQ lower than 50 ppb could be reached at -100°C. Therefore, signal enhancement at negative temperatures allows better detection and a lowering of quantification limits.

Finally, using air instead of helium as carrier gas can offer several advantages: air is readily available, it is more cost-effective as it does not require regular supply logistics to bring gas cylinders, and since helium is a non-renewable resource, replacing it contributes to more sustainable analytical practices. Therefore, the LOQ of THT using compressed air as carrier gas is currently being studied to assess the possibility of using systems facilitating online analysis for odorant quantification.

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MOLECULAR CHARACTERIZATION OF ABELMOSCHUS MANIHOT FLOWER AND ITS METABOLITES IN PLASMA BY 2D-CARBON MICROFIBER FRACTIONATION AND QTOF-HRMS

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Molecular characterization of 41 known substances and 6 compounds never detected before in *Abelmoschus manihot* flowers was performed by Two-Dimensional Microscale Carbon fiber fractionation combined with quadrupole time of flight high-resolution mass spectrometry (2D μ CFs-QTOF-HRMS) [1]. The comprehensive analysis was carried out in a single run of only 5 minutes and with a solvent consumption of 1.5 mL. Improved detection sensitivity compared with direct flow-injection MS analyses was achieved by the process of separations into fractions of different polarity (weak, medium, and high) before entering the mass spectrometer. The METLIN database was used to match the accurate experimental MS and MS/MS data. The main components of the flower extracts belong to flavonoids (quercetin, myricetin, quercetin 7-o-glucoside, hyperin, isoquercetin, quercetin 3'-o-glucoside, etc.) amino acids (γ -aminobutyric acid, serine, proline, valine, etc.), nucleosides (adenine, cytidine, 2'-deoxyadenosine, etc.), organic acids (5-(hydroxymethyl)-2-furancarboxylic acid, chlorogenic acid, etc.) and fatty acids (linoleic acid, oleic acid, etc.). The presence of 6 molecules (2-pyrrolidone-5-carboxylic acid, pipecolic acid, trigonelline, glucosamine, 1-aminocyclohexane-carboxylic acid, and 1,2-dioleoyl-sn-glycero-3-phosphocholine) not previously detected in *A. manihot* flower extracts was observed and confirmed by a deeper investigation based on molecular polarity and mass spectrum fragments.

From the dried flowers of *Abelmoschus manihot*, Huangkui capsule (HKC), a Chinese patent medicine, is prepared and used for the treatment of chronic kidney diseases, including diabetic nephropathy, chronic glomerulonephritis, and membranous nephropathy [2,3]. By 2D μ CFs-QTOF-HRMS, a rapid, high-throughput, and comprehensive screening of HKC metabolites in rat plasma was carried out, achieving high sensitivity and metabolite coverage. By excluding the *m/z* values of blank plasma and HKC extracts, information on HKC plasma metabolites was derived. Most of the metabolites come from the parent hyperoside, which is the main component in HKC. Finally, a comparison was performed between different parts of the *Abelmoschus manihot* plant. Principal Component Analysis (PCA) was performed using all the results obtained for flowers, buds, and stems, providing valuable insights into the active ingredients of the plant and creating a bridge between phytochemistry and clinical applications.

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THE POTENTIAL OF X-Ray FLUORESCENCE SPECTROSCOPY AS A UNIVERSAL, ELEMENTAL DETECTOR FOR HPLC: THE ANALYSIS OF ORGANOBROMINES

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Although many HPLC-detectors have been developed, most do not offer molecule-independent, universal responses whilst being selective. Elemental detection as performed by inductively coupled plasma mass spectrometry (ICP-MS) offers such responses but is hindered by its complexity and cost ^{1,2}. X-Ray Fluorescence (XRF), on the other hand, also has this potential in a more simple and robust way thanks to its element-specific, molecule-independent responses. XRF is currently extensively used as a static detector in many fields (including art-historical, biological, environmental and geological analysis ³⁻⁶). However, the use of XRF in a dynamic context has been limited to capillary electrophoresis (CE) whilst the hyphenation with HPLC has not been reported thus far ⁷⁻⁹. Therefore, XRF is demonstrated here for the first time as a complementary, flow-through, universal detector for HPLC and employed for the isocratic analysis of organobromines using reverse phase chromatography.

The main benefit that XRF offers HPLC is its universal response, allowing the use of element- based instead of molecule-specific calibration curves. This allows for the quantification of compounds for which standards are not (yet) available, too expensive or not economically sustainable to produce. Moreover, it allows for the quantification of complete compound groups instead of a few representative compounds, based on a single element in common, significantly simplifying the quantification process. HPLC-XRF also exhibits a broad linear range and has the potential of in-line hyphenation with other detection techniques such as MS because of its non-destructive nature and flow-through design.

In this work, the use of HPLC-XRF was tested through flow-injection analysis and isocratic, reverse phase separations of organobromines with varying numbers of bromine atoms. The set-up existed out of a polyethylene flow-through cell, a monochromatic Molybdenum X-ray source and a Silicon Drift Detector which proved to be simple and easily reproducible. A proof of principle exhibited good linearity between 0.2 and 20 mM with detection limits below 0.3 mM and confirmed the universal character of XRF. Sensitivity can be further improved through advanced set-up design using multiple detectors, dedicated software and HPLC techniques such as stop-flow chromatography and preconcentration.

This work provides an initial insight into the universal response abilities of XRF in combination with HPLC, whilst providing attainable detection limits with current lab-scale X-ray sources.

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P1-129**ANALYTICAL APPLICATION OF EDTA-STABILISED SILVER NANOPARTICLES****Świerczyna, Oliwia¹; Paluch, Justyna²; Kozak, Joanna².**¹Jagiellonian University, Doctoral School of Exact and Natural Sciences, Łojasiewicza 11, 30-348 Krakow, Poland.²Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland, Cracow, Poland; ²Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland, Cracow, Poland.

Silver nanoparticles (AgNPs) have gained great popularity in analytical chemistry due to their unique optical and chemical properties, including high reactivity and stability. AgNPs can be modified with various functional groups, increasing selectivity and sensitivity in analytical applications, making them valuable tools for the detection and determination of analytes [1].

The synthesis of AgNPs requires precise control of reaction conditions such as reagent concentration, pH, temperature, and reaction time to ensure reproducibility and desired physicochemical properties [2]. For this purpose, flow methods ensuring constant AgNP synthesis conditions have proven to be very effective [3].

For this study, EDTA-stabilized silver nanoparticles were synthesised in a Lab-in-Syringe flow system. During the study, the AgNO₃ concentration was selected for Fe(II) determination. The synthesised nanoparticles were examined using transmission electron microscopy (TEM), and an attempt was made to use them for analytical purposes. Research was conducted on developing a method for determining Fe(II) using synthesised silver nanoparticles with spectrophotometric and smartphone-based detection. The effect of possible interferences on the determination was investigated. Analytical parameters for both detection methods, such as linear range, limits of determination and quantification, precision, and accuracy, were determined and compared. The analytical usefulness of the method was confirmed by determining Fe(II) in white wine samples. The obtained results were compared with the results of determinations using the reference method for Fe(II) determination with 1,10-phenanthroline.

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P1-131**SIMPLE ANALYTICAL DEVICE FOR PHOTOMETRIC DETERMINATION OF ETHANOL IN ALCOHOLIC BEVERAGES****Trojanowski, Bartłomiej¹; Strzelak, Kamil²; Wasilewski, Krzysztof²; Koncki, Robert².**

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While numerous methodologies exist for ethanol estimation, each presents its own set of challenges: The conventional techniques (e.g. distillation, titration) are time-consuming and require significant effort, chromatographic methods demand expensive equipment and skilled personnel, and enzymatic tests are both costly and laborious.

This work introduces a simple low-cost optoelectronic detector, consisting of a LED and a photodiode, adapted to the photometric detection of the color complex of cerium (IV) ions with ethanol. However, this method of ethanol determination is highly susceptible to various interferences, primarily from reducing substances (sugars, vitamin C, etc.) and colored substances that are commonly found in various beverages. In order to address this issue, the detector was integrated with a membraneless separator that allows ethanol to be separated from the sample matrix using its volatility. The fabrication of the device was accomplished via 3D printing technology (SLA). The device is designed for use in non-laboratory format as an independently functioning device operated manually, but also as a separation-detection module in more advanced, mechanized analytical systems. An optimisation of the device construction and the proposed analytical procedure was carried out, and analytical characteristics (determination range, sensitivity, repeatability, selectivity) were performed. The device was successfully employed for the analysis of various alcoholic beverages. The analytical results obtained were consistent with both the beverage manufacturers' specifications and the results from the reference determination method.

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P1-132**ELECTROSPRAY VS. UniSpray - COMPARISON FOR THE ANALYSIS IN TERMS OF COMPOUNDS PROHIBITED IN SPORT**

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The presented study aimed to compare the use of different ion sources for the analysis of compounds present in urine from the World Anti-Doping Agency's (WADA) List of Prohibited Substances and Methods. These compounds involve the following groups: S1 (Anabolic Agents), S2 (Peptide Hormones, Growth Factors, Related Substances and Mimetics), S3 (Beta-2 Agonists), S4 (Hormone and Metabolic Modulators), S4 (Hormone and Metabolic Modulators), S5 (Diuretics), S6 9Stimulants), S7 (Narcotics), S8 (Cannabinoids) and S9 (Glucocorticoids) [1]. The evaluation covered the comparison of ionization sources in terms of the analysis of the identified substances and the influence of ionization on the signal responses for individual compounds. Instrumental analyses were performed in the Multiple Reaction Monitoring (MRM) mode with the positive ion detection.

As the ion source ESI (Electrospray) and, available only in Waters company, US (UniSpray) were used. Some of the advantages of using the UniSpray ion source are increased ionization efficiency and increased sensitivity for many compounds [2].

The results presented came from the analysis of at least 10 different quality control samples analyzed during the three-month time period.

In the course of the research, the ion intensity ranges for individual ion sources were compared, and the obtained results of the area values under the peaks of the analyzed compounds for the selected MRM transitions were compared, proving that the UniSpray ion source ensured a significant increase in sensitivity.

The use of the US source, ensuring a significant increase in sensitivity will allow the use of a lower amount of urine. As a natural consequence the reduced solvents usage and consumable materials will also be lower. This approach aligns with the concept of green chemistry.

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P1-133**DEVELOPMENT OF THE COMPACT AND LIGHTWEIGHT LIQUID HANDLING ROBOT****Lee, Jong Kwang; Pyo, Jae Young; So, Byoungjin; Park, Hwan Seo.***Korea Atomic Energy Research Institute, Daejeon, South Korea.*

Automated liquid handling is crucial for enhancing efficiency, precision, reliability, and scalability across a wide range of applications, from routine analyses to scientific experiments. There are various commercial or open-source robotic liquid handling systems available for use with column chromatographic systems.¹ The most common is a pipetting robot, where an automatic pipette is mounted on a mobile platform, such as a gantry system. However, it uses a relatively long pipette tip and requires a lengthy stroke to supply reagents from a low position to the top of the column, resulting in a considerably bulky and heavy system. Moreover, most of these systems are not suitable for radiochemistry applications, particularly for radioactive waste characterization, due to their limited volume handling capacity or low durability when exposed to strong corrosive acids or vapors.

In our previous research, we developed a novel liquid handling robot that employs a more compact and lightweight design using a gantry platform with a cantilever arm.² This arm moves a flexible PTFE dispensing line (1.6 mm O.D., 1.0 mm I.D.) extended from the pump and can precisely position its tip in arbitrary three-dimensional space. The vertical stroke of the outlet of the dispensing line was designed to be 130 mm, considering single or tandem column applications. To enhance corrosion resistance, the main components of the gantry system were constructed from polypropylene and assembled using Teflon-coated bolts. A peristaltic dispensing pump delivers reagents to the columns by switching a 15-port manifold valve. As the delivery rate of the pump affects both the volumetric accuracy and processing delay, the performance was evaluated at pump rotational speeds ranging from 40 to 80 RPM. A linear relationship ($R^2 = 0.9999$) between the angular speed of the dispensing pump and the flow rate was observed. The percent relative error (% RE) for accuracy and the percent relative standard deviation (% RSD) for precision of the pump in dispensing 1, 5, 10 mL of DI water were measured using the gravimetry method using an analytical balance (ME204, Mettler Toledo, ± 0.1 mg precision), revealing values below 1.4% and 0.9% ($n = 10$), respectively. Therefore, the developed system can be used with sufficient precision and accuracy compared to the ISO 8655-2 error limits for a single channel pipette with a volume range of 1–10 mL. The performance of the liquid handling system based on the dispensing line transfer is primarily influenced by the uncertainty of the quantity of the liquid hanging on the tip of the line. To effectively reduce this uncertainty, we applied a practical approach, in which the dispensing fluid is transferred with high flow rates during dispensing and then rapidly decelerates when stopping its flow.

When using a peristaltic pump, precise precalibration is essential due to uncertainty or unknown variation in the characteristics of the pump tubing, typically owing to aging or deformation. In this study, we have modified the existing liquid handling robot to reduce the operational burden for calibration. Specifically, we replaced the peristaltic pump and manifold valve used for liquid transfer with a multichannel syringe pump. The performance evaluation of the modified system is underway, and we are expanding its usage to transfer a liquid binder for ceramic 3D printing.

Acknowledgments

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FULL COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR ACHIRAL-CHIRAL SEPARATION OF PROTEINOGENIC AMINO ACIDS

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The analysis of amino acids is crucial due to their role in biochemical and pharmaceutical applications, such as chiral drug development and metabolic profiling. Traditionally, two separate analyses are required, or the purpose of the analysis differs. Reverse-phase liquid chromatography (RP-LC) is commonly used to identify amino acids within a complex mixture, allowing their characterization in a single run. On the other hand, chiral analysis resolves enantiomers but requires prior isolation of the amino acid or prior knowledge of its identity. These methods often rely on one-dimensional chromatography and require high concentrations of organic solvents. While effective, they have environmental and economic drawbacks. Developing techniques that minimize solvent consumption represents an innovative and sustainable alternative to chiral analysis. A potential solution to this is the use of comprehensive two-dimensional chromatography (2D-LC). 2D-LC is an advanced chromatographic technique that enhances resolution and separation power by coupling two distinct chromatographic dimensions. The effluent from the first dimension is divided into small fractions and sequentially transferred to the second-dimension column for further separation. This approach enables the analysis of complex mixtures with increased peak capacities and selectivity, unattainable through one-dimensional separations alone. In this work, the use of 2D-LC with Per Aqueous Liquid Chromatography (PALC) in the first dimension on a hydrophilic interaction (HILIC) column and fast chiral RP-LC in the second dimension was implemented for amino acid analysis. This method allows, in a single analysis, both the identification of amino acids in the sample and the separation of their enantiomers.

In the first dimension, the amino acids were separated using a commercial HILIC column, coupled with a PALC method. Unlike conventional HILIC, which relies on high acetonitrile concentrations, PALC employs water-rich eluents, reducing solvent use and offering a more environmentally friendly approach. A 100% aqueous mobile phase to 25% ACN gradient was applied over 50 minutes, ensuring optimal separation before enantioselective analysis in the second dimension.

One key advantage of PALC in the first dimension is that its aqueous eluent simplifies the transition to comprehensive 2D-LC, allowing direct injection into the second dimension. In contrast, conventional HILIC or RP-LC yield eluents with high organic solvent content, which makes direct injection into the second column difficult. As a result, heart-cutting 2D-LC is more commonly used in these cases, often requiring sample dilution or solvent exchange, which can lead to sample loss or reduced resolution.

After first-dimension separation, the effluent was fractionated and injected into a chiral reverse-phase column, allowing the separation of amino acid enantiomers. The method successfully resolved and identified all target amino acids within 90 minutes. Compared to conventional methods, this technique offers a more sustainable, cost-effective solution for chiral analysis, making it a promising tool in biochemical and pharmaceutical research.

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P1-135**OXYGEN ATTACHMENT DISSOCIATION-ENHANCED MALDI IMAGING FOR SPATIALLY RESOLVED ISOMER IDENTIFICATION**

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Introduction

Mass spectrometry imaging (MSI) is a powerful technique for visualizing the spatial distribution of biomolecules in biological tissues. However, conventional tandem mass spectrometry (MS/MS) methods, such as low-energy collision-induced dissociation (CID), often struggle to differentiate structural isomers, particularly in the analysis of natural compounds. Oxygen Attachment Dissociation (OAD) is a radical-induced fragmentation method that selectively cleaves carbon–carbon double bonds (C=C) and other heteroatom sites, enabling precise structural elucidation.

OAD has been successfully applied to lipidomics, providing superior differentiation of C=C positional isomers, such as ω 3 vs. ω 6 fatty acids, which are indistinguishable by CID alone [2]. In this study, we apply OAD-enhanced MSI to achieve high-spatial-resolution mapping of lipid isomers in biological tissues, offering deeper insights into lipid metabolism and cellular heterogeneity.

Methods

OAD utilizes charge-neutral oxygen radicals (O/OH•), generated from water vapor discharge, to induce selective fragmentation. MSI was conducted using MALDI-OAD-MS on an iMScope™ QT equipped with an OAD Radical Source I (Shimadzu, Japan). Mouse brain sections were coated with 2,5-dihydroxybenzoic acid (DHB) or 9-aminoacridine (9-AA) matrices using an iMLayer™ system.

MS images were processed with IMAGEREVEAL™ MS, and lipid structures were analyzed using LabSolutions Insight Explore™.

Results

Previous MSI studies detected coexisting C=C positional isomers, such as oleic acid 18:1(Δ 9) and vaccenic acid 18:1(Δ 11) in mouse brain sections, but lacked sufficient MS/MS specificity for clear spatial differentiation. In this study, OAD-MS/MS successfully distinguished these isomers, providing distinct spatial maps of lipid species.

For example, when analyzing phosphatidylcholine (PC) 16:0_18:1(Δ 11)/PC 16:0_18:1(Δ 9) at m/z 796.525 ([M+K]⁺), MSI revealed that PC 16:0_18:1(Δ 9) was enriched in the white matter, while PC 16:0_18:1(Δ 11) localized in the gray matter. These findings confirm that OAD enables precise visualization of lipid isomer distributions, which were previously unresolved using CID.

Additionally, OAD fragmentation patterns provided unique diagnostic ions for structural elucidation. Unlike CID, which primarily induces headgroup losses, OAD preferentially cleaves at C=C bonds, allowing accurate differentiation of isomeric lipid species. By integrating OAD-MS/MS with MSI, we successfully:

- Resolved C=C positional isomers in biological tissues.
- Achieved spatially distinct imaging of lipid isomers.
- Enhanced structural elucidation using radical-induced fragmentation.

Our findings demonstrate that OAD-MS/MS provides a more detailed and accurate representation of lipid metabolism, establishing it as a valuable tool for lipidomics and biomedical research.

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AN INTEGRATED APPROACH BASED ON HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF LEACHABLE SUBSTANCES FROM COSMETICS PACKAGING

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Plastic packaging for cosmetics is a source of human exposure to various chemicals potentially causing adverse health effects. Packaging materials contain both intentionally added substances (IAS), such as monomers, plasticizers, and antioxidants, as well as non-intentionally added substances (NIAS), which may be present as impurities in the raw materials used for the packaging production, oligomers, degradation products, or contaminants introduced during manufacturing processes. [1] These compounds can migrate to the cosmetic product, raising concerns about consumer health.

Comprehensive identification of leachables is challenging due to the wide range of chemical classes and their varied physicochemical properties. Therefore, an integrated analytical approach based on different platforms is required for the untargeted analysis of such substances. [2]

The aim of this research was to develop an untargeted, integrated workflow to evaluate compounds leached from cosmetic packaging, using high-resolution mass spectrometry (HRMS) in combination with different separation techniques—namely supercritical fluid chromatography (SFC) and ultra-high-performance liquid chromatography (UHPLC). HRMS analysis was performed using a Waters G2-Si Synapt Q-TOF using electrospray ionization in both positive and negative ion mode (ESI+ and ESI-). The samples of cosmetic packaging made of virgin and recycled polyethylene terephthalate (PET) were run in duplicate in data-independent acquisition mode. A 50% v/v ethanol solution was used as a food simulant: cosmetic packaging samples were filled with the food simulant and stored at 60 °C for 3 days. The samples were evaporated to dryness via vacuum evaporation concentration and redissolved in 1 mL methanol (up- concentration factor 100) and analyzed by both SFC-HRMS (normal phase) and UHPLC-HRMS (reverse phase).

Data processing and alignment were performed using the MS-Dial software v 4.9, resulting in the extraction of 20284 and 7199 features from UHPLC-HRMS and SFC-HRMS, respectively. To reduce the high number of extracted features, filtering and data reduction strategies were applied, including a 5-fold change intensity threshold compared to the blank samples and library match. A suspect screening list of 6130 substances, including plastic monomers, additives and processing aids, was used to support compound identification. Identification confidence was enhanced through MS/MS spectral matching using the MassBank of North America (MoNA) and in-silico spectra generated via Competitive Fragmentation Modeling. [3]

A total of 224 leachable compounds were annotated with confidence level 1 to 3 [4]: 147 by UHPLC-HRMS, 51 by SFC-HRMS, with 26 compounds annotated by both techniques. The annotated substances included plasticizers, dyes, intermediates, UV absorbers, flame retardants, antibacterial agents, flavouring agents, and antioxidants.

These findings highlight the complementarity of UHPLC and SFC coupled with HRMS for profiling semi-volatile and non-volatile substances, thus strengthening the importance of an integrated approach for a comprehensive safety assessment of substances migrating from packaging materials.

Further evaluation of semi-volatile components is currently in progress using in-situ derivatization followed by comprehensive 2D-Gas Chromatography (GC)-HRMS analysis. Finally, the complex data generated will be analyzed using multivariate statistical analysis techniques: unsupervised, supervised and data fusion approaches to improve the classification performance and identify the compounds capable of differentiating recycled and virgin PET.

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P1-137**A SEMI-QUANTITATIVE METHOD TO ANALYZE WALL PAINTINGS COUPLING SPECTROSCOPY AND IMAGING WITH MACHINE LEARNING****Sáez-Hernández, Roberto¹; Cruz, Jordi²; Alcalà-Bernàrdez, Manel³; Mauri-Aucejo, Adela R.¹; Morales-Rubio, Ángel¹; Cervera, M. Luisa¹.**

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This study introduces a semiquantitative method leveraging artificial intelligence (AI) and machine learning (ML) to analyze inorganic pigments in wall paintings through visible spectroscopy and digital imaging. In this work, historical red pigments were selected for investigation. In particular, three different inorganic species were analyzed: cinnabar, minium and hematite. Each one of those is characterized by a different metal: mercury, lead and iron, respectively. Following the original recipes, as compiled in the literature [1], these pigments were applied in frescoes laboratory mockups, covering a diverse color and hue palette. These samples were prepared to represent the potential variability in terms of visual appearance and elemental profile found in historical frescoes.

The analytical methodology involved measuring the samples through portable X-ray fluorescence spectrometry as a reference device. This instrument is one of the most commonly employed in archaeometric research, as it provides quantitative information on the elemental composition of samples for most of the elements in the periodic table. Thus, the metal content of the samples as obtained by this technique was considered the reference value.

Next, the samples were measured using two different colorimetric approaches. The first one involved visible reflectance spectroscopy, and the second one involved smartphone imaging. Each one of these techniques yields information about the color of the samples in different ways: visible reflectance spectroscopy provides a whole reflectance spectrum, while digital imaging provides colorimetric descriptors in RGB coordinates. These coordinates represent an information compression of the aforementioned spectra. In both instances, this data is representative of the color of the wall painting samples.

A first step involving the smartphone characterization was carried out to convert the device-dependent RGB coordinates to device-independent CIELAB descriptors. The quality of this characterization was assessed using the CIEDE2000 formulae. Results indicated that the corrected color coordinates were comparable to the real ones, ensuring the quality of the smartphone data.

In order to cross-reference the colorimetric information (either the spectra or the corrected color coordinates), two kinds of chemometric approaches were tested: Support Vector Machines (SVM) and Artificial Neural Networks (ANN). The dataset ($n = 102$) was split into a calibration ($n = 68$) and validation ($n = 34$). Making use of the calibration set, the models were built and internally validated using Venetian blinds (20 %). Subsequently, the validation set was employed to assess the predictive capacity of the models.

With these models, the colorimetric information was used to predict the metal concentration in each sample. Making use of the smartphone data, the Root Mean Square Errors (RMSE) of prediction were below 4.03 % in all instances, using SVM as regression models. Regarding the spectra-based models, the RMSE for the prediction set was below 2.99 %. In this latter case, Hg and Fe were modeled using SVM, while Pb was better modeled making use of ANN.

The study highlights the advantages of using affordable, non-invasive and portable techniques for analyzing cultural heritage samples. The innovative data treatment models developed in this research allow for the estimation of elemental content in inorganic pigments without damaging the samples. This approach is particularly valuable for the preservation and study of historical artifacts, where traditional invasive methods are not feasible.

Overall, this study demonstrates the potential of combining AI and ML with visible spectroscopy and digital imaging for the semiquantitative analysis of inorganic pigments in wall paintings. The developed methodology offers a reliable and non-destructive means of estimating elemental concentrations, contributing to the field of cultural heritage conservation. The results underscore the importance of innovative data treatment models in enhancing the accuracy and efficiency of pigment analysis, ensuring the preservation of valuable historical artifacts.

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P1-155**A MULTIFUNCTIONAL MICROFLUIDIC CHIP FOR SENSITIVE ABSORPTION AND REFRACTION MEASUREMENT****Tang, Jiukai; Wang, Jing.***ETH Zurich, Zurich, Switzerland.*

Integrated microfluidic chips, which combine multiple functional units, are being developed as highly compact analytical devices. In this work, we present a microfluidic chip comprising a detection channel and two microlens channels, enabling both absorption and refraction measurements through flexible operational modes. When samples are stably injected into the microlens channel, the refractive index can be measured with a resolution of 10^{-4} RIU. Stable injection into the 3.7 μ L detection channel allows for sensitive absorbance measurements, benefiting from an extended optical path length of up to 6 cm. Alternatively, when samples are introduced into the 3.7 μ L detection channel via fluid switching, a highly sensitive refractive interface is formed. This interface enables precise quantification of salinity and total dissolved solids (TDS), achieving a detection limit as low as 10 ppm. We envision that this microfluidic device could enable real-time, simultaneous monitoring of absorption spectra and TDS, offering a promising solution for intelligent environmental analysis.

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CHARACTERIZATION AND QUANTIFICATION OF FREE AMINO ACIDS IN ANCIENT DENTAL ENAMEL

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In recent years, there is a growing interest in the study of teeth as biomineralized archives of ancient biomolecules, including proteins. Intracrystalline proteins are encapsulated within nanometric voids in the bioapatite matrix. Despite the low abundance of proteins in enamel, the high degree of mineralization of this hard tissue offers significant protection from postmortem alteration, enabling the preservation of these biomolecules over extensive periods. Proteomic approaches have demonstrated the feasibility of analyzing proteinaceous residues preserved in tooth enamel to extract valuable information pertaining to sex, diet, and phylogenesis. Proteins within enamel, however, are subjected to degradation over time. As a result, in ancient samples, proteins may persist only as smaller peptides or individual free amino acids (FAAs). The few studies related to amino acid investigation were performed using high performance liquid chromatography (HPLC) in order to investigate the amino acid racemization. However, for pre-Quaternary samples there is little knowledge about how the biomineral-bound organic fraction is preserved, either as free amino acids or in the form of peptides.

To address the existing knowledge gaps in amino acid preservation in fossil samples, a recent investigation has led to the development of an advanced protocol for the detection and semi-quantification of free amino acids (FAA) using high-performance liquid chromatography (HPLC). The primary objective of this project is to detect free amino acids (FAA), which are not bound in peptides on samples for which the total hydrolysable amino acids were already determined. As final aim, this could allow to assess the temporal survival of amino acids either protein-bound (i.e., within peptides) or just as free amino acids. This will provide insights into the degree of peptide degradation over geological time scales and the conditions under which amino acids persist in different forms. We successfully detected free amino acids in the enamel of teeth and quantified the concentrations of each amino acid present. By analyzing both modern and fossil samples from two different mammals' clades. We were able to observe how the quantities of specific amino acids changed with age within each family. Our findings indicate that even considering the taphonomic setting, FAA preservation is mainly affected by the geological age. For each family, we identified which amino acids showed increased or decreased concentrations compared to modern enamel as a function of the sample's age. This analysis provided insights into the diagenetic processes affecting the abundance of free amino acids in enamel and their stability over geological timescales.

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CHARACTERISATION AND PROVENANCE OF RHYOLITE FROM CORSICA BY SMARTPHONE IMAGING COLORIMETRY

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Identifying the provenance of lithic tools is a crucial task for archaeologists to understand socio-ecological dynamics related to raw materials supply. In the last years, several methodological approaches have been developed to discriminate among outcrops and to link artefacts to potential sources, with a particular focus on protocols which are non-invasive, quick to implement and economically affordable.

Based on these criteria, our poster presents the first use of smartphone colorimetry as a viable method to characterise rhyolite in archaeological provenance studies of lithic tools at the prehistoric settlement of Basi (Serra-di-Ferro, France). This technique has previously been tested on other lithic and lithoid archaeological materials (Ramacciotti et al., 2022; 2023) and involves the extraction of colour data by the means of smartphone photo imaging.

Rhyolite has been one of the main lithologies exploited by the inhabitants of Corsica to make lithic tools. This rock outcrops within the geological levels linked to the volcanic events of the Carboniferous and Permian which cover a large portion of the island, making the study of rhyolite circulation particularly suitable to better understand mobility and settlement networks of its prehistoric inhabitants. Recently, Leck and colleagues (Leck et al., 2018; Leck, 2022) carried out an extensive sampling of rhyolite from several Corsica outcrops and built up a wide geochemical database by multielement analysis using energy dispersive X-ray fluorescence spectroscopy (ED-XRF).

In this work, the samples from different geological sources have been photographed with a smartphone under controlled lightning conditions and RGB data were extracted. The smartphone camera was characterised using a ColorChecker Classic Mini (Calibrite), whose CIELAB colour descriptors were previously measured by a spectrophotometer, and polynomial models transforming RGB values to CIELAB colour space coordinates were derived to fit the data. The imaging protocol was applied to Munsell Rock Colour Charts targets, previously measured by a spectrophotometer, and the CIEDE2000 Colour-Distance Formula has been employed to determine which was the best performing model. The obtained data were used to build discriminant analysis models, tested by k-fold cross-validation ($k = 10$) to distinguish local (< 20 km) and non-local potential sources in regard to the site and together with elemental data to differentiate among different areas of catchment of the island.

The results of the study indicate that colour data allow a good discrimination of allochthonous rocks, while their use together with elemental ones can improve the performance of classificatory models.

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P1-140**STANDARD ADDITION QUANTIFICATION OF ARSENIC IN POISONED BOOKS USING PORTABLE X-RAY FLUORESCENCE**

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Historical books often contain pigments formulated with toxic elements such as arsenic (As), lead (Pb), and mercury (Hg), originating from compounds like emerald green, orpiment, lead white and vermilion [1]. These substances pose health risks and conservation challenges [2]. Techniques like portable X-ray fluorescence (p-XRF) and ICP-OES typically identify and quantify these elements [2]. While p-XRF offers a non-destructive alternative, accurate quantification remains challenging due to the lack of reference materials, matrix effects, and heterogeneous nature of heritage objects. In this way, this study aims to develop a non-destructive strategy based on a modified standard addition method to quantify arsenic in historical books using portable XRF.[4]

Analyses were conducted using an Elio p-XRF spectrometer equipped with a Rhodium anode. Real book samples were analyzed by both ICP-OES and the proposed XRF approach. Several support materials were fortified with known concentrations of As and placed between the sample and the detector. Spectra were acquired for the sample alone and in combination with each spiked support. Calibration curves were then constructed to estimate arsenic concentrations.

Lint-free cotton cloth supports provided the best performance across medium and high concentration ranges, while cellulose showed improved results at low concentrations. Calibration curves demonstrated excellent linearity ($R^2 > 0.995$). The proposed method using non-destructive p-XRF yielded arsenic concentrations ranging from 0.025 to 10.954 $\mu\text{g}/\text{mm}^2$, with results closely matching those obtained by ICP-OES. Relative errors were generally below 15%, supporting the reliability and applicability for non-invasive quantification in heritage science.

The approach overcomes key limitations of traditional XRF quantification, such as matrix effects and the absence of suitable reference materials, by introducing external spiked supports. This strategy offers a practical non-destructive tool for heritage science, contributing to the safe characterization of toxic elements in valuable cultural artifacts.

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P2-001**DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN WASTE-DERIVED PYROLYSIS OILS USING GC-MS/MS****Vernerová, Andrea; Oravová, Lucie; Kurář, Pavel.***Department of Environmental Chemistry and Technology, Faculty of Environment, Jan Evangelista Purkyně University in Ústí nad Labem, Ústí nad Labem, Czech Republic.*

Polycyclic aromatic hydrocarbons (PAHs) are a group of hazardous compounds that are often present in pyrolysis oils derived from plastic waste. The presence of these compounds in the oils poses significant environmental and health risks, which highlights the importance of their identification and quantification.^{1,2} This study focuses on the separation of critical PAH compounds in pyrolysis oils obtained from plastic waste, using various chromatographic columns coupled with GC-MS/MS (Gas Chromatography-Tandem Mass Spectrometry). The aim is to optimize the chromatographic conditions for efficient separation of PAHs, enhancing resolution and sensitivity for low-concentration compounds typically present in complex pyrolysis oil matrices. In addition, the study will evaluate different extraction methods for pyrolysis oils, comparing their effectiveness in extracting PAHs while minimizing interference from other constituents, such as plastic additives, fillers, contaminations etc. The results of this research will provide valuable insights into best practices for PAH analysis in pyrolysis oils from waste materials, contributing to more accurate environmental monitoring and regulatory compliance in the recycling and disposal of plastic waste.

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P2-002

VISIBLE-LIGHT-DRIVEN BIOCHAR BASED $\text{CoFe}_2\text{O}_4/\text{nZVI}$ HETEROJUNCTION ACTIVATING PEROXYMONOSULFATE FOR SIMULTANEOUS IBUPROFEN, TETRACYCLINE OXIDATION AND Cr(VI) REDUCTION

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The rational design of heterojunction photocatalysts represents an effective strategy for improving pollutant degradation efficiency. In this study, a visible light-responsive $\text{CoFe}_2\text{O}_4/\text{nZVI}$ heterojunction was synthesized on biochar by hydrothermal chemical deposition, in which nano-zero-valent iron formed a heterojunction on CoFe_2O_4 . SEM and XRD characterization confirmed the uniform loading of $\text{CoFe}_2\text{O}_4/\text{nZVI}$ nanoparticles on biochar with intimate interfacial contact. The heterojunction extended the light absorption range to 580 nm and exhibited a photocurrent density 2.1 times higher than that of pristine CoFe_2O_4 . In the presence of persulfate (PMS), the BC/ $\text{CoFe}_2\text{O}_4/\text{nZVI}$ heterojunction achieved 98.9% degradation efficiency of tetracycline and 99.7% degradation efficiency of ibuprofen within 30 minutes under visible light irradiation, significantly outperforming individual CoFe_2O_4 (31.5%, 12.2%) and nZVI (28.2%, 18.7%). LC-MS analysis identified 12 and 5 degradation intermediates, and Fukui function calculations revealed that the C4-dimethylamino group and its adjacent conjugated region in tetracycline, as well as the tertiary carbon on the side chain of ibuprofen, are the preferential attack sites. At the same time, BC/ $\text{CoFe}_2\text{O}_4/\text{nZVI}$ can reduce 97.6% of hexavalent chromium, which is much better than CoFe_2O_4 and nZVI. DFT calculations verified the band alignment at the heterointerface, confirming the spatial separation mechanism of photogenerated carriers. This study provides theoretical foundations and technical references for developing efficient heterojunction photocatalysts.

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P2-003

PREPARATION OF A NEW MAGNETIC BIO-NANOPARTICLE ADSORBENT AND ITS APPLICATION FOR SEPARATION AND PRECONCENTRATION OF Cd(II) IONS IN ENVIRONMENTAL SAMPLES: OPTIMIZATION THROUGH RESPONSE SURFACE METHODOLOGY

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Cadmium is among the most potentially hazardous metals present in many effluents and can accumulate in the food chain through bioaccumulation, posing serious risks to both human health and the environment [1]. Since it is harmful even at extremely low concentrations (mg/kg), it is crucial to accurately and reliably determine at trace levels in complex matrices. For this reason, sample preparation steps, including separation and enrichment methods, are often required before analysis to handle complex matrices effectively.

In this study, magnetic CoFe_2O_4 was synthesized via the coprecipitation method, and *Escherichia coli* ATCC 25922 (EC) was loaded onto this magnetic bio-nanoparticle. The obtained adsorbent ($\text{EC\_CoFe}_2\text{O}_4$) was characterized by FTIR (Fig. 1), SEM-EDX and XRD techniques. Finally, the prepared adsorbent was utilized for the solid phase extraction of Cd(II) ions using the batch method before determination by FAAS. The central composite design (CCD) was used to investigate the most effective factors of pH, amount of adsorbent, desorption time, eluent concentration and their interactions. Also, CCD is used to optimize the process and to obtain response surface graphics (Fig. 2). After optimization, the obtained values were pH 6.97, adsorbent amount of 10.6 mg, desorption time of 3.3 min, and eluent concentration of 0.227 M. When the experiment was conducted under these conditions, the recovery of Cd(II) ions was found to be 97.9%, whereas the model predicted a recovery of 100.2%. Also, the adsorption capacity of $\text{EC\_CoFe}_2\text{O}_4$ for Cd(II) ions was calculated as 97.4 mg/g. The detection limit of the method for Cd(II) ions was 0.40 $\mu\text{g/L}$, with a relative standard deviation (RSD%) of 0.80% for ten replicates and an enrichment factor of 40. The method was successfully applied for the determination of Cd(II) ions in various water samples including stream water and wastewater.

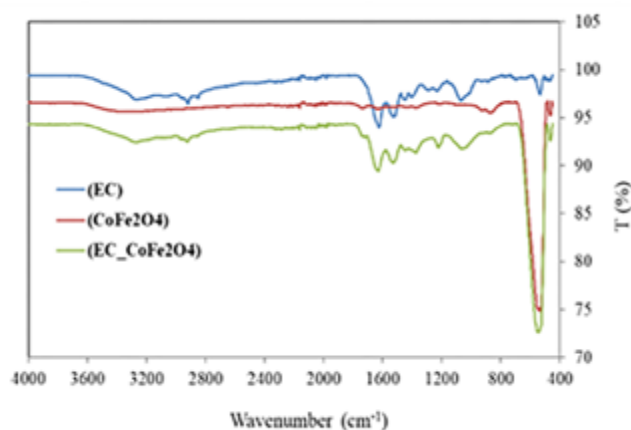


Figure 1 FTIR Spectrum of EC, CoFe_2O_4 and $\text{EC\_CoFe}_2\text{O}_4$

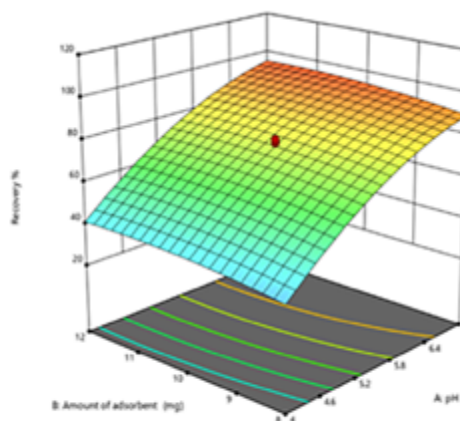


Figure 2 Three-dimensional surface response diagram for Cd^{2+} recovery in terms of pH and amount of adsorbent

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P2-004

PREPARATION OF Co(II) PHTHALOCYANINE COATINGS AND INVESTIGATION OF THEIR ANTIBACTERIAL AND SURFACE PROPERTIES

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Phthalocyanines are macrocyclic compounds composed of four iminoisoindole units, possessing a large central cavity capable of coordinating various metal ions. With an 18- π electron system, they share structural similarities with porphyrins but differ due to the presence of benzo units and meso nitrogens[1]. Among them, cobalt phthalocyanine stands out as an efficient catalyst in organic reactions due to its high activity and selectivity in oxidation processes [2]. Metal phthalocyanines have also been investigated as potential corrosion inhibitors for different metal alloys in various corrosive environments. They exhibit strong chemisorption onto metal surfaces through metal-ligand coordination bonds, making them promising candidates for corrosion inhibition coatings[3].

In this study, firstly, Co(II) phthalocyanine compound was synthesized and characterized. After dissolving the phthalocyanine compound in DMSO, it was coated on titanium surfaces using the dip method. After coating, XRD, SEM, EDX, FTIR and contact angle measurement tests were performed. Phthalocyanine-coated surfaces showed hydrophobic character compared to uncoated surfaces. Activity tests of the coated surfaces against E. coli and S. aureus bacteria were performed under dark and daylight conditions. Phthalocyanine-coated titanium surfaces showed over 90% inhibition against these organisms under daylight conditions. **This study was supported by Karadeniz Technical University Scientific Research Projects. (Project no: 11045)]**

Keywords: Phthalocyanine, cobalt, coating, antibacterial, surface properties.

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P2-005**LIPIDOMIC ANALYSIS AND NEUROTOXIC EFFECTS OF DINP AND DEHP PHTHALATES IN SH-SY5Y CELLS AND BRAIN ORGANOIDS****Ayala, Esmeralda¹; Porte, Cinta²; Bedia, Carmen².**

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Phthalates, such as diisononyl phthalate (DINP) and di(2-ethylhexyl) phthalate (DEHP), are widely used plasticizers found in various consumer products, such as food packaging, personal care items (like shampoos and lotions), medical devices, and children's toys [1]. Due to their potential neurotoxic effects, there is growing concern about their impact on brain development and function [2]. This study aims to evaluate the neurotoxic effects of DINP and DEHP using in vitro models, including SH-SY5Y human neuroblastoma cells and brain organoids, which offer a more physiologically relevant three-dimensional (3D) structure.

To assess cellular toxicity in 2D SH-SY5Y cultures, we performed a series of assays, including cell viability, membrane permeability, reactive oxygen species (ROS) production, acetylcholinesterase (AChE) activity assay, and gene expression analysis for XRE, ARE, and P53 reporter genes, which are involved in xenobiotic response, antioxidant defense, and apoptosis regulation, respectively. Additionally, to investigate the metabolic disruptions induced by phthalate exposure, we performed a lipidomic analysis on brain organoids treated with sub-toxic concentrations of DEHP and DINP (1, 10, and 20 μ M). Lipid extracts from the organoids were analyzed using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) in data-independent acquisition (DIA) mode, employing both positive and negative ionization. An untargeted lipidomic approach was used, and lipid species were identified based on MS1 and MS2 spectral accuracy by comparison with the MS-DIAL database. Multivariate statistical analyses, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were applied to assess the impact of phthalates on the lipidomic profiles of brain organoids and to identify the key lipids driving these changes.

Our results showed no neurotoxic effects in SH-SY5Y cells under the conditions tested, as assessed by cell viability, AChE activity, oxidative stress, and gene expression analysis. However, lipidomic analysis of brain organoids revealed alterations in lipid composition, mostly upon DEHP exposure. The multivariate analyses indicated significant increases in triglycerides, gangliosides, and glycerophospholipids, suggesting lipid reprogramming induced by phthalate exposure. These lipid classes are critical for membrane integrity, signaling, energy storage, and neural development, and their dysregulation may reflect early neurotoxic events. These findings highlight the importance of using advanced 3D models to detect subtle neurotoxic effects that may not be evident in traditional 2D cell cultures.

Given the widespread exposure to phthalates and their potential impact on neural function, further studies are needed to elucidate the underlying mechanisms of lipid metabolism disruption and its implications for neurodevelopmental disorders. Our study underscores the necessity of incorporating organoid models into neurotoxicity assessments to better predict the potential risks associated with environmental contaminants like DINP and DEHP.

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P2-006**ANALYSIS OF ANTIBIOTIC RESIDUES IN SEWAGE SLUDGE: OPTIMIZATION OF EXTRACTION TECHNIQUES AND OCCURRENCE IN WWTPs**

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Every year, between 100,000 to 200,000 tons of antibiotics are consumed worldwide in several sectors, including human medicine (both in hospitals and households) and veterinary applications. This extensive use has raised significant concern regarding the presence of antibiotic residues in different environmental compartments [1], and the subsequent antibiotic resistance.

Sludge management constitutes a challenge at wastewater treatment plants (WWTPs), particularly due to the huge production volume and concerns related to potential uses of this residue [2,3]. Sewage sludge is considered as a sink for different pollutants due to its ability to accumulate substances that are not removed during the treatment of wastewaters, such as antibiotics, which may pose a risk to the environment [4]. Some families, such as fluoroquinolones, can reach concentrations up to several mg/Kg in this type of sample [5].

The analysis of antibiotic residues in sludge is particularly complex due to the diverse physicochemical properties of different antibiotic families, and the complexity of the matrix. In this study, different sample preparation methodologies, including QuEChERS and Pressurized Liquid Extraction (PLE), have been investigated for the extraction of different families of antibiotics. Key extraction parameters -such as extraction solvent, and sample and sorbent amount- have been evaluated. Once optimized, the extraction conditions were validated in terms of accuracy, precision, and repeatability. Finally, the proposed methodology was applied to the analysis of antibiotic residues in sludge samples collected from different WWTPs in Galicia (Northwest of Spain).

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P2-007**PHARMACEUTICAL RESIDUES IN SLUDGE FROM WASTEWATER TREATMENT PLANTS**

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During the last decades, the presence of contaminants of emerging concern (CECs) in the different compartments of wastewater treatment plants (WWTPs) has been extensively investigated [1,2]. Among these compartments, sludge represents the most challenging residue due to its complex composition and high pollutant retention capacity. However, research on the presence of CECs in sludge, and particularly pharmaceuticals, remains limited, primarily due to the analytical challenges associated with its complex matrix [3].

To address this gap, several analytical techniques have been explored for extraction of pharmaceuticals and personal care products (PPCPs) in sludge, including Pressurized Liquid Extraction (PLE) [4], Matrix Solid Phase Dispersion (MSPD) [3] and QuEChERS [5]. Despite these advancements, there is still need for robust analytical methodologies capable to efficiently extracting and determining a broad range of contaminants with different physicochemical properties.

Herein, we present a novel and validated analytical methodology based on a two-step QuEChERS approach (extraction plus purification) for the simultaneous determination of a relevant number of pharmaceuticals. Target compounds were selected based either on their inclusion in the latest EU Watch List [6], the Wastewater Directive, or their occurrence in the environment.

The proposed methodology was validated in terms of repeatability and reproducibility, global extraction efficiency and limits of quantification. The optimized methodology was applied to quantify the selected contaminants in sludge samples collected from different WWTPs across Galicia (Northwest of Spain), providing valuable insights into the presence and distribution of these pollutants in wastewater treatment residues.

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P2-008

DEVELOPMENT AND OPTIMIZATION OF GREEN COLORIMETRIC METHODS FOR CHLORATE DETERMINATION IN AQUEOUS MEDIUM

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The disinfection stage is important in diverse fields, from the food industry to the treatment of swimming pool water, as it aims to eliminate pathogenic microorganisms and improve product quality. However, the use of chlorinated disinfectants in processing water can lead to contamination of water with disinfection by-products (DBPs), such as chlorate, which can be absorbed by food and cause hormonal problems. . Halogenated disinfectants, particularly chlorine derivatives such as hypochlorous acid or chlorine dioxide, are commonly used due to their low cost and high efficiency in reducing the presence of microorganisms. Both give chlorate and many other disinfection byproducts, such as trihalomethanes.^{1, 2}

This project aimed to develop simple, rapid, and cost-effective colorimetric methods for chlorate. The study successfully demonstrated the viability of an indigo carmine-based detection method on homemade paper supports, proving its robustness through satisfactory validation parameters and real samples applications. Additionally, an alternative UV-Vis spectrophotometric method using manganese sulfate, orthophosphoric acid, and diphenylcarbazide showed high selectivity, surpassing traditional ortho-tolidine-based techniques.

To improve efficiency, the indigo carmine method was adapted to a microplate format, significantly reducing reagent consumption while maintaining high analytical performance. For increased portability, the method was further transferred to a paper-based analytical device. The method using manganese sulfate was transferred to slides with a hydrophobic teflon barrier. In this way, the difficulties encountered with paper could be overcome, since the drop did not need to be completely dried and the teflon barrier allowed the addition of diphenylcarbazide dissolved in acetone. The colorimetric response was analyzed using smartphone-based digital imaging, providing a practical and accessible alternative for field applications. Validation with real water samples confirmed the reliability of these methods, showing strong correlation with reference technique.

Future research will also investigate the potential of microfluidic methods to integrate these detection systems into ready-to-use commercial kits. Lastly, automation of image capture and result processing will be prioritized to enhance usability and reliability.

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P2-009

Cu, Fe AND Zn ISOTOPIC ANALYSIS OF PARASITISED EDIBLE FISH: A STEP FORWARD IN UNDERSTANDING HOST-PATHOGEN METALLOMICS

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Recent studies on natural variations in the isotope ratios of essential metals in living organisms have provided valuable insights into metabolism. Factors influencing isotopic variations of Cu, Fe, and Zn remain unclear but seem to be driven by changes in their absorption, metabolic processes, and/or excretion. [1] It is well established that isotopic fractionation is linked to kinetic reactions and differences in binding energies, which are influenced by ligand coordination and redox conditions.

The goal of this work was the study of the elemental and isotopic compositions of Cu, Fe and Zn in *Brama brama* tissues and the parasites (*Gymnorhynchus gigas*), commonly present in such specie, for the study of the body isotopic distribution of parasitized fish and intra-individual variations. Concentrations were determined by time-of-flight inductively coupled plasma mass spectrometry (ICP-TOF-MS) and isotope ratio measurements by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS).

The method consisted on the acid digestion of the samples followed by the sequential purification of Cu, Fe and Zn using the anion exchange resin AG-MP1. Quantitative recoveries (ranging from 89 to 103 %) were obtained for the three elements in the subsequent pure fractions, guaranteeing the absence of on-column isotopic fractionation. The concentrations of Cu, Fe and Zn in the certificated reference materials (CRMs) of fish tissues were in good agreement with the certified values, and the Cu, Fe and Zn isotope ratios were consistent with previously reported data. [2] Furthermore, the fractionation lines constructed using the CRMs, fish tissues and parasites followed the theoretical mass-dependant fractionation lines. The parasites showed elevated concentrations of Cu, Fe and Zn compared to the host (adjacent muscle), following the trend Zn > Fe > Cu. The intra-individual isotopic variability was 0.6 ‰ for Cu, 0.5 ‰ for $\delta^{56}\text{Fe}$ and 1.6 ‰ for $\delta^{66}\text{Zn}$. Parasites showed lighter Cu and Fe but heavier Zn isotopic compositions than these of the fish muscle potentially attributed to the preferential absorption of the light ^{63}Cu and ^{54}Fe isotopes and heavy $^{66,67,68}\text{Zn}$ isotopes by the parasite.

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P2-010**ENHANCING SENSITIVITY AND ACCURACY IN PFAS ANALYSIS: METHOD DEVELOPMENT AND OPTIMIZATION FOR RELIABLE MONITORING OF ENVIRONMENTAL AND BIOLOGICAL MATRICES**

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Per- and polyfluoroalkyl substances (PFAS) are a broad and diverse class of synthetic chemicals, distinguished by their highly fluorinated carbon chains and diverse functional groups [1]. Their unique chemical properties, such as thermal stability, hydrophobicity, and resistance to degradation, have led to widespread industrial and commercial use since the 1940s. However, these same properties make PFAS highly persistent in the environment and prone to bioaccumulation, raising significant concerns for both ecosystems and human health. PFAS have been detected globally in environmental compartments, wildlife, and human biological fluids, earning them the classification of emerging contaminants [2]. Human exposure to PFAS primarily occurs through dietary intake, including contaminated drinking water and food [3]. Once in the body, PFAS accumulate in biological matrices such as serum, plasma, milk, and tissues, due to their high affinity for proteins and inability to be metabolized [4]. Prolonged exposure to PFAS has been associated with adverse health effects, including immunotoxicity, endocrine disruption, and reproductive disorders [5]. Given their persistence and bioaccumulation ability, there is an urgent need for reliable, sensitive analytical methods to monitor PFAS contamination in biological and environmental samples.

This study focused on developing and optimizing two analytical methods for determining PFAS in human serum and water, using online solid-phase extraction (online SPE) and ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). These methods enable sensitive and accurate quantification of more than 30 PFAS congeners, including both legacy and emerging compounds. For water analysis, the instrumental method was carefully optimized, starting with mass spectrometry parameters and extending through chromatography to online solid-phase extraction. Different separation columns and online SPE cartridges were tested, evaluating all possible combinations. Subsequently, parameters such as mobile phases, flow rate, and sample diluent were optimized to achieve the best analytical compromise, ensuring optimal performance for all PFAS congeners. The method was validated according to ISO/IEC 17025 and demonstrated excellent linearity, precision, and accuracy, with limits of quantification ranging from 1.2 to 24 ng/L and recoveries ranging from 74% to 120%. A significant effort was made to expand the number of compounds analyzed simultaneously, including more emerging PFAS, overcoming the limitations of previous studies that focused on a more restricted set of compounds.

For serum analysis, the developed method has been recently published [6]. Particular attention was given to sample pre-treatment, essential for protein elimination and efficient PFAS extraction. Key steps in the pre-treatment process include protein precipitation, pellet rinsing, centrifugation, solvent evaporation for preconcentration, and online SPE using a weak anion-exchange polymeric sorbent. The protein precipitation and pellet-rinsing procedures were optimized through an in-depth exploration of solvent combinations, with optimal conditions identified using Principal Component Analysis. The method was further refined by optimizing chromatographic and mass spectrometry conditions and validated according to ISO/IEC 17025, with limits of quantification ranging from 8.9 to 27 ng/L and recoveries ranging from 80% to 120%, achieving excellent reproducibility. This method is the first to apply online SPE for the simultaneous determination of a wide spectrum of PFAS, including ether congeners such as perfluoro(2-ethoxyethane) sulfonic acid and Nafion byproduct 2. Control charts were used to monitor instrument performance during routine analyses and implement corrective actions as necessary.

The developed methods were successfully applied to real serum and water samples, demonstrating their reliability and robustness in quantifying PFAS. These findings improve current analytical capabilities for PFAS monitoring and could support future environmental assessments. Additionally, they may help shape regulatory measures, especially for emerging PFAS compounds.

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P2-011**EXPLORING THE DETECTION LIMITS OF A THERMO-ANALYTICAL METHOD BASED ON IMAGE ANALYSIS OF MICROPLASTICS**

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Microplastics are an ever-increasing concern in the context of human pollution. The extended use of plastic products has caused an ubiquitous presence of said contaminants across natural environments. Given that the smaller fractions of MPs can easily enter the food chain and end up in the human body, developing techniques for detecting their presence is paramount. In previous work, we developed a new automated analytical system to assess the extent of MPs contamination in the environment by performing an image-based analysis of stained MPs during a heating ramp between 25 and 220 °C. This system, though low-cost, simple, and easily replicable, could only resolve particles as small as 250 µm. In this work, we present an optimization of said system which can now be used to detect even smaller particles. By filtering the MPs which were previously stained with a combination of Coomassie Blue and Methylene Blue dye, the whole filter containing the MPs can be exposed to the same heating ramp. This allows for better manipulation of small particles which would otherwise be difficult to manage. Images taken with a smartphone camera and a magnifying lens before and after the heating process can be analyzed to determine the presence of MPs by studying changes in superficial area and colour of the particles. This system detected the presence of MPs as small as 20 µm or less.

P2-012**FORMALDEHYDE DETERMINATION IN PARTICLEBOARDS: THE POTENTIAL OF THE GDME-HPLC-DAD METHODOLOGY AS AN ALTERNATIVE TO CURRENT STANDARDS**

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Particleboards (PBs) are a type of wood-based panels (WBP) made by combining wood particles with a thermosetting adhesive through the application of heat and pressure. These products can release volatile organic compounds (VOC) to indoor spaces, such as formaldehyde [1], a compound classified as carcinogenic by the International Agency for Research on Cancer.

For the determination of formaldehyde in WBP different international standards are available. From these, EN ISO 12460-3 (gas analysis) and EN ISO 12460-5 (perforator method) are used for quantifying formaldehyde emissions and content in WBP, respectively. Briefly, the Perforator method involves extracting formaldehyde in WBP samples through boiling toluene, allowing the evaporated formaldehyde to be retained in an aqueous phase for subsequent spectrophotometric analysis following derivatization with acetylacetone. The Gas Analysis method, in contrast, measures formaldehyde released from a panel within a controlled chamber, where airflow directs the emissions into several water impingers for posterior spectrophotometric analysis after acetylacetone derivatization. Despite their standardization, these methods present limitations for routine analysis. The Perforator method requires a considerable volume of toluene for each procedure, a hazardous solvent that poses risks to both human health and the environment. Meanwhile, the Gas Analysis method, requires a sophisticated and complex chamber equipment, which increases both cost and general availability [2].

Recently, the application of gas-diffusion microextraction (GDME) in the extraction of volatile carbonyl compounds, including formaldehyde, from different WBP was proposed as a simpler and faster alternative [3-4]. GDME is an efficient and versatile sample preparation technique designed to simultaneously extract, derivatize, and concentrate volatile compounds from both liquid and solid matrices [5]. This method operates on the principle of selectively capturing gaseous analytes as they diffuse through a gas-permeable membrane into an acceptor solution. The captured analytes are then retained in the acceptor solution for subsequent analysis.

In this study, the GDME-HPLC-DAD methodology coupled with 2,4-dinitrophenylhydrazine (DNPH) derivatization was used to extract and analyse formaldehyde emitted from PBs produced with different resin formulations and wood particles (pine and recycled). The formaldehyde-DNPH results obtained using the GDME-HPLC-DAD methodology were compared to those obtained from the Perforator and Gas Analysis standard methods, from which good correlation was observed ($R > 0.99$ and $R > 0.94$, respectively), showcasing the potential of the GDME-HPLC-DAD methodology as a greener alternative for formaldehyde screening in WBP.

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P2-013**ICP-MS RECORDING OF RARE EARTH ELEMENT SORPTION ONTO ALGINATE-ENCAPSULATED IRON NANOPARTICLES****Ilić, Nikolina¹; Bogdan, Karla²; Perić, Kristina¹; Nemet, Ivan¹; Rončević, Sanda¹.**¹Faculty of Science, University of Zagreb, Zagreb, Croatia; ²Ruđer Bošković Institute, Zagreb, Croatia.

Rare earth elements (REEs), a group of 17 chemically similar elements, are indispensable in the production of advanced technological devices such as magnets, batteries, sensors, and catalysts.[1] However, their widespread usage, coupled with inefficient recycling practices, has led to environmental pollution, particularly in aquatic systems. The contamination of water resources with REEs has raised significant environmental concerns due to their persistence and potential toxicity, especially at high concentrations. To reduce water and soil pollution by these elements, it is necessary to develop methods and techniques that enable their successful removal.

Recent research has highlighted the potential of using iron nanoparticles as an innovative and effective material for the removal of REEs from contaminated environments. Iron nanoparticles (nZVI), due to their high reactivity, large specific surface area, and environmentally and economically favorable characteristics, differ from traditional iron particles (ZVI). These differences make them a valuable tool for the removal of heavy metals and organic pollutants from water and aqueous solutions. The removal process is based on mechanisms of adsorption, reduction, and complexation.[2] However, despite their numerous advantages, the handling of nanoparticles is limited by their strong agglomeration and sedimentation. To minimize these issues, the surface of iron nanoparticles is often functionalized. Subsequently, various stabilizers can be added to iron nanoparticles to improve stability and reactivity, and one method for enhancing their stability is encapsulation in alginate hydrogel which was used in this study.[3]

In this study, iron nanoparticles were prepared in two forms: neutral and modified with pyridine-2,6-dicarboxylic acid. The synthesis was carried out by reducing iron (III) chloride hexahydrate with sodium borohydride, during which a portion of the nZVI particles was functionalized through a "one-pot" synthesis, where PDCA was added to the reaction mixture during the synthesis. The characterization of synthesized nanoparticles was performed by infrared spectroscopy using the attenuated total reflection (ATR) technique. Furthermore, nanoparticles were encapsulated in alginate hydrogel via a spherification process using sodium alginate and calcium chloride. Solutions of selected lanthanide ions (La^{3+} , Pr^{3+} , Nd^{3+} , Eu^{3+} , Gd^{3+} , Dy^{3+} , Er^{3+} , Tm^{3+}), were prepared, and the sorption of lanthanides onto iron nanoparticles was monitored by inductively coupled plasma mass spectrometry (ICP-MS). Sorption of ions was observed over different time periods and at varying initial concentrations of the solutions. Both, neutral and modified iron nanoparticles encapsulated in calcium alginate demonstrated exceptional sorption efficiency for all elements, particularly in the low concentration range, where the sorption efficiency ranged from 95.0% to 99.8%. However, functionalized iron nanoparticles exhibited slightly lower sorption efficiency in the low concentration range compared to the neutral iron nanoparticles. Furthermore, neutral nZVI showed higher efficacy in adsorbing the individual lanthanides from aqueous solutions whereas functionalized nanoparticles demonstrated better performance in adsorbing a mixture of the eight lanthanide ions.

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P2-014**DEVELOPMENT OF ANALYTICAL METHODS FOR THE MULTIRESIDUAL DETERMINATION OF FOOD AND LIFESTYLE BIOMARKERS IN WASTEWATER USING UHPLC-MS/MS**

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Wastewater-based epidemiology (WBE) is increasingly applied to assess population habits of drug, alcohol, and nicotine use, but its potential for assessing dietary patterns remains largely unexplored. Since WBE is based on the chemical analysis of specific urinary biomarkers excreted by the human body, one of the major challenges is the development of reliable, multiresidual analytical methods for the determination of these biomarkers in wastewater.

The aim of this study was to develop analytical methods for the quantitative determination of over 50 biomarkers in raw municipal wastewater, including biomarkers of alcohol, nicotine, caffeine, artificial sweeteners, vitamins, and plant-based food constituents such as flavonoids, lignans, and phenylethanoids. The biomarkers were analyzed by ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) in multiple reaction monitoring (MRM) mode with electrospray ionization in both polarities. Chromatographic separation was performed on a C18 HSS T3 column with gradient elution using 0.1% acetic acid and acetonitrile. The analytical run times were 13.5 minutes in negative polarity and 8 minutes in positive polarity.

Due to the quite different physicochemical properties of the target compounds, various solid-phase extraction (SPE) sorbents were tested, including hydrophilic-lipophilic balance (HLB), mixed-mode anion exchange, and mixed-mode cation exchange. Optimal recoveries for most analytes (70-121%) were obtained with HLB cartridges at pH 2, while direct injection of diluted wastewater samples was used for compounds present at higher concentrations (in the $\mu\text{g/L}$ range) in wastewater. More than 20 isotopically labeled analogs were used as internal standards to ensure accurate quantification. The accuracy of the method was generally acceptable, with a trueness ranging from 51 to 126% and a precision (RSD) of $\leq 23\%$, while the method quantification limits were in the low ng L^{-1} range. Model stability tests were also conducted to evaluate biomarker degradation under different storage conditions. Significant degradation was observed at room temperature ($25\text{ }^{\circ}\text{C}$), particularly for vitamins and flavonoids, while biomarker integrity was mostly maintained when samples were stored at $4\text{ }^{\circ}\text{C}$, emphasizing the need for controlled sampling and storage conditions.

The method was successfully applied to wastewater samples from three Croatian cities, where most of the target biomarkers were detected at concentrations between 30 ng L^{-1} and $50\text{ }\mu\text{g L}^{-1}$. The most prominent compounds were artificial sweeteners, and metabolites of alcohol, nicotine, and caffeine, while concentrations of dietary biomarkers were generally lower. The UHPLC-MS/MS method proved to be robust in the analysis of lifestyle and dietary biomarkers, with an optimized SPE protocol and isotopically labeled internal standards ensuring precise quantification in the complex wastewater matrix. This analytical approach will be used to study spatial and temporal variations in lifestyle and dietary habits in Croatian regions.

P2-015

INVESTIGATING MICROPLASTIC-CONTAMINANT SORPTION USING SPME COUPLED TO LEI-QqQ

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Water quality in the European Union is increasingly threatened by the presence of emerging pollutants (EPs) and microplastics (MPs) in freshwater systems. MPs, ranging from 100 nm to <5 mm[1], are ubiquitous environmental contaminants that adsorb hazardous organic pollutants, including Polycyclic Aromatic Hydrocarbons (PAHs), Per- and Polyfluoroalkyl Substances (PFASs), and pesticides, posing significant risks to ecosystems and human health[2], [3], [4]. This study introduces an innovative analytical approach combining Solid Phase Microextraction (SPME) with Microfluidic Open Interface (MOI) and Liquid Electron Ionization Mass Spectrometry (LEI-MS) to investigate pollutant adsorption onto MPs. LEI-MS offers reduced matrix effects, allowing external calibration for a direct-MS technique and providing an effective tool for continuous monitoring of organic contaminants in aquatic environments[5], [6].

Experimental procedures were conducted in a 500 mL glass bowl, containing water fortified with PAHs (50 ppb), pesticides (80 ppb) and microplastic pellets (200 pellets of Low Density Poly Ethylene (LDPE) and 254 pellets of Polypropylene (PP)), with their mass recorded prior to the experiment. Adsorption dynamics were assessed by collecting 1 mL aliquots from the solution at specific time intervals. After had determining the equilibration time in the kinetic experiment, the thermodynamic experiment was performed in a scaled down version of the kinetic experiment. After reaching the equilibrium at 30 hours, 8 aliquots of 1 mL were taken and extracted with Hydrophilic-Lipophilic Balance/Polyacrylonitrile (HLB/PAN) and C₁₈/PAN SPME fibers. The MOI enables direct coupling of SPME with LEI-MS. The MOI-LEI-MS system facilitates rapid analysis of liquid samples by integrating direct sample introduction into the ionization source. MOI operates on the principle of flow-isolated desorption, allowing the insertion of the SPME fiber into a chamber, where analytes are desorbed and transferred to the ionization region. LEI generates gas-phase analytes before they reach the electron ionization source, ensuring efficient ionization.

The research focuses on the kinetic and thermodynamic aspects of pollutant adsorption on PP and LDPE MPs. Kinetic analysis of PAH adsorption (4-n-nonylphenol, naphthalene, anthracene, pyrene) revealed that PAHs followed pseudo-second-order kinetics. Similarly, the adsorption of pesticides (metalaxyl, chlorpyrifos, alachlor, chlorfenvinphos, dichlorvos, atrazine) on LDPE MPs adhered to pseudo-second-order kinetics. Thermodynamic analysis, including equilibrium adsorption capacity (Q_e) calculations, demonstrated higher adsorption of chlorpyrifos (6.0 µg/g), anthracene (3.6 µg/g), pyrene (3.6 µg/g) and chlorfenvinphos (3.2 µg/g) compared to other compounds, while naphthalene (2.7 µg/g), 4-n-nonylphenol (2.2 µg/g), alachlor (1.2 µg/g), metalaxyl (1.0 µg/g), dichlorvos (1.0 µg/g), and atrazine (<1.0 µg/g) exhibited lower adsorption. These findings provide critical insights in MP-EP interactions, enhancing understanding of contaminant transport and persistence in freshwater ecosystems. This study informs microplastic pollution dynamics and supports strategies to mitigate emerging chemical threats to water quality.

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P2-016**ANALYTICAL POTENTIAL OF X-RAY FLUORESCENCE SPECTROMETRY BASED TECHNIQUES IN STUDIES DEALING WITH THE PRESENCE AND ADSORPTION OF METALS IN MICROPLASTICS**

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At present, water pollution by microplastics (MPs) is a significant environmental issue, affecting aquatic ecosystems and human health. These plastic fragments, smaller than 5 millimetres, result from the degradation of larger plastic items and can persist in the environment for centuries. Most used plastics are polystyrene (PS), polyethylene (PE) and polypropylene (PP) due to their properties and low cost.

From an analytical point of view, it is of special interest to have analytical techniques for not only properly identifying the polymer type of MPs, but also for obtaining information about the element content of these particulate materials. Metals and organometallic compounds (i.e., Fe, Zn, Ti, Ba, Br, Cd, Pb) are commonly blended with polymer matrix to improve plastic items qualities and lower production costs. As these additives are not chemically bound to polymer molecules, they can gradually leach into the environment becoming a source of inorganic chemical pollution. In addition, the presence of MPs in aquatic environments raised concern about possible enrichment of organic and inorganic pollutants due to their large specific surface and chemical properties. In particular, the role of metals in this context is still poorly understood.

In this contribution, the analytical potential of different X-ray fluorescence (XRF) systems in studies dealing with the presence and adsorption of metals in MPs has been evaluated.

On the one hand, a m-XRF method for the multielemental analysis of plastic samples was developed. The calibration procedure was established by employing a set of three plastic certified reference materials and several secondary standard calibrators, previously characterized by other analytical techniques. A thickness correction model applied to the spectra evaluation was employed to compensate thickness variances in real plastic samples. The developed m-XRF method seems to be adequate for the determination of most elements present in plastics with limits of detection in the range of 10-50 mg/kg. The method was applied for the multielemental analysis of a set of MPs collected in the Atlantic Ocean (Azores) and the Mediterranean Sea. Results showed the presence of environmental relevant metals such as Pb and Cr in some of the target samples.

On the other hand, in a second set of experiments, the analytical potential of Total reflection X-ray fluorescence spectrometry (TXRF) and energy dispersive X-ray fluorescence spectrometry (EDXRF) was evaluated to monitor the metal content adsorbed to the MPs in batch adsorption studies performed to enhance the understanding of metal sorption processes in MPs. In this type of experiments, usually commercial, pristine and spherical MPs prepared at specific sizes are considered. Some recent publications have showed up the limitations of using these commercial materials as environmentally representative MPs. For that, more “real-to-life” MPs, prepared from micronized fragments of single-use plastics, were used instead. Specifically, MPs made of PE, PP and PS with different sizes (<63 µm and 63-80 µm) were prepared and used. It is interesting to mention that MPs in the natural environment can undergo aging processes that can lead to changes in the surface and physicochemical properties of MPs and their bioavailability and adsorption capacities. For that, the same MPs were also submitted to an aging process with UV light (8 weeks at 42 °C) to simulate the effect of sunlight on MPs once they have entered to the aquatic environment. After the metal adsorption experiments, the supernatants were analysed by TXRF and the MPs collected on filters directly analysed by EDXRF, without any sample treatment. Results demonstrated that metal adsorption depends on the polymer type but mostly on the aging processes, being the aged MPs the ones that exhibited a higher metal adsorption capacity.

P2-017**NOVEL TECHNIQUES FOR ULTRA-SENSITIVE DETECTION OF PFAS IN LC-MS/MS****Kawakami, Kazuhiro¹; Iwasa, Nami¹; Higashi, Yui¹; Kitano, Riki¹; Fujito, Yuka¹; Moreau, Stephane².**¹Shimadzu Corporation, Kyoto, Japan; ²Shimadzu Europa GmbH, Duisburg, Germany.**Introduction**

Per- and polyfluoroalkyl substances (PFAS) have been widely used in various fields and industries. Due to their structural stability and resistance to decomposition, they easily accumulate in the environment and are suspected of being harmful to humans. Recently, PFAS regulations and surveys have strengthened, and standardization of analytical methods has been discussed.

In April 2024, EPA set Final Maximum Contaminant Levels (MCLs) for PFAS in drinking water: 4 ng/L for PFOA and PFOS, and 10 ng/L for PFHxS, PFNA, and HFPO-DA. With the strengthening of regulatory limits, there is a growing demand for analytical methods that can perform measurements with higher sensitivity and greater stability.

In this study, we report a highly sensitive PFAS analysis using newly designed LC-MS/MS techniques.

Methods

The sample pretreatment followed EPA 533 and 537.1. Semi-automated solid phase extraction method was used for the sample pretreatment. The eluate from the SPE cartridge was completely dried, dissolved in 1 mL of the specified solvent, and the internal standard solution was added for measurements. Direct injection of water samples was also performed. Methanol and internal standards were added to the water samples for the direct injection. The measurements were carried out for the compound panels of EPA 533 and 537.1 using a LC-MS/MS.

In addition to the analytical column, a delay column was installed between the mixer and autosampler for these analyses to control the effects of PFAS contamination from the LC system and the mobile phases.

Preliminary Data

Solid Phase Extraction samples: The lowest concentration minimum reporting level (LCMRL) was set at 0.2 ng/L in water (analytical concentration 50 ng/L) by the results of the measurement of the standards. The reproducibility (%RSD) of the main compounds at 0.2 ng/L in water were observed in EPA 533: PFOA 3.1%, PFOS 2.7%, PFHxS 5.0%, PFNA 7.6%, HFPO-DA 8.6%, PFBS 7.6%, in EPA 537.1: PFOA 2.3%, PFOS 3.9%, PFHxS 9.6%, PFNA 7.6%, HFPO-DA 5.8%, PFBS 1.4%, respectively. Both repeatability and accuracy at each concentration of the standards met EPA requirements for all compounds. The calibration curves showed good linearity with $R > 0.995$ for all compounds in both EPA methods 533 and 537.1. In addition, the recovery rates of drinking water spiked with 4 ng/L were within 80 to 120 %. The recovery rates of the main compounds were observed in EPA 533: PFOA 91%, PFOS 94%, PFHxS 109%, PFNA 98%, HFPO-DA 106%, PFBS 105%, in EPA 537.1: PFOA 93%, PFOS 100%, PFHxS 101%, PFNA 99%, HFPO-DA 91%, PFBS 92%, respectively.

Direct injection samples: The sample was analyzed as a 50% methanol solution to prevent adsorption to the vial. Concentrations below the MCLs were successfully detected with excellent reproducibility with %RSD < 15 for the target compounds listed in EPA 533 and EPA 537.1. The calibration curves showed excellent linearity with $R > 0.995$ within the required dynamic range. We confirmed that the newly developed LC-MS/MS method provided higher signals and lower noise compared to conventional techniques, supporting high-sensitivity analysis of PFAS.

Novel Aspect

We developed a highly sensitive LC-MS/MS analytical method of target compounds listed in EPA 533 and EPA 537.1.

P2-018**MINERAL COMPOSITION OF PELOIDS IN SOUTHWESTERN SERBIAN SPAS****Mutić, Tijana¹; Vukićević, Emilija²; Veselinović, Gorica¹; Roganović, Jovana²; Gajica, Gordana¹.**

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Serbia hosts over 50 spas with more than 1,000 mineral water sources, offering a wide variety of geological, hydrogeological, and geothermal characteristics. Some spas provide therapeutic waters, mud, and gases, while others function as air spas due to their geographic location. Despite this diversity, the systematic study of Serbian peloids remains limited. The PELAS project (Peloids in Serbia: Geochemical Characterisation, Quality Assessment, and Ecosystem Services of Peloid-Rich Areas) seeks to investigate the geochemical and ecochemical characteristics of peloids from 15 commercial spas across Serbia. Peloids, or healing matured mud, have been used for therapeutic, muscle, bone, and skin treatments and relaxation purposes since ancient times. These muds are composed of natural materials of geological or biological origin and enriched thermal, mineral, or seawater [1,2]. Peloids are considered economically valuable natural resources, utilized in healing, therapy, or cosmetics, depending on their thermal, chemical, and mechanical properties [3]. Peloids contain essential, trace, and potentially toxic elements. Some essential elements, when absorbed through the skin, ingested, or inhaled, may cause health issues or toxicity in humans. Since peloids are used in pelotherapy (direct skin application), it is crucial to investigate the content, mobility, and bioavailability of elements present in these clays, particularly in therapeutic and cosmetic applications.

This study involves a comprehensive elemental analysis of peloids using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). The peloids were collected from five spas in Southwestern Serbia: Jošanička, Niška, Lukovska, Kuršumlijska, and Prolom Spa. The analyzed elements include macroelements (Al, Ca, Fe, K, Mg, Na, P, and S) and microelements (As, B, Ba, Bi, Cd, Co, Cr, Cu, Hg, Li, Mn, Ni, Pb, Sr, and Zn). The samples of peloids were prepared using an acid digestion procedure with HNO₃: HCl (1:3 v/v). The pseudo-total concentrations of metals in the peloids were determined after aqua regia digestion. Results indicate that Niška spa samples contained higher macroelement concentrations, while Lukovska spa exhibited elevated levels of microelements. These findings provide valuable insights into the composition and applications of Serbian peloids in the therapeutic and cosmetic industries.

Acknowledgments

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P2-019**RADIOLOGICAL ASSESSMENT OF THERAPEUTIC PELOIDS FROM SERBIAN SPAS**

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The PELAS project (Peloids in Serbia: Geochemical Characterisation, Quality Assessment, and Ecosystem Services of Peloid-Rich Areas) aims to investigate the geochemical and ecochemical characteristics of peloids from 15 commercial spas across Serbia [1]. Serbia has a rich tradition of balneotherapy, with numerous spas utilizing peloids for therapeutic purposes. Peloids, commonly known as healing mud, are used in pelotherapy by direct skin application for their thermal, chemical, and mechanical properties, particularly in the treatment of rheumatic diseases, dermatological conditions, and musculoskeletal disorders, as well as in cosmetic applications [2]. However, concerns have been raised regarding the natural radioactivity of peloids due to the presence of radionuclides in their clay composition, necessitating a comprehensive assessment of their radiological safety.

In this study, 16 samples of peloids from 15 Serbian spas were analyzed to determine their radionuclide activity concentrations. The concentrations of ²²⁶Ra, ²³²Th, ⁴⁰K, ²³⁸U, ²³⁵U, ¹³⁷Cs, and ²¹⁰Pb were determined using a gamma ray spectrometer with a high-purity germanium (HPGe) detector. Results showed that ¹³⁷Cs activity was below the detection limit, except in four samples, indicating no significant contamination from anthropogenic sources. The levels of naturally occurring radionuclides varied within expected natural ranges, where ⁴⁰K exhibited the highest average activity concentration of 600 Bq/kg. Despite the absence of specific regulatory limits for radionuclide concentrations in medicinal peloids, a comparative analysis with international studies revealed that the measured values are consistent with those found in peloids worldwide [3]. These findings contribute to the radiological characterization of Serbian peloids, supporting their continued safe use in therapeutic and cosmetic applications while providing a baseline for future geochemical and radiological monitoring.

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P2-020**DETERMINATION OF IMPACTS OF METAL POLLUTANTS ON WATER FROM VRANSKO LAKE CROATIA BY ICP-MS****Nemet, Ivan; Ilić, Nikolina; Rončević, Sanda; Fajković, Hana; Marković, Frane.***Faculty of Science, University of Zagreb, Zagreb, Croatia.*

In recent times we can witness changes of continuous pressure on the lake's ecosystem status which facilitate the accumulation of emerging, non-regulated, chemical contaminants and nutrient enrichment.^{1,2} Scientifically-based Action Plan for the restoration and protection of European Natural Lakes (ENL) ensure its adoption by relevant decision-makers, such as lake managers, local authorities, and policymakers.³ This plan is developed as a part of project ProCleanLakes, funded by the European climate, infrastructure and environment executive agency (CINEA) within the framework Horizon Europe Climate. Among wide categories of solutions this project is focused on enhancing the ecological and chemical status of the lakes, increasing biodiversity, and utilizing new nature-based solutions. Vransko lake in Croatia is an area of special interest within the project, where it is acting as a monitoring site for project goals. Vransko Lake is the largest natural lake in Croatia, covering an area of 30.2 km². It's 13.6 km long and between 1.4 and 3.4 km wide. The lake runs parallel to the sea coast in a northwest-southeast direction and is separated from the sea by a limestone ridge that ranges from 800 to 2,500 meters wide, with the highest point at 113 meters above sea level. Because it's shallow, the water temperature can change quickly depending on the air temperature, ranging from 2.9°C in February to 25.2°C in July. The lake has slightly brackish water with a salinity of 0.16-0.86‰. As part of the project, a comprehensive sampling process was carried out, followed by the development of a detailed analysis strategy. Key parameters for the analysis were carefully selected, with a focus on measuring metal content within the samples. These metal concentrations were then compared with previously collected data, allowing for a thorough assessment of any significant changes or trends over time. This comparison provided essential insights into the current levels of metals, helping to identify potential environmental or health-related implications. Based on historical data, a slight but noticeable increase has been observed in the concentrations of several elements, including aluminum (Al), cobalt (Co), chromium (Cr), iron (Fe), and manganese (Mn). Despite these increases, the measured concentrations remain well within the maximum permissible limits established for environmental and public health safety. The chemical composition of water and sediment samples collected from Vransko Lake in October 2024 was determined using both standard classical and spectrometric analytical methods. Metal content was analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), while classical chemical techniques were employed to measure chlorides, ammonia, water hardness, and chemical oxygen demand. The distribution of the measured components in the water and sediment samples will be used to monitor changes in the chemical composition of the aquatic system and assess potential environmental impacts. The analysis of the obtained results will serve as the basis for the development of natural-based solutions models.

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P2-021**ELEMENTAL COMPOSITION AND BIO-BASED CONTENT OF POLYMERS USING ICP-OES AND ¹⁴C TECHNIQUES**

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Plastics are one of today's dominant factors, widely used in many areas of industry and life over the years thanks to their versatility and low production costs. More than a third of the world's plastic production is used for packaging that comes into contact with food and the human body. Environmental concerns and the limited availability of fossil resources mean that plastics from renewable sources are becoming increasingly important. The present study presents a rather new perspective on the analysis of partly fossil and partly bio-based polymers using accelerator mass spectrometry radioactivity (C-14) and inductively coupled plasma optical emission spectrometry techniques. In our research, different types of polymer samples were analysed, including raw materials, commercially available finished products and selected materials from recycling plants. Elemental analysis was used to determine the amounts of selected elements (Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cu, Cr, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Pd, S, Sb, Sn, Sr, Ti, Zn). The measured concentration values give a good indication of the quantitative relationships of the additives added during the manufacturing processes and the importance of Ca. When the concentrations of lead and cadmium in the samples were compared with the limit values of the EU legislation, it was found that only one bin liner with a lead concentration of 294 mg kg⁻¹ exceeded the limit value of 60 mg kg⁻¹. The bio-based carbon content of each fossil and bio-based polymer tested by our C-14 measurements was within the expected quantitative ranges. In the case of the PLA-based samples, we observed products that differed from the expected value and were found to be a mixture. By comparing the two analyses, it was concluded that despite the lack of a close relationship, a much more comprehensive picture of the polymers can be obtained by combining the two measurement techniques.

P2-022**OCCURRENCE AND ASSESSMENT OF PER- AND POLYFLUOROALKYL SUBSTANCES IN AIR AND WATER SAMPLES**

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Per- and polyfluoroalkyl substances (PFASs) are a group of persistent organic pollutants comprising over 9,000 chemicals. The extensive production and use of these compounds, combined with their chemical stability, led to their bioaccumulation in both the environment and humans [1,2]. Recent studies revealed harmful health effects associated with PFASs exposure, such as reproductive disorders, neurotoxicity and carcinogenicity among others, highlighting the need for regulating their levels in the environment.

Drinking water is a major exposure route, prompting the European Directive 2020/2184 to limit 20 PFASs to a sum of 0.1 µg/L, with specific limits for PFOA (perfluoro-n-octanoic acid), PFOS (perfluoro-1-octane sulfonate), PFNA (perfluoro-n-nonanoic acid), and PFHxS (perfluoro-n-hexanoic acid) set at 0.07 µg/L each. The presence of PFASs in other matrices, such as dust and indoor/outdoor air, has yet to be regulated. Nevertheless, the atmospheric distribution of PFASs is particularly concerning due to their long-range transport potential, which facilitates human exposure through inhalation, ingestion, and dermal contact [1].

PFASs are generally found in environmental samples at trace levels, highlighting the need of robust and reliable analytical methods for long-term monitoring and a better understanding of their behavior in the environment. Here, is described a liquid chromatography tandem mass spectrometry (LC-MS/MS) based method for the determination of the 20 most characteristic PFASs in both drinking water and airborne particulate matter. Off-line solid phase extraction (SPE) was used to preconcentrate PFASs from 100 mL drinking water samples [2]. 24-hour 700 m³ airborne particulate matter samples were collected with a MCV-PM₁₀ High Volume Air Sampler. More specifically, PM₁₀ particles were deposited on quartz fibre filters (150 mm Ø). Pressurized liquid extraction (PLE) with methanol was applied for the extraction of PFASs before LC-MS/MS.

PFAS in drinking water have mostly been found under the LOQ of < 0.005 µg/L. From 2023 to 2025 only 2 samples exhibited a concentration of 0.025 µg/L for the sum of 20 PFAS being the perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-pentanoic acid (PFPeA) and perfluoro-n-heptanoic acid (PFHpA) over the LOQ. However, the mean of the 50 samples (0.001 µg/L) does not reach the LOQ. Surface water exhibited a relatively higher presence of PFAS, mean concentration for the sum of 20 PFAS is 0.003 µg/L, with only one sample reaching the 0.067 µg/L.

The analysis of PM₁₀ air samples from two urban locations in the area of Tarragona (Spain) revealed the widespread occurrence of PFASs, with average concentrations between <LOQ and 106 pg/m³. PFBA and PFPeA were the most frequently detected compounds with maximum concentration peaks of 202 pg/m³ for PFBA and 178 pg/m³ for PFPA.

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P2-023

ENHANCING MICROPOLLUTANT REMOVAL EFFICACY THROUGH THE MICROLOG CONCEPT: NOVEL OZONATION APPROACHES IN WASTEWATER TREATMENT SYSTEMS

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Population growth and increased freshwater consumption have significantly raised the amount of contaminants discharged into water sources [1]. Two of the families that have raised concern are pharmaceuticals and pesticides, which are not effectively removed in conventional wastewater treatment plants (WWTP). In response to this issue, additional treatments to conventional methods have been proposed to ensure reduced environmental contamination [2]. Recently, Dantas et al. [3] introduced the innovative Microlog concept, which establishes a quantifiable correlation between indicator bacteria inactivation and organic micropollutant (OMP) removal during ozonation processes, offering a practical alternative to expensive analysis by using commonly measured disinfection parameters as surrogates for monitoring MP removal efficiency. This study evaluates the ozonation process effectiveness for the OMP removal in treated wastewater in Brazil, applying the Microlog concept. For this, three different secondary wastewater (SW) samples and a total of 36 OMP, including 20 pharmaceutical, 2 lifestyle-related, and 14 pesticides, were analyzed. The SW samples were collected from three different WWTP, after secondary treatment, and the OMP were spiked to the SW at 1 µg/L and then, subjected to an ozonation process, at a constant ozone dose in a stirred reactor containing the spiked solution sample. Samples were taken at different time intervals (0, 2, 4, 7, 10, 15, 30, and 60 minutes) to determine the optimal ozone dose for the OMP removal. The collected samples were analyzed using two approaches: one fraction was processed by solid-phase extraction (SPE) to assess the OMP removal, while the other fraction was analyzed for disinfection efficiency, specifically evaluating the elimination of total coliforms and *Escherichia coli* (*E. coli*). The SPE-processed samples were analyzed using ultra-high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UPLC-MS/MS). Additionally, this study assessed the treatment efficiency at different temperatures: ambient temperature ($T = 25^{\circ}\text{C}$), representing autumn and spring conditions; elevated temperature ($T = 36^{\circ}\text{C}$), simulating summer conditions; and low temperature ($T = 14^{\circ}\text{C}$), representing winter conditions. By calculating Microlog80 values, which indicate the bacterial log reduction required to achieve 80% removal of target OMP, it is possible to provide the estimation of MP removal using standard disinfection measurements. The results will help assess the viability of ozone as an additional treatment for wastewater treatment to mitigate the environmental impact of OMP, while demonstrating the practical application of the Microlog concept in real-world treatment systems.

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P2-024**PRESENCE OF PFAS RESIDUES IN ALL HYDROGRAPHIC BASINS OF CATALONIA****Rodríguez Murillo, Natalia¹; Agulló Chaler, Núria²; Díaz-Ferrero, Jordi¹; Gómez-Canela, Cristian¹.**

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PFAS (per- and polyfluoroalkylated substances) are synthetic compounds characterised by carbon-fluorine (C-F) bonds, which provide them high chemical stability, heat resistance, and water and oil repellence (1). Due to these properties, they have been widely used in various industrial applications, including firefighting foams, agriculture, cosmetics, textiles, and food packaging, among others. However, their persistence in the environment, particularly in the aquatic environment, is a significant concern (1). In river ecosystems, PFAS can be transported over long distances, redistributing among different compartments such as water, sediments, and biota. Short- and ultra-short-chain compounds are highly soluble in water, which enhances their mobility and potential infiltration into groundwater. In contrast, longer-chain PFAS tend to adsorb onto particles and sediments, increasing their potential for bioaccumulation in aquatic organisms. Despite growing concerns, most studies have prioritised the detection of long-chain PFAS. At the same time, emerging substitutes, such as perfluoroalkyl carboxylic acids (PFCA) and shorter chain perfluoroalkyl sulphonic acids (PFSA), remain insufficiently characterised in terms of toxicity and environmental distribution (2).

To assess the potential adverse effects of these compounds on the aquatic environment, prior knowledge of their concentration levels is essential. Thus, the aim of this study is to determine the presence of 30 PFAS across all hydrographic basins in Catalonia. As the author knowledge, this is the first time that PFAS are monitored in all the rivers of Catalonia. Target compounds include different families of PFAS such as PFCA, PFSA, fluorotelomers (FTS), perfluorooctane sulphonamide (FOSA), and perfluorooctane sulphonyl fluoride (FOSE). Their chain lengths vary between 1 and 18 carbon atoms, influencing their solubility, persistence, and bioaccumulation potential in the aquatic environment. Samples were collected from 14 rivers in Catalonia—(Noguera Pallaresa, Noguera Ribagorçana, Tordera, Muga, Fluvià, Besòs, Llobregat, Segre, Ebro, Ter, Francolí, Gaià, Daró, and Foix)—with a total of 52 sampling points, including the source, intermediate points, and the mouth of each river.

For the analysis, 500 µL of pre-filtered sample was taken using regenerated cellulose (RC) syringe filters, spiked with deuterated internal standards, and mixed with 500 µL of methanol with 0.2% formic acid. Subsequently, 100 µL was directly injected without further sample treatment. Instrumental analysis was performed using an HPLC-MS/MS system. Chromatographic separation was conducted on an Exion LC system (SCIEX, MA) with a Luna Omega PS C18 analytical column (3 µm, 100 × 3 mm). The mobile phase consisted of ultrapure water with 2 mM ammonium acetate (Solvent A) and acetonitrile: methanol (2:1) mixture (Solvent B) in negative-mode electrospray ionisation (ESI⁻). MS/MS detection was carried out with a Triple Quad™ 7500 MS/MS system (SCIEX, MA) in MRM mode, acquiring two transitions per analyte (quantifier and qualifier), except for TFMS, PFPrA, and PFBA, where only one transition per target compound was used.

The results of this study provide the first detailed characterisation of PFAS distribution in Catalan rivers, revealing the widespread presence of these pollutants in the superficial waters. These findings reflect diverse contamination patterns depending on compound type and river characteristics. Moreover, the previously developed methodology enabled the simultaneous detection of ultra-short-, short-, and long-chain PFAS using a single analytical strategy, without requiring sample pre-treatment due to direct injection. This represents a key advantage in terms of analytical simplicity for large-scale studies such as this one.

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P2-025**PRESENCE AND CHARACTERISATION OF MICROPLASTIC RESIDUES IN ALL HYDROGRAPHIC BASINS OF CATALONIA**

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Plastic pollution in rivers has emerged over recent decades as a critical global environmental concern. It is estimated that more than 70% of the plastics found in the environment originate from terrestrial sources. These plastics undergo degradation through physical, chemical, and biological processes, resulting in the formation of microplastics (MPs; 1 µm–5 mm) (1). These emerging contaminants, primarily generated by urban runoff, industrial activity, and the degradation of larger plastic, can be transported via water and air, persisting in diverse ecosystems over extended periods (2). Their high specific surface area and hydrophobic nature facilitate the adsorption and transport of other pollutants. This increases their toxicity and promotes their entry into the trophic chain when ingested by aquatic organisms (3).

Rivers play a crucial role as transport routes for these MPs. This study aimed to evaluate, for the first time, their presence and distribution along the fluvial network of Catalonia. Fourteen representative rivers were selected (Noguera Pallaresa, Noguera Ribagorçana, Tordera, Muga, Fluvià, Besòs, Llobregat, Segre, Ebro, Ter, Francolí, Gaià, Daró and Foix), with a total of 52 sampling points, including the source, middle section and mouth of each river.

The samples were filtered and analysed to quantify the concentration of MPs per cubic metre of water. Sample pretreatment was applied to the samples where interferences, such as organic matter, was found. Characterisation of the MPs was carried out using optical microscopy (Nicolet™ In10MX) and Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet™ iZ10 FT-IR), which allowed the identification of their chemical composition and size distribution.

This research is the first comprehensive characterisation of MPs in the main rivers of Catalonia, revealing a widespread presence of these new pollutants throughout the river network. The findings allow the identification of the areas with the highest microplastic loads and potential emission sources, which is crucial for prioritising environmental management strategies. The results emphasise the need to extend this line of research through seasonal studies, which would help to assess the influence of factors such as river discharge, rainfall and anthropogenic activity on the spatial and temporal variability of microplastic pollution in river systems.

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P2-026**UNVEILING CONTAMINANTS OF EMERGING CONCERN: A STUDY ON WATER QUALITY IN THE MAPOCHO RIVER BASIN (CHILE)**

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Water is a vital compound for sustaining life and is essential for various daily activities. However, due to human activities, a multitude of chemical compounds can contaminate water sources, ultimately finding their way into wastewater treatment plants (WWTPs) via the sewerage system. These compounds, known as Contaminants of Emerging Concern (CECs), lack regulatory guidelines dictating maximum allowable concentrations for detection. WWTPs, originally not designed for the removal of such recalcitrant compounds, serve as the primary conduit for their release into the environment. Despite typically occurring in water at concentrations ranging from ng/L to µg/L, reports suggest their potential adverse effects on the environment, though many of these effects remain largely unexplored.

The objective of this study is to conduct a comprehensive screening for CECs, with a specific focus on the presence of pharmaceutical compounds, pesticides, and drugs of abuse. This screening will involve analyzing water samples collected from both the Mapocho River (Chile) and Aguas Santiago Poniente wastewater treatment plants.

In this study, the presence of CECs was assessed using liquid chromatography coupled with mass spectrometry (LC-MS). The water samples were collected between the months of May and June of 2022 in the vicinity of the Aguas Santiago Poniente Wastewater Treatment Plant. Sampling points included the Mapocho River both before and after the tertiary treatment discharge of the WWTP, as well as the influent, secondary effluent, and tertiary effluent (post-chlorination). To enhance detection sensitivity, the samples underwent pre-concentration through solid-phase extraction cartridges before undergoing LC-MS analysis. Additionally, various physicochemical parameters, such as pH, conductivity, total dissolved solids, turbidity, and chemical oxygen demand, were measured.

The study allowed the identification or tentative identification of 73 CECs in the water samples analysed. This set of contaminants includes drugs of abuse, pesticides, pharmaceuticals, and metabolites of pharmaceuticals. Among the pharmaceutical compounds detected, antibiotics, antihypertensives, and anti-inflammatory drugs were the most prevalent, constituting 28%, 12%, and 12% of the total compounds identified, respectively. These results highlight the diversity and complexity of the compounds present, providing a detailed insight into the chemical composition of the effluent and the need to comprehensively address the management of these emerging contaminants. The river samples and the effluent samples treated by the WWTP comply with the water quality standards established by Chilean legislation for freshwater bodies.

The analysis revealed the presence of various CECs in all examined water samples, with the WWTP identified as the primary source of entry into the Mapocho riverbed. Notably, a significant proportion of these contaminants belong to diverse categories of pharmaceuticals employed for treating various medical conditions, with antibiotics constituting the majority (31%). This finding raises concerns due to the potential development of bacterial resistance to antibiotics in the presence of these chemical compounds and aligns with the World Health Organization's recognition of antimicrobial resistance as a top-ten global threat to public health³.

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P2-028**DETERMINATION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN BIOFILMS AND *LEMNA* BY A MODIFIED QuEChERS-based UPLC-MS/MS METHOD AND ITS APPLICATION TO NATURAL WATERS AND NATURE-BASED WATER TREATMENT SYSTEMS**

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Biofilms are microbial communities that adhere to surfaces in aquatic environments, forming matrices composed of cells and extracellular substances such as polysaccharides and proteins. These biofilms play a crucial role in the adsorption, accumulation, and degradation of organic microcontaminants in natural waters, as well as facilitating their removal in wastewater treatment systems. This study presents an innovative method for the determination of selected pharmaceuticals and personal care products (PPCPs) in biofilms and *Lemna* (duckweed), based on a modified QuEChERS procedure.

The developed methodology employs focused ultrasound solid-liquid extraction (FUSLE) followed by dispersive solid-phase extraction (dSPE) clean-up, coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode. This approach is optimized for the quantitative determination of five classes of pharmaceuticals: non-steroidal anti-inflammatory drugs (NSAIDs), antiepileptics, antidepressants, a lipid regulator, and antibiotics. The target compounds include sulfamethoxazole, clofibric acid, naproxen, ibuprofen, carbamazepine, diclofenac, citalopram, gemfibrozil, paroxetine, fluoxetine, and triclosan.

Various sample pre-treatment approaches were evaluated, including freezing, dry freezing, and grinding with liquid nitrogen, which was the most effective. For the extraction of target analytes from biofilm and *Lemna*, several solvents (acetonitrile, acetone, and methanol) were tested, with methanol giving the best results. The solid-liquid extraction process was further enhanced by focused ultrasonication, with optimization of key parameters such as sonication time, amplitude, pulse, and frequency. The best results were achieved with three cycles of 3 minutes each, using an amplitude of 25%, a pulse duration of 2 seconds (ON) and 1 second (OFF). After each sonication cycle, the samples were centrifuged at 4000 rpm for 5 minutes.

For the clean-up of extracts by dSPE, various sorbents and combinations were tested. The optimized procedure used 150 mg of Septra™ C18-E sorbent and 15 mg of activated carbon powder. The limits of detection (LODs) and limits of quantification (LOQs) for the targeted compounds ranged from 0.9–9 ng/g and 0.9–18 ng/g, respectively. Recovery of spiked samples ranged from 75% to 120% for biofilm samples and 70% to 130% for *Lemna* samples, demonstrating satisfactory accuracy and precision.

The developed methodology was successfully applied to the analysis of biofilms from the Onyar River, Spain, and a pilot nature-based wastewater treatment system, consisting of a cascade photobioreactor tank that uses microalgae and biofilm, in combination with solar UV radiation, which has shown to be very effective in removing nutrients, pathogens, and emerging contaminants. In the samples analysed, the values found for the PPCPs targeted ranged from 0.05 to 4.5 µg/g in both biofilm and *Lemna* samples.

This proposed method represents an innovative tool for the quantification of PPCPs in biofilms and plants such as *Lemna* and offers a robust solution for determining emerging contaminants highlighting the key role of biofilms in enhancing the removal of PPCPs in both natural waters and nature-based water treatment systems, optimizing treatment efficiency and reducing environmental impact.

P2-029**DETECTION OF POTATO DISEASES VIA VOLATILE ORGANIC COMPOUND ANALYSIS**

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The potato is an important staple food in Germany and Europe. Numerous pests e.g. stem and stolons canker, potato wart disease, potato brown rot, potato ring rot, black scurf and others cause crop losses every year. To minimize these losses, early detection of an infestation is of crucial importance. One method to ensure such early detection is the so-called volatile organic compound (VOC) analysis.¹

Plants emit a variety of VOCs, e.g. to communicate with each other or when they are exposed to biotic and abiotic stress.² As a result, the composition of the emitted VOCs (VOC profiles) changes as the plant tries to counteract the stress. By investigating these changes in the VOC profiles during pest infestation, patterns and markers can be identified that are specific to the pest. This allows early detection and enables control and containment strategies to be implemented.³

In this study, gas chromatography-mass spectrometry (GC-MS) will be used to analyse the VOC profiles of potatoes exposed to various potato pests. Therefore, a Python® script was written which combines pre-processing of the raw data, data merging of train and test data, data reduction using principal component analysis (PCA) and classification using linear discriminant analysis (LDA). With the help of this multivariate evaluation method possible patterns and markers specific to the respective pest infestation are to be identified. The functionality of this evaluation method was already tested and proven using a dataset that had already been evaluated manually, as the results not only matched the manually evaluated ones but also refined them.

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P2-030

STRATEGIES FOR THE ISOLATION OF ARSENOSUGARS EXTRACTED FROM ALGAE

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Arsenosugars, ribose derivatives of arsenic, are becoming more significant because of their predominance in algae and the increasing incorporation of such vegetal products into the diet. Although these compounds do not appear to be acutely toxic, they have potential for mild chronic toxicity. Therefore, given the increasing consumption of seaweed, assessment of arsenosugars exposure becomes a need. On the other hand, the analysis of these compounds is hampered by their similar physical and chemical properties and the difficulty to obtain the calibration standards needed for their unambiguous identification and quantification. The reported methods in the literature to obtain several standards are diverse and have limitations. Chemical syntheses of some arsenosugars are quite complex and by now needs further yield improvements. As alternative, separation and purification of complex mixtures coming from algae have been also attempted.

This communication addresses three approaches assessed in our research group to achieve the isolation of arsenosugars, the first based on the use of high-resolution preparative chromatography (Yu et al., 2020), the second based on solid-phase extraction (Morales-Rodríguez et al., 2022), and finally a third one based on countercurrent chromatography (Morales-Rodríguez et al., 2024) in centrifugal partition mode (CPC).

A preparative anion exchange column (Hamilton PRP-X100) with an ammonium bicarbonate mobile phase was used for fractionation of aqueous extracts of a selected sample containing the three anionic arsenosugars. Several peaks containing arsenic were obtained, but only SO₃-Sug and SO₄-Sug were completely isolated, whereas PO₄-Sug fractions contained little amounts of MMA. When using a mobile phase containing ammonium formate the isolation of the PO₄-Sug is achievable. In all cases, purity of obtained fractions was higher than 99%.

The use of strong anion exchange SPE cartridges allows the isolation of SO₃-Sug, SO₄-Sug and PO₄-Sug as the only arsenic species present in the solution. In all cases, recoveries over 75% were obtained for SO₃-Sug and SO₄-Sug, whereas for PO₄-Sug recoveries were around 45%. Similar arsenosugar recoveries were obtained when different elution conditions are used.

Finally, CPC was also evaluated for the separation of arsenosugars present in algae. After testing several partitioning systems (presence of strong acids, formation of ionic pairs or presence of salts at high ionic strength), adequate distribution constants were obtained only for the systems 1-BuOH/MeOH/TFA 0.2% (8:4:18) and 1-BuOH/EtOH/sat. (NH₄)₂SO₄/water at a volume ratio 0.2:1:1:1. Only this latter could be used because of the lack of retention of the stationary phase in the column for the former. Using the 1-BuOH/EtOH/sat.(NH₄)₂SO₄/water solvent system, it was possible to obtain the three arsenosugars with a purity of 98.7%, 90.4% and 96.1% for PO₄-Sug, SO₃-Sug and SO₄-Sug, respectively. These results are comparable to those obtained when preparative chromatography was applied.

The results obtained show that it is possible to isolate anionic arsenosugars using the proposed techniques, although further improvements are still required. These may be considered the first steps toward achieving the analytical standards for arsenosugars that are demanded in the literature from different research fields, such as toxicology, environment, and food sciences.

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P2-031**EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM COCOA BEAN SHELL USING GREEN APPROACHES****Boni, Sk Fahim Tahmid¹; Sentellas, Sonia²; Saurina, Javier².**¹*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona, Barcelona, Spain;*²*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona, Barcelona, Spain.*

Cocoa (*Theobroma cacao* L.) is one of the most important crops worldwide, particularly in tropical regions, where it serves as a critical source of income for millions of smallholder farmers. The global cocoa industry is valued not only for its contribution to food products such as chocolate but also for its role in economic development. However, cocoa processing generates a considerable volume of by-products, including cocoa bean shells (CBS), which holds significant potential for value-added recovery within a circular economy framework.

This study aims to recover methylxanthines, polyphenols, pectin, and lignin using sustainable extraction techniques and environment-friendly solvents. Firstly, the extraction of methylxanthines and polyphenols was carried out using ethanol/water mixtures as the solvents. Conventional (mechanical stirring extraction) and advanced (microwave-assisted extraction and ultrasound-assisted extraction) techniques were assessed. The influence of ethanol percentage, temperature, and extraction time was evaluated to determine optimal conditions. Quantitative analysis was performed using high-performance liquid chromatography (HPLC-UV) and spectrophotometry to assess the methylxanthine content and antioxidant activity. Additionally, major polyphenols, such as catechin, epicatechin, and procyanidins, were identified and quantified by LC-MS/MS. Results indicate that mechanical stirring extraction at 50°C for 30 min with 50% ethanol/water yielded the highest recovery while maintaining energy efficiency and scalability.

Following polyphenol recovery, pectin extraction was performed under acidic conditions and optimized using response surface methodology (RSM) to maximize yield and quality. The obtained product was characterized by IR, SEM, TGA, and XRF. The ongoing research aims to extend this sequential valorization approach to extract lignin, cellulose, and hemicellulose from CBS. These findings highlight CBS as a promising raw material for food, pharmaceutical, and nutraceutical applications, supporting sustainable waste valorization and resource efficiency.

P2-032**GENERIC SUPRAS-BASED MICROEXTRACTION METHOD FOR THE DETERMINATION OF MYCOTOXINS IN SOLID AND LIQUID FOOD PRODUCTS****Muñiz-Bustamante, Luis; Caballero-Casero, Noelia; Rubio, Soledad.***University of Córdoba, Córdoba, Spain.*

Mycotoxins rank among the top three most frequently reported hazards in the latest Rapid Alert System for Food and Feed (RASFF) report, affecting both EU and non-EU food products. The International Agency for Research on Cancer (IARC) classifies these compounds based on their carcinogenic potential in humans (Groups I, IIA, and IIB), which has prompted the implementation of regulatory limits for their presence in various food matrices, depending on their composition. Consequently, it is necessary the development of robust analytical methods capable of simultaneously quantifying multiple mycotoxins at regulated concentrations in both solid and liquid foods.

Nowadays, extraction techniques such as QuEChERS, solid-phase extraction, and immunoaffinity columns are widely applied. However, these methods have several drawbacks, such as the requirement of different protocols for solid and liquid matrices and the need for additional purification steps, which increase the consumption of organic solvents and overall analytical costs.

Supramolecular solvents (SUPRAS), are nanostructured liquids generated from colloidal solutions of amphiphilic molecules through self-assembly and coacervation processes. SUPRAS exhibit regions of different polarity within their structure, allowing for the extraction of compounds across a wide range of polarities. They possess mixed interaction mechanisms, which increase the solubilization of analytes. Additionally, they have a high number of available solubilization centres, enabling efficient extraction using low volumes of SUPRAS. Moreover, the structure of SUPRAS can be tailored to favour the extraction of compounds of interest and eliminate interferences from the matrix.

This study introduces a SUPRAS-based microextraction approach for the simultaneous extraction of 12 EU-regulated mycotoxins, and their subsequent quantification using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The developed method was optimised and validated for five different categories of liquid and solid food products, in accordance with the performance criteria outlined in Regulation (EU) No. 519/2014. Owing to the unique properties of SUPRAS, analyte extraction and matrix cleanup were achieved in a single step. The method demonstrated excellent sensitivity (quantification limits ranging from 0.07 to 25.48 ng/mL), high extraction efficiency (recoveries between 69% and 115%), and strong precision (relative standard deviations of 0.3–20% for repeatability and 3–19% for reproducibility).

In addition to effectively extracting the 12 target mycotoxins from diverse food liquid and solid matrices, this method aligns with the principles of green chemistry, offering a simple, rapid, economical, and environmentally conscious alternative for routine mycotoxin analysis in food safety control.

Acknowledgements

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P2-033**ANALYSIS OF PESTICIDE RESIDUES AND MYCOTOXINS IN FREEZE-DRIED FRUIT: POTENTIAL RISK FOR CHILDREN**

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Freeze-dried fruit products (strawberries, raspberries, blueberries, mango, pineapple,...) are widely marketed as a "healthy" snack for children and adults. However, due to their high water and sugar content, fresh fruits are highly susceptible to mold growth, which is why various agrochemicals, such as pesticides, are commonly used. Pesticides play a crucial role in preventing plant diseases caused by bacteria, viruses, and fungi that could otherwise lead to significant crop losses. Freeze-drying is a modern method of fruit processing that allows the extension of the shelf life of fruit while preserving its taste, aroma, texture, and most of its nutritional value. During the freeze-drying process, substances with positive biological activity and possible harmful substances (e.g., pesticide residues) are concentrated and can pose a certain health risk.

The aim of this study was to assess the safety of various types of freeze-dried fruit available on the Czech market, with a particular focus on pesticide residues and mycotoxins. Freeze-dried fruit products—such as strawberries, raspberries, blueberries, mango, and pineapple—were analysed for pesticide residues and mycotoxins, with some products examined across different batches. The processing factor (PF) of the freeze-drying process was experimentally determined for the analysed fruit species. The processing factor represents the ratio of pesticide residue levels in the processed product compared to the raw material, and it is an important parameter when comparing residue levels to legislative limits. In the case of freeze-drying, the removal of water significantly increases the concentration of residues, resulting in higher PF values.

The QuEChERS extraction method was used for multi-residue analysis of 470 pesticide residues, followed by LC-MS/MS and GC-MS/MS analysis. Mycotoxin contamination was also assessed through LC-MS/MS analysis (57 mycotoxins). QuPPe methodology was used for the extraction of 10 polar pesticide residues followed by LC-MS/MS analysis. LOQ for pesticide residues ranged between 0.01-0.2 mg kg⁻¹. LOQ for mycotoxins was between 0.001-0.5 mg kg⁻¹. A high matrix effect was seen between individual types of fruit; matrix-matched calibration or standard addition was necessary.

In total, more than 100 different pesticide residues (or their metabolites) were detected. Several samples of freeze-dried fruit had levels of pesticide residues above the MRL. The most frequently detected pesticide was phosphonic acid (fosetyl metabolite). Prohibited substances such as carbofuran, omethoate, and haloxyfop residues were also detected, which pose a serious health risk. Among the mycotoxins, three were detected: alternariol, tenuazonic acid, and tentoxin.

Processing factor must be considered when assessing dietary exposure to pesticides and mycotoxins, as it may amplify potential health risks despite initial residue levels in fresh fruit being within regulatory limits. It is crucial to consider the potential health risks, particularly for vulnerable groups such as young children who are more sensitive to chemical exposures.

P2-034

DETERMINATION OF THE IMPROVEMENT OF PHENOLIC COMPOUNDS RECOVERY FROM MANGO BY-PRODUCTS BY HPLC-ESI-TOF-MS AFTER LACTIC ACID FERMENTATION

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Mango (*Mangifera indica* Linn.) is a tropical fruit wide consumed. When processing for obtaining juice around 35-60% of the whole fruit is discarded as by-products (peels and seeds) that have reported several bioactivities attributed to phenolic compounds. Thus, the aim of this study was to investigate the fermentation of mango peels and seeds by different lactic acid bacteria (LAB) strains and its impact on phenolic composition and antioxidant and antidiabetic activities (1,2). LAB used were *Pediococcus acidilactici* CECT 5765T, *Pediococcus pentosaceus* CECT 923, two strains of *Lactiplantibacillus plantarum* (CECT 748T and 9567-C4), *Levilactobacillus brevis* CECT 5354 and *Lecunostoc mesenteroides* CECT 219T. Phenolic compounds were determined by HPLC-ESI-TOF-MS, the antioxidant activity by DPPH and FRAP methods, and the antidiabetic activity by alpha-amylase inhibitory capacity. All LAB strains exhibited good growth and good survival rates. It was revealed significant increases of 6 and 4% ($p < 0.05$) in phenolic compounds in seeds fermented by *L. mesenteroides* CECT 219T and *L. brevis* CECT 5354, respectively, compared to non-fermented seeds. Otherwise, mango peels also showed significant increases in the phenolic compounds content of 8 and 6% ($p < 0.05$) when fermented by *L. plantarum* 748T and *L. mesenteroides* 219T, respectively. Antioxidant and antidiabetic assays demonstrated strain and time-dependent variations with the highest increment in mango peels fermented by *L. plantarum* and in mango seeds fermented by *P. acidilactici*. Positive correlations were found between evaluated antioxidant and antidiabetic activities and the phenolic compounds, both for mango peels and seeds. The study highlights how carefully selecting bacteria can greatly influence the phenolic composition and its *in vitro* bioactivities in mango peel and seed extracts. This process has the potential to enhance the recovery of phenolic compounds in mango peel and seed, offering exciting possibilities for their application in food and beverage processing.

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P2-035

NOVEL MICROWAVE-ASSISTED CLOUD POINT EXTRACTION PRIOR TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR DETERMINATION OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES IN FRUIT JUICES

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The current research aims to develop a green analytical method applying microwave radiation to assist cloud point extraction (MW-CPE), using Triton X-100 as a surfactant, in combination with GC-MS/MS, for the group extraction of 19 organochlorine and organophosphorus pesticides. The heating program for extraction was optimized to increase energy transfer efficiency. Compared to conventional hot plate heating, the MW procedure was faster and the energy consumption was lowered more than four times (17 W/sample for MW-CPE vs 70 W/sample for conventional CPE). The main experimental factors affecting the CPE (i) initial concentration of Triton X-100; (ii) sample pH; (iii) ionic strength (as content of MgSO_4) have been optimized. Due to the expected negative effect of surfactants on the GC system, a re-extraction of the target analytes in a small volume of hexane was applied. To increase the efficiency of the re-extraction, two agitation methods were compared (vortex or ultrasound) and the experimental factors affecting the re-extraction (i) agitation time; (ii) centrifugation time; (iii) volume of organic solvent and (iv) addition of MgSO_4 have been studied.

Calibration by matrix-matched standards (0.09% m/m Triton X-100 in hexane medium) has been found adequate and applied in the current study. Under the optimized extraction conditions, the limits of quantification (LOQ) of the evolved MW-CPE-GC-MS/MS combined technique ranged between 5 and 47 ng L^{-1} for all studied pesticides. The proposed method showed good linearity, accuracy and precision when applying only 0.25 mL hexane for re-extraction.

The developed MW-CPE-GC-MS/MS has been successfully applied for pesticide analysis in fruit juices (lemon concentrate and red apple juice diluted with water 5 and 1.25 times, respectively). The accuracy has been assessed by spike recoveries at 10 $\mu\text{g L}^{-1}$ level. For lemon juice ($n=3$) the obtained values for absolute recoveries were within the range of 72 % and 114 %, with RSD varying between 5 % and 26 %. Exceptions were evident only for beta and gamma-HCH (62 % $\pm$ 6 %, mean $\pm$ standard deviation) and Heptachlor-endo-epoxide A (65 % $\pm$ 8 %). For apple juice ($n=3$) were obtained recoveries within the interval of 71 % and 88 % with RSD values between 5 % and 10 %, except for alpha-HCH (51 $\pm$ 4 %); beta and gamma-HCH (41% $\pm$ 4 %); Chlorpyrifos-methyl (60 % $\pm$ 4 %); Heptachlor-endo-epoxide A (64 % $\pm$ 6 %); o,p-DDD (57 % $\pm$ 4 %), p,p-DDD and o,p-DDT (58 % $\pm$ 5 %), and p,p-DDT (66 % $\pm$ 3 %). Additionally, we found that the developed method combines a pre-concentration step with a sample clean-up step. The inherent pigments in the fruit juices were extracted into the non-polar core of the micelles during the extraction step, but were deposited in an intermediate layer of MgSO_4 during the re-extraction step, resulting in a colorless hexane phase.

The "greenness" of the proposed methodology was evaluated, yielding a score of 0.50 via the AGREE prep software [1], indicative of a low to moderate ecological impact. The main factors negatively affecting the calculated metric are the need for sample preparation in the laboratory, the volume of final waste generated (including the sample volume and the water for dilution), and the GC-MS/MS technique. In comparison with the routine method for analysis of pesticide residues in foods of plant origin (extraction with acetonitrile and clean-up by d-SPE-QuEChERS EN 15662:2018), a higher score has been obtained for MW-CPE-GC-MS/MS due to the minimization of the hazardous solvents and the reduction of the total amount of waste.

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P2-036**DETERMINATION OF TRACE ARSANILIC ACID IN FISHERY AND LIVESTOCK PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY****Kim, Jinhong; Bahn, Kyeongnyeo; Kim, Jiyong; Jeon, Seongsil.***Hazardous Substances Analysis Division, Daejeon Regional Korea Food & Drug Administration, Daejeon, South Korea.*

Arsanilic acid, a derivative of arsenic, is an organoarsenic compound commonly used in veterinary medicine. Primarily as a feed additive in fishery, livestock products to promote growth and prevent diseases. This study was conducted to develop an analytical method to determine Arsanilic acid residues in fishery and livestock products. Arsanilic acid was confirmed and quantified via liquid chromatography tandem mass spectrometry (LC-MS/MS) in the positive ion mode using multiple reaction monitoring (MRM). The sample was extracted with 0.1 M EDTA- Na_2 and acetonitrile solution, and purified with C_{18} powder. Chromatographic separation was performed on a C18 column (3.5 μm , 2.1 x 150 mm), and quantification was carried out by AB sciex 4500 mass spectrometer. The method validation was performed based on the Codex Alimentarius Commission (CAC) guideline.

The limit of quantitation for Arsanilic acid were 0.01 mg/kg. The linearity of the matrix (Flat fish, Eel, Shrimp, Beef, Pork, Chicken, Egg, Milk, Fat) matched calibration curve (R^2) were ≥ 0.99 in the calibration range of 0.0025 and 0.08 mg/kg. The average recovery rates were 72.4% to 120.4% at three fortification levels (1 x LOQ, 2 x LOQ, and 10 x LOQ) and the maximum coefficient of variation (C.V.) was 11.3%. This method is expected to improve the efficiency of monitoring veterinary drugs residues in fishery and livestock products, thereby enhance food safety.

P2-037**NIAS AND IAS MIGRATION FROM DRY FOOD: SYSTEMATIC OPTIMIZATION AND VALIDATION OF CONTACT, EXTRACTION AND DETERMINATION STEPS**

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The food packaging can act as potential sources of contaminants due to the migration or transfer of substances into food during product storage and transport. These substances include additives such as light and heat stabilizers, release agents, flame retardants, plasticizers, antioxidants, lubricants, blowing agents, and antistatic agents. They are known as Intentionally Added Substances (IAS). Although these additives are used in small quantities and are intended to remain within the polymer matrix, they may still migrate into food under various conditions (acidic, lipophilic, hydrophilic, or high temperatures) over prolonged periods. Additionally, substances used in manufacturing can contain impurities or result in degradation products, referred to as Non-Intentionally Added Substances (NIAS)¹. The potential migration of these substances necessitates the establishment of regulatory limits and monitoring measures to prevent the contamination that may pose health risks or negatively affect the composition of food and sensory properties.

The aim of this study is to optimize a comprehensive analytical method that allows the identification and quantification of the migration of Non-Intentionally Added Substances (NIAS) that may occur due to the contact of a polyurethane gasket from a silo intended for contact with dry foods. The migration of aniline, acetyltributylcitrate, bis(2-ethylhexyl) adipate, 2,6-tert-butylhydroxytoluene (BHT), and 1,4-butanediol from this polymeric material has been studied. Tenax[®] was used as simulant for dry food matrices², and the study was then applied to real dry foods (rice, flour, powdered milk, cocoa powder, sugar, corn starch). Migration tests were conducted in compliance with EU Regulation 2020/1245³, which establishes specific migration limits corresponding to the maximum amount of a substance allowed in food products, simulating the worst-case conditions inside the real application of the material under study (in our case, in the silo)⁴.

During the optimization of the pre-treatment procedure, several analytical challenges were identified. In particular, the presence of matrix effects for various real dry foods, statistically confirmed by the application of a t-test, and very low recovery yields of the analytes were identified. To address these issues, the extraction method was optimized to minimize the necessary steps and prevents analyte losses. Finally, thanks to the optimised pre-treatment method and the use of the standard addition method, when the matrix effect was present, improved migration values and extraction yields were achieved. The final optimized method thus includes three steps: migration, extraction of target analytes, and instrumental analysis using GC-MS. This approach emphasized minimizing procedural steps and maximizing analyte recovery, resulting in a consistent method for evaluating potential migration risks.

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P2-038**CHALLENGES AND STRATEGIES IN ANALYZING PFAS IN FOOD PRODUCTS**

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Perfluorinated alkyl substances (PFAS) represent a group of synthetic chemicals extensively utilized in various industrial and commercial sectors due to their distinctive characteristics, including repellence to water and oil, heat stability, and chemical durability. However, the persistent nature of PFAS in the environment, along with their potential negative health impacts on humans, has generated significant concern. PFAS contamination in food products has been primarily linked to the bioaccumulation of these substances within the aquatic food chain. The presence of PFAS in food samples poses serious health risks, as these compounds are known to accumulate in living organisms and can remain in the body for extended periods. Research has associated PFAS exposure with numerous adverse health effects, including liver dysfunction, endocrine disruption, and immune system impairment. Therefore, monitoring PFAS levels in food is essential for ensuring food safety and safeguarding public health.

While PFAS analysis in environmental samples typically employs solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [1-3], a methodology tailored for food samples has been developed that utilizes QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction, followed by SPE clean-up (if necessary) and LC-MS/MS analysis [4].

In this study, we report the findings from testing milk and fish as representative food samples, utilizing the FDA method C-010. We will discuss the application of QuEChERS extraction and clean-up, as well as the use of various HPLC columns and modifications to the mobile phases. Furthermore, a weak anion exchange (WAX) SPE clean-up was employed for the more complex fish samples.

One of the primary challenges in PFAS analysis is the widespread presence of these compounds. During our analysis, we detected background contamination from a PFAS compound, and we will detail the measures taken to identify and eliminate the source of this contamination.

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P2-039**ASSESSMENT OF VOLATILE AROMATIC COMPOUNDS IN DEALCOHOLIZED WINES USING SBSE-GC-MS AND MACHINE LEARNING MODELLING**

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The processes for dealcoholizing wine have multiplied in recent years due to factors such as the increase in alcohol content caused by global warming, as well as changes in drinking habits. There is now a growing consumer demand for beverages that are free from calories, alcohol, or additives. One of the latest advancements in this area is the GoLo technology, a very low-temperature vacuum distillation method. This technique aims to produce beverages while maintaining the organoleptic characteristics of the final product. According to its manufacturer, GoLo can remove nearly 100% of the volatile aromatic compounds from wine, which can later be reincorporated into the dealcoholized product. Furthermore, it can reduce the alcohol content of wine to just 0.05% (v/v). In addition to GoLo, other dealcoholisation technologies, collectively known as NoLo, include methods such as nanofiltration, reverse osmosis, osmotic distillation —also known as evaporative perstraction—or pervaporation, non-filtration techniques like vacuum distillation, spinning cone columns, multi-stage membrane systems, supercritical fluid extraction with CO₂, solid CO₂ extraction, microbial fuel cells, and freeze-drying can result in the loss of up to 20% of volatile compounds during the process (Veiga-del-Baño et al., 2024).

The aromatic compounds of 18 different wines (comprising 6 whites, 6 rosés, and 6 reds) were analyzed using the Stir Bar Sorptive Extraction (SBSE) method, coupled with Gas Chromatography and a mass detector. This analysis identified a total of 7 aroma groups: esters, alcohols, aldehydes, acids, terpenes, norisoprenoids, and phenols, with multiple compounds present in each group. We measured their concentrations both before and after the dealcoholisation process, along with other relevant oenological parameters such as the total polyphenol concentration and turbidity (Cuenca-Martínez, J.J. et al., 2025).

Based on the results and the physical-chemical properties of the compounds obtained, such as their molecular weight and polarity, several machine learning models were developed to predict the percentage of volatile compound loss during the GoLo dealcoholization process. These models identified significant dependencies on total polyphenols, turbidity, and notably, the aromas of alcohols, aldehydes, acids, norisoprenoids, and phenols. The best-performing model achieved an adjusted R² value of 0.828, with a p-value of 0.00029.

Keywords: Dealcoholisation; GoLo, Machine learning; Volatile compounds

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P2-040**ON-GOING METHOD PERFORMANCE VERIFICATION IN FOOD PESTICIDE ANALYSIS THROUGH A SINGLE CONTROL CHART**

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Pesticide analysis in food is a crucial aspect of food safety. To reduce the presence of pesticide contaminants in food, the European Commission has established measures to control harmful chemical levels and ensure consumer safety. The Maximum Residue Levels (MRLs) for both plant and animal products are specified in Regulation (EC) No 396/2005 (EC 2005). The analytical methods employed to detect pesticides must adhere to the criteria outlined in document SANTE/11312/2021v2 (SANTE 2021). One of the significant challenges in food analysis is the wide variety of pesticides and the numerous products that fall within a single commodity group. All products within the same commodity group must demonstrate a routine recovery rate of 60-140%, with a maximum relative standard deviation (RSD) of 20%, as specified in sections C39-C44 of the SANTE document.

To facilitate rapid visualization of both Recovery and RSD criteria, a graphical tool called Fast Risk Estimation and Analysis (FREA) was developed as an alternative to Shewhart charts. This chart employs a bubble graph for each pesticide, providing three pieces of information simultaneously: recovery, historical RSD, and the risk associated with the pesticide based on the MRL and the concentration detected in the sample (Gonzalez, F.J.E. et al., 2004; Hill, A.R. et al., 2005; Alminderej, F.M. et al., 2024).

The FREA tool was created by integrating data from commercial software with SQL databases managed through a PHP program, which enabled a graphical HTML display. It was specifically utilized for routine sampling of paprika and saffron, which are part of the spice commodity group. This tool enables quick and visual identification of pesticides that exhibit high RSD values and assesses the risk based on the MRLs established for paprika and saffron.

When comparing review times with and without the FREA chart, it was found that the review time decreased by up to 80%. Additionally, the FREA chart identified 100% more pesticides with potential risk based on the values obtained compared to the MRL, in contrast to the tools provided by the commercial chromatographic software.

Keywords: Control chart; FREA; Recovery; RSD; Pesticide residues

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P2-041**DETERMINATION OF ACRYLAMIDE CONTENT IN KEY FOOD SOURCES WITHIN THE MEDITERRANEAN FRAMEWORK**

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Acrylamide (AA) is a low molecular weight chemical compound formed principally by the Maillard reaction in carbohydrate-rich and low-protein foods subjected to high temperatures (above 120°C) and low humidity¹. This reaction involves interactions between free amino acids, particularly asparagine, and reducing sugars such as glucose and fructose that are naturally present in foods. Classified as a “probable human carcinogen” by the International Agency for Research on Cancer (IARC)², AA is receiving a lot of attention from European authorities, especially in the main food sources of AA exposure: fried potato products, coffee and processed foods such as crackers³.

In this context, the following study focused on the assessment of AA exposure in 85 Spanish and Italian samples (potato chips, roasted coffee and crackers). In addition, an assessment of the potential toxicological risk following the consumption of products containing AA was carried out. The AA content varied widely, ranging from 30 to 1905 µg/kg. However, only some of the potato samples analysed exceeded the maximum limit fixed in the European regulation⁴. The Estimated Daily Intakes (EDIs) obtained represented approximately 21 – 33% of the minimum mean EDI value reported by the European Food Safety Authority (EFSA) for the European population (0.4 µg/kg-bw/day). Based on the conducted risk assessments, the findings suggest that dietary AA exposure could pose a potential health risks.

Therefore, continuous monitoring of AA levels in food products and regular evaluation of dietary exposure are essential to enhance better mitigation and prevention measures to minimize its exposure among citizens.

Acknowledgements

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P2-042**GREEN EXTRACTION OF CAROTENOIDS FROM ORANGE PEEL USING CO₂-EXPANDED ETHANOL: A SUSTAINABLE APPROACH****Plaza, Merichel; Marina, María Luisa.***Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona Km. 33,600, 28871, Alcalá de Henares, Spain.*

Carotenoids, naturally occurring pigments with potent antioxidant properties, are widely recognized for their health benefits. Among them, β -carotene, lutein, and astaxanthin exhibit significant bioactivity, conferring a great interest to their extraction from natural sources. The food industry generates substantial agro-industrial by-products, such as orange peels, which contain high concentrations of carotenoids, particularly in the flavedo layer. However, conventional extraction methods rely on organic solvents like hexane and acetone, raising concerns regarding toxicity, environmental impact, and carotenoid degradation. The aim of this study was to develop a sustainable and efficient extraction method to obtain antioxidant carotenoids from fresh orange peel. CO₂-expanded ethanol (CXE) was investigated as a green alternative, providing the benefits of both CO₂ and ethanol while reducing solvent consumption and improving extraction efficiency. A theoretical solubility assessment using Hansen solubility parameters (HSP) guided the solvent selection to optimize carotenoid solubility. A D-optimal experimental design was employed to optimize key extraction parameters, including CO₂ molar fraction, temperature, pressure, and flow rate, maximizing carotenoid recovery using CXE and pressurized liquid extraction (PLE) technologies. The optimal extraction conditions were 57.6 °C, a pressure of 6.3 MPa, a solvent flow rate of 1 mL/min, and a CO₂ molar fraction in ethanol of 50% (v/v). The results demonstrated that CXE enhances mass transfer, reducing the need for high temperatures and allowing the extraction of carotenoids while preserving their native composition. Statistical modelling indicated that molar fraction of CO₂ in ethanol and solvent flow rate significantly influenced total carotenoid content, while temperature and pressure modulated the solubility and diffusion of carotenoids. CXE extraction was compared to conventional organic solvent extraction, revealing the higher efficiency and improved antioxidant recovery of CXE. The sustainability of the method was evaluated using the AGREEprep metric, confirming its green profile compared to conventional solvent-based approaches. This study highlights CXE as a promising green technology for extracting carotenoids from agro-industrial by-products, contributing to circular economy principles and sustainable development. The integration of theoretical solubility models and experimental optimization underscores the potential of CXE for scalable, environmentally friendly extraction processes.

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P2-043**ADVANCED ANALYTICAL STRATEGY FOR MONITORING PYRROLIZIDINE AND TROPANE ALKALOIDS IN HONEY****Ponz-Perelló, Paula; Pardo, Olga; Gallart-Mateu, Daniel; Esteve-Turrillas, Francesc Albert.***Universitat de València, Valencia, Spain.*

Pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) are naturally occurring toxins produced by specific plant species, including *Boraginaceae*, *Asteraceae*, and *Fabaceae* (for PAs) and *Solanaceae* (for TAs). These alkaloids pose a significant risk to human health, primarily through the consumption of contaminated plant-derived food products, such as tea, infusions, and honey. The 1,2-unsaturated PAs are of particular concern due to their hepatotoxic, genotoxic, and carcinogenic properties. Chronic exposure has been linked to hepatic veno-occlusive disease (HVOD), a severe condition characterized by liver fibrosis and hepatic failure. Furthermore, studies suggest that long-term ingestion of these alkaloids may increase the risk of DNA damage and tumor formation. Several cases of foodborne intoxications have been reported, such as the Afghanistan flour contamination incident in 2008, where PA-contaminated wheat caused widespread poisoning. (EFSA Journal, 2017) On the other hand, TAs, such as scopolamine and atropine, exert their toxicity by inhibiting acetylcholine receptors in the nervous system. Acute poisoning symptoms include blurred vision, dry mouth, tachycardia, hallucinations, and, in severe cases, respiratory failure. Young children are particularly vulnerable, with several fatal intoxications reported after ingestion of TA-containing berries. (EFSA Journal, 2013) Due to these serious health concerns, the European Commission Regulation (EU) 2023/915 has established maximum residue limits (MRLs) for these alkaloids in food products, including cereals, pollen, tea, and spices, necessitating the development of sensitive and reliable analytical methodologies. (European Commission (EC), 2023a)

Traditional methods for the determination of PAs and TAs rely on gas chromatography-mass spectrometry (GC-MS), which often requires a derivatization step to improve volatility and detectability. (Fuente-Ballesteros et al. 2024) However, these procedures are time-consuming, labor-intensive, and may introduce variability in quantification. In contrast, this study presents a novel, efficient, and validated ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method, which enables direct analysis of alkaloids without derivatization. The method is based on a quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction followed by extract preconcentration, enhancing sensitivity while maintaining simplicity.

The proposed methodology was validated following the Commission Implementing Regulation (EU) 2023/2783, which establishes guidelines for the sampling and analysis of contaminants in food. The method demonstrated excellent linearity ($R^2 > 0.99$) with residuals $\leq 25\%$, precision below 25%, and recoveries between 89–127%. The limits of quantification (LOQs) ranged from 5 to 10 $\mu\text{g}/\text{kg}$, ensuring compliance with regulatory requirements. A total of five honey samples were analyzed, with one sample containing lasiocarpine-*N*-oxide and senkirkine at levels of 5.2 and 5.7 $\mu\text{g}/\text{kg}$, respectively, exceeding the LOQ. Compared to existing methodologies, this approach offers several key advantages, including faster sample preparation, elimination of derivatization, reduced solvent consumption, and enhanced sensitivity and reproducibility. This makes it a cost-effective and robust alternative for routine monitoring of PAs and TAs in honey. The implementation of this validated method will contribute to improve food safety and public health protection.

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P2-044**ORANGE JUICE FORTIFICATION WITH PHENOLIC COMPOUNDS FROM FOOD BY-PRODUCTS: SENSORIAL AND CHEMICAL EVALUATION**

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Given the high consumption of orange juice, enriching it with bioactive compounds sourced from food by-products presents a dual advantage: boosting its health benefits while simultaneously reducing food waste through a circular economy approach. This research explores the fortification of orange juice with specific bioactive compounds—hesperidin, catechin, and hydroxytyrosol—extracted from orange peel, grape peel and seed, and olive leaves, respectively. To determine an appropriate fortification level, a sensory acceptability test ($n = 15$) was conducted, leading to the selection of 2 mg/mL as optimal concentration level of fortification. The fortified juice's stability was then monitored over five days, evaluating the impact of pasteurization on pH, total soluble solids (TSS), color, phenolic compounds (analyzed via HPLC-ESI-TOF-MS), vitamin C (assessed using HPLC-UV/VIS), and antioxidant activity (measured with DPPH and FRAP assays). Pasteurization had a noticeable effect on the juice's color parameters, initially increasing brightness before causing a darkening effect that later reversed over time. Although pasteurization led to an initial reduction in natural flavonoids ($\approx 49\%$) and vitamin C ($\geq 10\%$), the fortified juice demonstrated stability throughout the five-day storage period. Compared to the control, fortified juices retained 30% more phenolic compounds and maintained consistent vitamin C levels. Additionally, the fortified juice exhibited greater antioxidant activity than the non-fortified version. In conclusion, this study highlights that fortifying orange juice with extracts from food by-products is a viable strategy to enhance and preserve health benefits over time while ensuring sensory acceptability.

P2-045

PRESSURIZED LIQUIDS EXTRACTION OF PROTEINS FROM CRICKET (*ACHETA DOMESTICUS*) FLOUR. CHARACTERIZATION OF PROTEIN EXTRACTS AND THEIR HYDROLYSATES

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The growing demand for proteins has boosted the search for new and affordable protein sources. *Acheta domesticus* is an edible insect with a high nutritional value and sustainable farming, considered a suitable protein source for human consumption. Recovery of proteins from *Acheta* is commonly performed using conventional alkaline extraction, which results in a low yield, co-extraction of compounds such as chitin, and low-quality proteins. The aim of this work was to evaluate the potential of pressurized liquids extraction (PLE) as an alternative for a more efficient and holistic extraction of proteins from *Acheta*.

A Box-Behnken experimental design was employed to optimize the recovery of proteins by PLE while minimizing the co-extraction of chitin and the formation of potentially harmful Maillard products, using as solvent a combination of water and ethanol. Temperature and time enhanced the extraction of proteins, while a 100% water enabled the larger extraction of proteins. This extraction yield was similar to that obtained when using conventional alkaline extraction, while the chitin content was significantly reduced. Different strategies were employed to further increase the extraction yield. A slight improvement in the extraction yield was observed when using 2 cycles, while 3 cycles had no further effect. Sample defatting prior to extraction showed no significant improvement in the yield. The combination of PLE with enzyme-assisted extraction was also addressed to further increase the recovery of proteins. Extracted proteins were characterized by sodium-dodecyl sulfate gel electrophoresis, off-gel isoelectric focusing, and reversed-phase chromatography. The bioactive properties of proteins hydrolysates obtained with alcalase and flavourzyme enzymes were also evaluated. Results were compared with those obtained for proteins extracted using conventional alkaline extraction.

P2-046**DEVELOPMENT OF A RAPID UHPLC-UV METHOD FOR RED WINE CLASSIFICATION AND AUTHENTICATION BASED ON PHENOLIC COMPOUND PROFILING****Calvo, Júlia¹; Ahumada, Diego¹; Sentellas, Sònia²; Saurina, Javier³.**

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Red wine, one of the most consumed beverages worldwide, has significant economic and cultural importance, making its accurate characterization essential for determining its quality and ensuring authenticity. Wine is composed mainly of water and ethanol, however, other minor components, including phenolic compounds, are associated with several wine sensory properties, such as color, aroma, bitterness, and astringency. Additionally, polyphenols are linked to various physiological benefits due to their antioxidant and anti-inflammatory properties. The phenolic compound composition can be affected by several factors, namely grape variety, geographical origin, or wine aging; hence, they can be used as potential descriptors to differentiate among these features, making their analysis particularly relevant for wine characterization and authentication.

This study focuses on developing an analytical method using Ultra High-Performance Liquid Chromatography with UV-visible detection (UHPLC-UV). The elution gradient was optimized, establishing conditions that maximize the separation of chromatographic peaks in the shortest analysis time. As a result, a fast 10-minute chromatographic method with high reproducibility between days was developed, which has been used to analyze 198 red wine samples. The chromatograms at 280 nm were exported, and, using an R script, they were aligned, baseline-corrected, and compiled into a single data matrix for chemometric analysis of the entire fingerprint.

First, exploratory models using Principal Component Analysis (PCA) revealed sample clustering mainly based on geographical origin and grape variety. Additionally, multivariate classification models, using Partial Least Squares Discriminant Analysis (PLS-DA) and Soft Independent Modeling of Class Analogy (SIMCA), discriminated the enological traits with acceptable sensitivity, specificity, and error rates. In general, PLS-DA models provided better specificity and error rates, higher than 0.9 and lower than 0.1, respectively, while SIMCA models were more sensitive for most of the observed classes, with sensitivity rates ranging from 0.88 to 1. However, some samples were misclassified due to the simultaneous influence of both geographical origin and grape variety, suggesting that a classification model integrating both variables could enhance the accuracy of wine classification.

P2-047

TF-SPME: A POWERFUL TECHNIQUE FOR ANALYZING ODORANTS IN ALCOHOLIC BEVERAGES

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A major challenge in analyzing odorants in food arises from their extremely low odor thresholds, sometimes reaching the parts-per-trillion (ppt) level. Additionally, many odorants exhibit high polarity ($\log P < 1$), further complicating their analysis.

This study explores the application of Thin-Film Solid-Phase Microextraction (TF-SPME) for analyzing odorants in alcoholic beverages, highlighting its advantages over other SPME techniques, such as SPME fibers and Stir Bar Sorptive Extraction (SBSE). It also compares different TF-SPME variants characterized by various sorbents. The study evaluates the technique's performance in both headspace and direct sampling modes.

Furthermore, it presents strategies developed to address common SPME-related challenges, such as a limited linear range and low sensitivity to polar analytes. These strategies include sequential SPME and the standalone application of TF-SPME, demonstrating its effectiveness as a solution to these analytical limitations.

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P2-048

MICROBEADS FROM RECYCLED POLYSTYRENE POLYMER WASTE: A SUSTAINABLE SORBENT FOR IN-SYRINGE MICRO SOLID-PHASE EXTRACTION

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Plastic pollution of water resources is an issue of worldwide concern, empowering the development of strategies for integrating end-of-life materials into a sustainable cycle of reuse, thus reducing their environmental impact. Polymers possess good adsorption capacity towards different analytes, rendering them ideally suited materials as sorbents in analytical sample preparation. Current trends in this field are devoted to minimizing the environmental impact of this unavoidable step of the analytical procedure [1] by e.g., using natural sorbents [2] or those recycled from polymeric waste, minimizing sample, chemical and material amounts, as well as energy consumption, among other principles of Green Sample Preparation [3].

In this poster, an approach for the preparation of microbeads from second-hand regenerated polystyrene from yogurt cups is presented based on an optimized emulsion solidification technique [4]. The production of spheres of micrometric size boosts the sorption capacity of the sorbent material as it ensures a good surface area. The polystyrene microbeads, deposited on a rounded piece of paper, have been used as a sorbent material in an in-syringe extraction device. Under the optimum conditions, four opioids (i.e., methadone, tramadol, codeine, and morphine) were determined by direct infusion triple quadrupole mass spectrometry in environmental water as well as biofluids such as saliva and urine. Limits of detection lower than $8 \mu\text{g L}^{-1}$ and precision better than 14.9 % have been obtained for all the analytes in saliva and environmental water samples.

Moreover, recycled polystyrene microbeads have further been modified to broaden the spectrum of analytes that can be extracted. Sulfonated polystyrene microbeads were prepared via sulfuric acid treatment for different times. 6 hours of acidic incubation revealed an improved sorption capacity of the target analytes, i.e., sulfamerazine, sulfaguanidine, sulfadiazine and sulfanilamide. A microextraction strategy with comprises an in-syringe dispersive solid-phase microextraction step was applied to the extraction of the analytes in biological samples (saliva and urine) and their determination upon elution by liquid chromatography-mass spectrometry. Full validation study, following ICH guidelines for bioanalytical method validation, proved the applicability and reliability of the procedure, ensuring satisfactory analytical features in light of a sustainable and fully recycled extraction method. Limits of detection lower than $5 \mu\text{g L}^{-1}$ (with the only exception of sulfanilamide, LOD lower than $25 \mu\text{g L}^{-1}$) secure a trustworthy detection at low biological concentrations.

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P2-049

ELECTROCHEMICAL BIOSENSING PLATFORMS BASED ON NON-COVALENTLY FUNCTIONALIZED MULTI-WALLED CARBON NANOTUBES WITH BIOTIN-BINDING PROTEINS

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The development of highly specific, sensitive, and reliable detection methods for the identification of exosomes is of great clinical importance for cancer diagnosis, progression monitoring, and prognosis prediction. In this context, electrochemical biosensors have emerged as powerful analytical tools for profiling exosomal surface proteins due to their intrinsic advantages, including simplicity, rapid analysis, miniaturization potential, high selectivity and sensitivity, low detection limits, and cost-effectiveness.

In the present study, we focus on the development of electrochemical aptasensors for the rapid and reliable analysis of cancer-related exosomes. These aptasensors are constructed using novel hybrid materials based on multi-walled carbon nanotubes non-covalently functionalized with biotin-binding proteins such as avidin, streptavidin, and neutravidin. The resulting sensing platforms were thoroughly characterized by cyclic voltammetry, electrochemical impedance spectroscopy, scanning electrochemical microscopy, and scanning electron microscopy.

The selective recognition capacity at low biomarker concentrations was found to be directly correlated with the enhanced electrocatalytic surface area and improved electrical conductivity of the platforms, thereby enabling maximum sensitivity. The results demonstrate a simple yet highly effective electrochemical approach for the early detection of exosomes, providing rapid and accurate diagnostic information—an achievement not previously reported in the literature.

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P2-050

DEVELOPMENT OF NiMoO_4 AND CoMoO_4 -BASED HYBRID MATERIALS FOR ELECTROCHEMICAL DETECTION OF HYDROGEN PEROXIDE

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To develop highly sensitive and stable sensors for hydrogen peroxide detection, this work reports the synthesis of NiMoO_4 and CoMoO_4 multioxides via hydrothermal methods followed by thermal treatment. XRD analysis confirmed that NiMoO_4 required an additional calcination step, while CoMoO_4 was successfully obtained by doubling the reaction temperature reported in the literature.

SEM analysis revealed nanowire morphology for NiMoO_4 and nanorod morphology for CoMoO_4 . Elemental composition confirmed by EDX showed atomic percentages of O 57%, Ni 11%, Mo 11% for NiMoO_4 , and O 64%, Co 13%, Mo 11% for CoMoO_4 . Composites were prepared with 70% multioxide and 30% reduced graphene oxide (rGO). Contact angle measurements indicated hydrophilic behavior in both, with $\text{CoMoO}_4/\text{rGO}$ being more hydrophilic than $\text{NiMoO}_4/\text{rGO}$.

Electrochemical studies using modified glassy carbon electrodes showed that both composites exhibit catalytic activity for H_2O_2 oxidation, with no response for reduction. Optimal working potentials were 0.6 V ($\text{NiMoO}_4/\text{rGO}$) and 0.4 V ($\text{CoMoO}_4/\text{rGO}$). The highest sensitivity ($47.7 \mu\text{A} \cdot \text{mM}^{-1}$, $R^2 = 0.99956$) was achieved with $\text{CoMoO}_4/\text{rGO}$ using a 2.5 mM H_2O_2 stock solution. Stability tests showed a $34.1\% \pm 6.6\%$ current loss after 2700 s. Glucose interference tests confirmed its non-interfering effect under the studied conditions.

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P2-051**ANALYTICAL STRATEGIES FOR THE STUDY OF BACTERIAL VOLATILE: A COMPARISON OF HS-GC-IMS AND HS/SPME-GC-MS**

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Microbial volatile organic compounds (mVOCs) are low-molecular-weight metabolites that play crucial roles in microbial communication, both within and across species and kingdoms. Due to their growing relevance in fields such as agriculture, food safety, biotechnology, and clinical diagnostics, the development of robust analytical methodologies for their detection and characterization has become increasingly important. However, the low abundance of many mVOCs pose significant analytical challenges.

In this study, we present a comparative study of different analytical approaches for the detection and identification of mVOCs emitted by *Pseudomonas simiae* strain PICF7, a rhizobacterium isolated from olive roots. Specifically, we evaluated gas chromatography (GC) coupled to ion mobility spectrometry (IMS) and to mass spectrometry (MS) detectors, in combination with two sampling/pre-concentration techniques: headspace (HS) extraction and solid-phase microextraction (SPME). The main goal was to assess the strengths and limitations of each configuration, both in terms of analytical performance (e.g., sensitivity, selectivity, precision, number of detectable signals) and in their capacity for qualitative compound identification.

Solid cultures of PICF7 were analyzed using HS-GC-IMS, HS-GC-MS, and SPME-GC-MS. The technique HS-GC-IMS enabled the detection of 37 signals, whereas SPME-GC-MS and HS-GC-MS detected 18 and 7 peaks, respectively. A first tentative identification was performed based on NIST spectral library matching in the MS-based methods, while in GC-IMS, identification relied on both retention index and drift time of the signals. Using these criteria, 11, 7, and 4 compounds were tentatively identified by HS-GC-IMS, SPME-GC-MS, and HS-GC-MS, respectively. These identifications were subsequently confirmed by the injection of analytical standards. The results highlight the complementary nature of HS-GC-IMS and SPME-GC-MS due to their differing mVOC detection profiles. Furthermore, GC-IMS demonstrated excellent sensitivity and lower quantification limits, making it particularly suitable for detecting low-concentration volatiles.

Overall, this study provides a critical evaluation of analytical strategies for bacterial mVOC profiling, highlighting the importance of selecting the appropriate technique based on specific analytical objectives; whether for qualitative or quantitative analysis, or for non-targeted analysis. The results support the growing relevance of GC-IMS in microbial volatile studies and offer valuable insights into its potential application in areas such as microbial biocontrol, food contamination monitoring, and the non-invasive diagnosis of microbial infections.

P2-052

CONCURRENT EVALUATION OF A PANEL OF BIOMARKERS PERTINENT TO CARDIOVASCULAR DISEASES

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The work lies in the design of a 3D stochastic microsensor obtained by modifying a single-wall carbon nanotube (SWCNT) with gold nanoparticles (AuNP) and quinine (QN) for the qualitative and quantitative determination of a panel of biomarkers like C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and vascular cell adhesion molecule-1 (VCAM-1) for the prognosis and diagnosis of cardiovascular diseases from blood samples.

The cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) tests were used to study the bare SWCNT, Au-NP/SWCNT, and QN-Au-NP/SWCNT electrodes' electrochemical behavior in a 5 mM potassium ferricyanide solution made in 0.1 M KCl. Also, the morphology of the pastes was realized by scanning electron microscopy (SEM).

For this study, the stochastic method was used to quantitatively and qualitatively analyze one or more analytes in any sample. The stochastic sensor is based on the principle of channel/pore conductivity, which occurs in two phases. The benefits of using this microsensor for stochastic analysis come from the way it works: the signature of the analytes (t_{off} values in the diagrams), which is a qualitative parameter, and the t_{on} value, which is related to the concentration and is not affected by the matrix composition. High sensitivities and selectivities are achieved; the signature depends only on the size and shape of the molecules, and wide linear concentration ranges are obtained.

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P2-053

MINIATURIZED GRAPHENE FIELD-EFFECT TRANSISTOR FOR LABEL-FREE DETECTION OF SARS-CoV-2 SPIKE PROTEIN

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Numerous studies have reported the use of graphene electrolyte-gated field-effect transistors (GFETs) as transducer elements in electrochemical biosensors. Graphene's high sensitivity to electric fields and charge enables electrochemical transduction with output signals acquired at low operating voltages. Essentially, when a target molecule is recognized at the sensor surface, the ionic atmosphere over the graphene channel is disturbed. This physicochemical change in the Debye layer can be detected and eventually linearized by monitoring shifts in the graphene's Dirac point. To evaluate the electroanalytical performance of the graphene channels, we monitored their transfer curves. From these curves, we derived two key parameters: the shift in the Dirac Point potential (ΔEDP) and the corresponding change in drain current (ΔIDP). Ultrasensitive biodetection using GFETs undoubtedly holds significant potential for clinical diagnostics and monitoring; however, it also faces major challenges, such as high variability and considerable drift in the electroanalytical signal. This work provides new bioanalytical perspectives on the application of GFETs functionalized with eukaryotic bioreceptors through a novel and robust GFET design and a practical approach to managing signal instability. Initially, and in relation to our group's previous work [1], we propose the use of miniaturized GFETs (mGFETs) as an alternative platform for multichannel (bio)electrochemical transduction. These devices offer the primary advantage of enhanced analytical reliability due to a higher quality graphene channel and improved inter-channel reproducibility. To study the mGFETs, a "Graphenea card" was used as an interface between the graphene chip and the electronic instrumentation (Keithley 2200). The combination with mGFETs yields more robust and reliable analytical results, owing to superior electrical connections that provide an improved signal-to-noise ratio as well as reduced risks of breakage and trace contamination of the graphene. The measurement system also incorporates an external Ag/AgCl reference electrode to provide a more stable electrochemical reference potential for data acquisition. In addition to these incremental improvements, a stabilization protocol—comprising a triple-cycle transfer curve—was implemented prior to signal acquisition. Graphene channel functionalization was achieved using angiotensin-converting enzyme 2 (ACE2) and 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBSE) as a linker. When evaluating the affinity biosensor by incubating with 300 fg/mL of the SARS-CoV-2 Spike protein, both analytical parameters showed a significant response relative to the blank and the controls. A signal was observed in the presence of BSA, which is attributable to nonspecific adsorption in the absence of blocking. Notably, the change in drain current (ΔIDP) relative to the shift in Dirac point potential (ΔEDP) is significantly higher when considering the signal-to-noise ratio.

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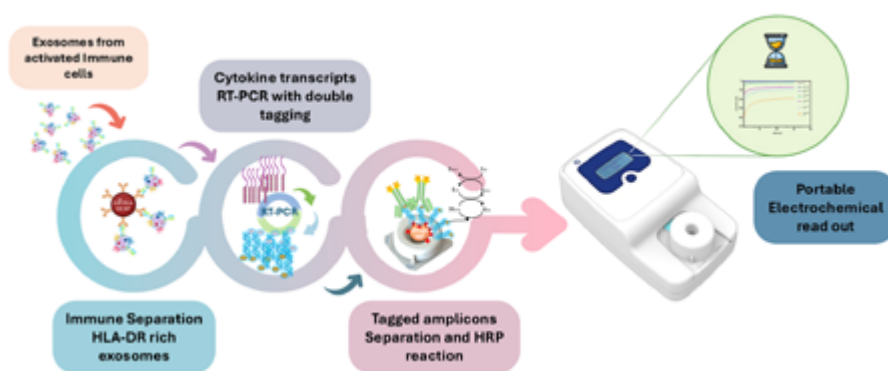
P2-054

EXOSOME-BASED ELECTROCHEMICAL GENOSENSING FOR SENSITIVE DETECTION OF IFN γ IN IMMUNE MONITORING APPLICATIONS

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Interferon-gamma (IFN γ) is a key cytokine involved in regulating immune responses, particularly in the context of infections, autoimmune diseases, and cancer. Its dual role in pro-inflammatory and regulatory pathways makes it a critical biomarker for immune monitoring. While conventional detection methods such as ELISA and RT-PCR are well established,



emerging strategies using exosomes as biomarker carriers offer enhanced sensitivity and early diagnostic potential. Exosomes are nanoscale extracellular vesicles that mediate intercellular communication and transport biologically active molecules, including mRNA transcripts of cytokines. In this study, we present a novel system for the capture and molecular profiling of exosomes derived from peripheral blood mononuclear cells (PBMCs), aiming to quantify IFN γ expression at the transcriptomic level. The experimental approach takes the advantage that MHC class II molecules (named HLA in humans) are only expressed in dendritic cells, macrophages, and B lymphocytes at low levels, but during the immune response those cells including activated T-cells, increase their expression at the membrane. Activated immune cells are good exporters of exosomes to plasma, therefore capture of exosomes by their expression of HLA-DR (one of the genes of HLA-II) and the analysis of IFN-g mRNA cargo, can be an alternative way of measuring the cellular immune response in plasma samples. Exosomes were selectively isolated using immunomagnetic particles functionalized with anti-HLA-DR antibodies to target MHC class II+ vesicles. This approach enabled effective preconcentration of immune-related exosomes, bypassing the need for ultracentrifugation. Captured exosomes were analyzed via electrochemical genosensing, incorporating reverse transcription and double-tagging endpoint PCR to detect IFN γ and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. The method demonstrated enhanced sensitivity in samples enriched with HLA-DR+ exosomes compared to bulk, non-enriched preparations. These findings underscore the potential of exosome-based diagnostics for real-time immune monitoring, offering a simplified, rapid, and sensitive platform suitable for point-of-care settings. This work paves the way for personalized immunodiagnostics, with broad applications in managing cancer, infectious diseases, and autoimmune conditions through dynamic, non-invasive biomarker tracking.

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P2-055**HOLISTICALLY UNCOVERING THE CHEMICAL EXPOSOME IN PREGNANT WOMEN: CONTRIBUTIONS OF LC-HRMS FOR AN ENVIRONMENTAL JUSTICE PERSPECTIVE**

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The human exposome is defined as the totality of exposures an individual experiences from conception onwards [1]. One of its important components is the chemical exposome, which encompasses lifelong exposure to environmental chemicals. These exposures are considered major risk factors in the development of a wide range of developmental disorders and chronic diseases. Perinatal period is a critical vulnerable window, when adverse effects of these exposures can have lasting impacts across the life course. Consequently, it is essential to define and characterize the perinatal chemical exposome in a comprehensive manner to better understand its life-course implications for human health.

This study aimed to comprehensively assess organic contaminants in pregnant women through the application of liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) [2]. To this end, we analyzed 294 serum samples collected during the first trimester of pregnancy from participants in the Barcelona Life Study Cohort (BiSC), Spain (2018-2021). As a first step, we employed a validated wide-scope screening methodology that enabled the detection of over 700 potentially hazardous chemicals in these samples. We detected a broad spectrum of chemicals—over 150 substances were found in at least one sample, and more than 35 had a detection rate exceeding 50%. These included plasticizers, tire additives, UV filters, PFAS, and personal care product ingredients, with concentrations ranging from low ng/L to low mg/L. When reference standards were available, concentrations were quantified directly; otherwise, we applied semiquantitative estimations using logIE-based machine learning models, developed and validated by our team. These models demonstrated strong predictive performance, with estimated concentrations typically falling within a threefold range of the true value, opening new possibilities for risk assessment of chemicals lacking analytical standards.

Subsequently, chemical levels were statistically cross-referenced with individual-level metadata, including age, maternal education, ethnicity, economic self-assessment, adherence to the Mediterranean diet or use of different products (i.e. cosmetics, personal care products, etc), among others. Among them, we found interesting associations, such as different plasticizers or preservatives and socioeconomic level, which demonstrate the importance of these studies to reduce sociological disparities.

Furthermore, we geospatially mapped chemical concentrations across neighborhoods in Barcelona, highlighting specific areas with elevated levels of certain contaminants. These spatial patterns also reflect underlying socioeconomic disparities, while focusing specific areas for further studies in order to test new hypothesis about their possible effects of children in their first years, and offer valuable insight into environmental injustice within the urban landscape.

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P2-056**ANTITUMORAL PROPERTIES OF POMEGRANATE SEED PROTEIN AND PEPTIDE ISOLATES. *IN VITRO* AND *IN VIVO* STUDIES IN PROSTATE AND BREAST CANCER MODELS**

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Pomegranate seeds are a cheap and sustainable source of proteins. Different sustainable strategies have been already developed for the extraction of these proteins, namely high intensity focused ultrasounds (HIFU) and pressurized liquids (PLE) [1]. Moreover, this work also demonstrated the potential of pomegranate seed proteins and peptides as antioxidant molecules, although the use of different extraction methodologies can affect these properties [1]. Against this backdrop, in the present work we have evaluated the antitumoral capacity of pomegranate seed protein and peptide isolates obtained under these different extraction conditions. To study that, we employed two different cell lines: a prostate cancer cell line (PC-3), and a breast cancer cell line (HCC1187). Several cancer-related processes were studied to assess the anticancer potential of pomegranate seed extracts, including migration, viability, clonogenicity, adhesion, cell cycle, metalloproteinase (MMP) activity, vascular endothelial growth factor (VEGF) expression, and physical tumor parameters (weight and volume). *In vitro* results were confirmed using *in vivo* models with immunodeficient (Hsd:Athymic Nude-Foxn1^{nu}) mice.

All samples significantly reduced cell viability in a concentration ranging from 0.1 to 15 mg/mL, observing lower IC₅₀ values in breast cancer cells than in PC3 cells, in all cases. Based on these results, concentrations of 4 mg/mL (PLE PI), 2 mg/mL (HIFU PI), and 2.4 mg/mL (hydrolysates) were selected for further studies. Cell migration and proliferation were significantly reduced in both cell lines by all treatments. Cell adhesion was enhanced with all extracts in HCC1187 cells, but only with HIFU hydrolysate in PC-3 cells. Moreover, MMP9 (active and pro-forms) activity decreased in HCC1187 cells after treating with all samples, whereas Pro-MMP2 was not affected. In PC-3 cells, MMP9 activity was also reduced by all samples, whereas hydrolysates were able to reduce Pro-MMP9 and Pro-MMP2 activity. Cell cycle analysis revealed S-phase arrest with HIFU samples and apoptosis with PLE samples in the two cell lines. *In vivo* experiments allowed to observe significant reductions in tumor volume and weight after being treated with the pomegranate seed hydrolysates. Additionally, VEGF expression was also affected, as well as the activity of MMP2 and MMP9 (active and pro-forms).

These promising results pave the way for the development of new antitumoral molecules. However, further studies focusing on the molecular mechanisms of these compounds are needed to fully understand the potential of these extracts.

Acknowledgements

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P2-057

ANALYTICAL SUPPORT FOR THE TOXICOLOGICAL STUDY OF ATRACTYLOSIDE AND ANALOGUES

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Atractyloside and its analogues are diterpene compounds belonging to the kaurene family, primarily found in plants of the Asteraceae family. These compounds have been associated with the toxicity of *Atractylis gummifera*. The toxic components of *A. gummifera* have been identified as gummiferin and atractyloside, which were first structurally characterized in 1972 by Danieli et al. and Defaye et al. Later, the presence of atractyligenin derivatives, structurally similar to atractylosides, were reported in edible plants.

To facilitate a toxicokinetic study of these compounds, we developed a quantitative method for analyzing atractyloside-like compounds across various in-vitro assays. Our approach employs Reverse-Phase Ultra High Pressure Liquid Chromatography coupled with High Resolution Mass Spectrometry (RP-UHPLC-HRMS). This method specifically targets atractyloside, gummiferin, and two structurally similar chemicals in water, HBSS, and DMEM cell culture media.

The method underwent comprehensive in-house validation for the three matrices spiked with the four analytes, utilizing a third atractyloside-derivative as an internal standard. Quantification was performed through aqueous calibration or matrix-matched calibration, ensuring accurate and reliable results by incorporating quality control samples throughout the analytical process.

The analytical campaign aimed to determine the levels of the four compounds in different aqueous solutions and cell culture media as part of an in vitro ADME (Absorption, Distribution, Metabolism, Excretion) study.

All samples were successfully analyzed, and the results were validated through quality control measures. The method was successfully applied to samples from ADME assays, demonstrating its suitability for the intended application. The analytical method also leveraged HRMS technology for tentatively identifying rat hepatocyte metabolites of the tested compounds.

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P2-058

RAPID STRATIFICATION OF FEVER SYNDROMES USING A TRAFFIC LIGHT-BASED BIOSENSOR FOR sTREM-1

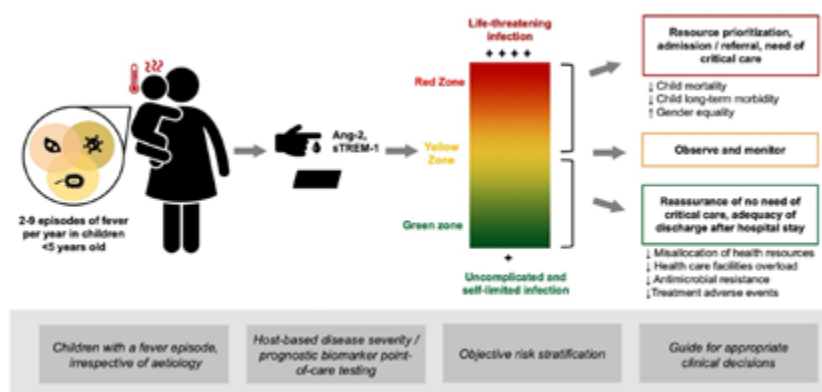
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Recent evidence highlights the significance of the soluble fragment of the Triggering Receptor Expressed on Myeloid cells 1 (sTREM-1) and the growth factor Angiopoietin-2 (Ang-2) as quantitative novel biomarkers for assessing disease severity, treatment response, and outcomes in various conditions, including malaria, pneumonia, COVID-19, and others fever syndromes. Some studies have demonstrated that sTREM-1 has shown superior prognostic performance^{1, 2, 3} compared to any previously used predictors involved in host immune and endothelial activation, such as C-Reactive Protein and Procalcitonin. Elevated sTREM-1 levels have shown strong correlations with disease severity, multiple organ dysfunction, and mortality. To aid in risk stratification, patients in this study were classified into three groups based on their sTREM-1 levels using the WHO-proposed traffic light color system⁴.

A threshold value of 239 pg/mL represented the "green light," indicating low risk according to uncomplicated and self-limited infections, while levels exceeding 629 pg/mL were designated as "red light," signifying an urgent need for admission due to life-threatening infections. An intermediate "yellow light" indicated further monitoring¹. In this work, we present a rapid test integrating magnetic separation and electrochemical biosensing on a portable device operated by batteries. Several pairs of commercial anti-TREM-1 antibodies were tested in a magneto-actuated immunoassay, and operating conditions were optimized as proof of concept. We demonstrated that our detection system can distinguish sTREM-1 levels associated with low, moderate and high risk of death, including in spiked serum and plasma samples. The development of our own monoclonal antibodies is currently underway, and various enzymatic labeling strategies are being evaluated. The laboratory prototype comprises two components: (1) a disposable cartridge containing magnetic particles conjugated with antibodies specific to the biomarker of interest, and (2) a digital reader equipped with an interface for quantitative electrochemical readout. The cartridge's microfluidic system facilitates magnetic actuation, while excess sample and reagents are removed. Within less than one minute, the digital reader provides quantitative readout of the biomarker levels in pg/mL, which is then displayed on the device's screen and transmitted to the accompanying App via Bluetooth.

The device's performance in classifying sTREM-1 levels is presented, demonstrating excellent readout results with a short 15-minute incubation step and a swift 2-minute readout process. This innovative point-of-care test holds great promise for aiding clinicians in rapid risk stratification and timely decision-making, potentially enhancing child survival outcomes and improving patient management in a variety of fever syndromes and specific diseases.



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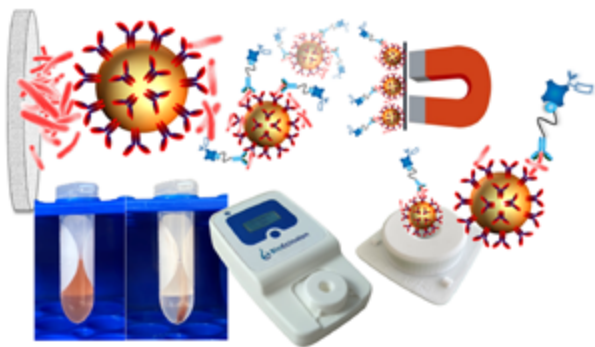
P2-059

MYCOBACTERIUM DETECTION METHOD COMBINING FILTRATION, IMMUNOMAGNETIC SEPARATION, AND ELECTROCHEMICAL READOUT IN A PORTABLE BIOSENSING DEVICE

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One of the main drawbacks in rapid diagnostic tests (RDTs) is the detection of targets at very low concentration levels in complex specimens. Novel developments in RDTs that are needed involve solid-phase preconcentration procedures which can be easily integrated in emerging technologies, including biosensors. Biomarkers in complex samples can be thus preconcentrated while the interfering matrix is removed at the same time, increasing the sensitivity and the specificity of RDTs. Since the early reports on magnetic separation technology, magnetic particles (MPs) have been used as a powerful and versatile preconcentration tool in a variety of analytical and biotechnology applications. Besides the amazing properties of MPs, the main drawback is that magnetic separation is usually performed in less than 1 mL of sample, due to i) the high cost of biological-modified MPs and ii) the issues related to the magnetic actuation of large volume of samples. Due to the low concentration, the detection of waterborne and environmental pathogenic bacteria requires processing up to 100 mL¹, or in some instances, up to 1000 mL of sample², thus making the use of biologically-modified MPs rather impractical for this purpose. The standard method for detection of waterborne pathogens relies on the isolation in pure culture, which includes the tandem: i) filtration from large volumes of sample, and ii) the time-consuming culture enrichment. This procedure has not significantly changed over the last years, constituting a bottleneck for the application of rapid methods such as PCR as a replacement for classical culturing. Due to the potential health hazards caused by waterborne pathogens, a robust, rapid, and easy-to-use method for the rapid preconcentration of these bacteria at low concentration level from large volume of samples has been developed.



This study addresses a straightforward and highly sensitive approach for detecting *Mycobacterium fortuitum*, as a model of Pathogenic Environmental Mycobacteria (PEM)³. PEM are a frequent cause of infection and water is a significant vehicle for the transmission of these organisms⁴. The method involves a combination of filtration and direct immunomagnetic separation to isolate the bacteria retained on filters for further electrochemical magneto immunosensing in a handheld device. Unlike conventional methods involving preenrichment by culturing, this approach employs a simplified preconcentration technique, which includes

filtration of large water samples, up to 100 mL, followed by a one-step process of immunomagnetic separation and labeling. After a 60-min, during which the filter with the retained bacteria and all the reagents (including modified magnetic particles and enzymatic conjugates) are incubated, the resulting product is directly dropped into a cartridge, capable of performing magnetic actuation and washing. The electrochemical readout is carried out on a portable battery-operated device within 30 sec. Remarkably, the immunosensor demonstrates an outstanding limit of detection of 5 CFU mL⁻¹ in hemodialysis water processing 100 mL of sample⁵. This achievement is remarkable considering the short and simplified analytical procedure, compared to the traditional isolation and culturing of mycobacteria, which typically takes 2 weeks.

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P2-060

RAPID DIAGNOSTIC TEST FOR CELIAC DISEASE BASED ON DEAMIDATED MAGNETIC PEPTIDE AS A NOVEL BIOMARKER

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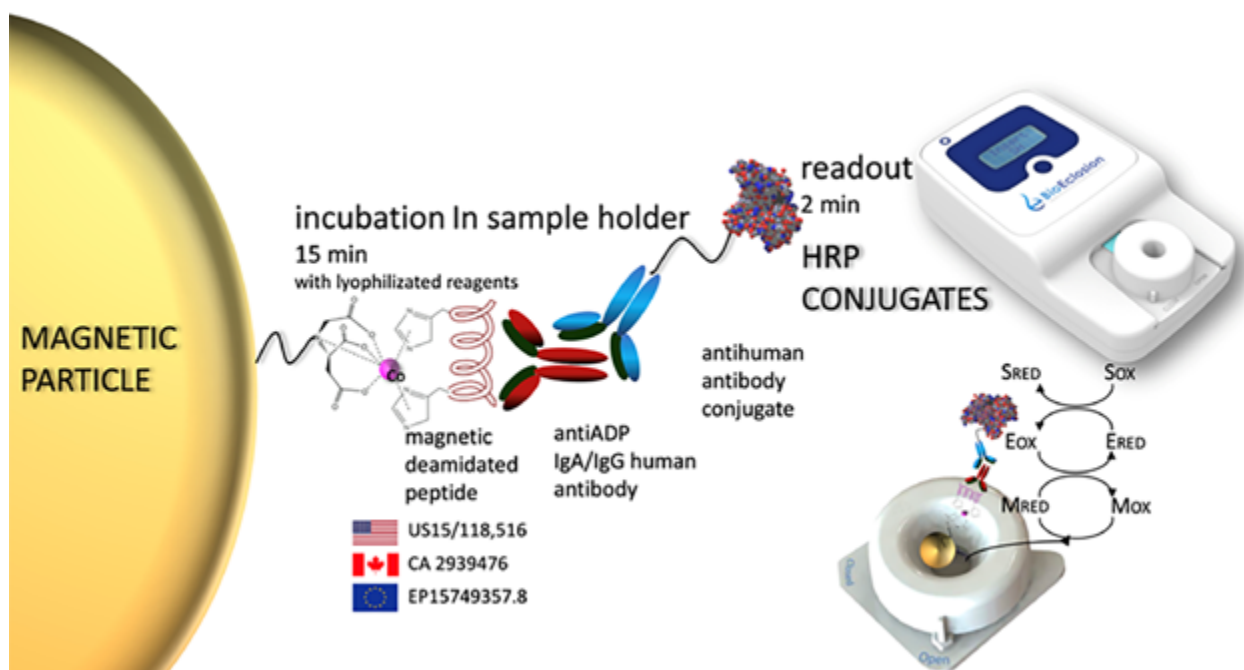
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Celiac disease (CD) has emerged as an increasingly recognized public health concern over the last half-century and is now acknowledged as a global phenomenon contributing to avoidable morbidity and mortality, as it is triggered by the ingestion of gluten proteins found in certain cereals [1]. The symptoms of CD can vary over time in a given individual and often mimic other conditions. Combined with low global awareness, this leads to many cases remaining undiagnosed or being ineffectively treated [2]. Although the global prevalence of CD is estimated to be around 1%, for every diagnosed case, an estimated 5 to 10 cases remain undiagnosed—primarily due to a lack of recognition by healthcare professionals [3]. From a public health standpoint, this undiagnosed population represents a significant at-risk group. Currently available diagnostic tests for CD are limited to specialized laboratories, and the gold standard—intestinal biopsy—is an invasive procedure requiring anesthesia [4]. Given the high prevalence of the disease, the rapid and accurate identification of CD patients at the primary care level remains a major global health challenge. The burden of CD could be significantly reduced if diagnostic tests were more accessible at the community and primary-care levels. In addition, such tests would be valuable for patient follow-up, as relapse can occur later in life, and for the screening of first-degree relatives of diagnosed individuals.

This study focuses on the development of portable rapid diagnostic test for CD, based on deamidated gliadin-related peptides as a novel biomarker, which improve the sensitivity of current diagnostic methods.

Various gliadin peptide sequences, including deamidated modifications, were studied for their conformational similarity to the native gliadin protein. The candidates with the highest conformational matches were synthesized and used as bioreceptors in the biosensing platform. The deaminated peptides were integrated on a device platform composed by two main components: A) Disposable cartridge, including i) the sample holder containing reagents (including magnetic particles modified with peptide and the labeled-antibody which binds specifically the patient antibodies in 15 minutes), and ii) the cartridge containing the microfluidic and the electrode in which the magnetic actuation is performed, while the excess of sample and reagents are removed. B) Digital reader which allows the electrochemical readout in less than one minute. The output of the device is a quantitative response.

The resulting test, named CeliFast, has demonstrated great potential due to its low cost, speed, sensitivity, minimal handling requirements, and suitability for point-of-care screening by general practitioners. Moreover, the prototype was evaluated using anonymized blood samples, showing a high capacity to distinguish between healthy individuals and CD patients.



In summary, CeliFast combines all the competitive advantages of existing diagnostic tests: it is point-of-care, rapid, user-friendly, quantitative, and highly predictive.

Keywords: Celiac Disease; deamidated peptide; magnetic particles.

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P2-061**ADVANCES IN BIOANALYTICAL TECHNIQUES FOR MONITORING INFLAMMATION BIOMARKERS****Přivratská, Nikola; Kujovská Krčmová, Lenka.***Department of Analytical Chemistry, Faculty of Pharmacy; Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Hradec Králové, Czech Republic.*

Tryptophan and kynurenine serve as inflammatory markers due to their roles in the kynurenine pathway, which is activated during immune responses. Together, these metabolites can reflect immune activity and have been linked to inflammation and disease severity in conditions such as SARS-CoV-2 infections [1].

The accurate quantification of kynurenine and tryptophan in urine requires specific and sensitive analytical techniques to ensure reliable data. Our method involves a straightforward three-step extraction process—dilution, centrifugation, and filtration—making it suitable for high-throughput clinical laboratories. The method employs a fully validated approach using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) that utilises Multiple Reaction Monitoring (MRM) mode to optimise detection sensitivity and specificity. Chromatographic separation is achieved using a polar C18 core-shell column, which offers flexibility for easy transition from UHPLC to HPLC if needed. Additionally, the method is environmentally friendly, requiring only 3 mL of mobile phase and a small injection volume of 5 µL per sample. This combination of efficiency and sensitivity makes our method ideal for routine clinical analysis of these metabolites.

In addition to the high demands on the analytical phase, there is an increasing focus on optimising the pre-analytical aspects of bioanalysis. This includes introducing less invasive sampling methods and improved sample handling and storage. For this reason, our method for analysing kynurenine and tryptophan was adapted to the Dried urine spot (DUS) format. The DUS is a microsampling technique that allows convenient home sampling with minimal volume, is environmentally friendly and leads to cost savings. Unlike liquid urine, which is prone to bacterial contamination and requires refrigeration or freezing for transport and storage, DUS eliminates these logistical problems, making it a more practical and cost-effective option for large-scale studies and clinical diagnostics [2].

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P2-062**LC-Q-ORBITRAP-HRMS UNTARGETED SCREENING OF MIGRANT SUBSTANCES FROM POST-CONSUMER AND RECYCLED POLYOLEFINS - EVALUATION OF DECONTAMINATION FOR FOOD-CONTACT APPLICATIONS**

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Ensuring the safety of food contact materials (FCMs), particularly those made from recycled plastics, is essential for protecting public health. Regulation (EU) No 2025/40 [1] mandates the progressive incorporation of recycled plastic FCMs, which must also comply with the safety aspects and requirements established in Regulation (EC) No 10/2011 [2]. This regulation establishes the framework to evaluate FCM safety, and sets the requirements for compliance testing, contact conditions and maximum migration levels.

Ideally, only substances complying with Regulation (EU) No 10/2011 should be found during migration testing. These substances, known as intentionally added (IAS), include starting monomers, production aids, and additives such as stabilizers, plasticizers, and antioxidants. In reality, other substances can also be found. These substances are classified as non-intentionally added (NIAS) and may originate from different sources, including impurities from raw materials, by-products from polymerization and degradation reactions or contaminants absorbed during the material's life cycle (in the case of recycled plastics).

In this work, to assess the potential migration of post-consumer and recycled plastics, migration tests were conducted on three different plastic batches using three food simulants (10 % ethanol, 95 % ethanol and 3 % acetic acid) at 60 °C for 10 days. The first batch consisted of high-density polyethylene (HDPE) obtained from post-consumer milk bottles, while the second and third batches were sourced from the HDPE and polypropylene (PP) collection streams of a sorting plant. The migration of all batches was evaluated before and after being treated by a novel recycling method [3].

The food simulants were analysed using liquid chromatography coupled to high-resolution mass spectrometry (LC-Q-Orbitrap-HRMS), which provides high sensitivity and accurate mass measurements. This approach, combined with spectral library and database searches via Compound Discoverer software, enables the tentative identification of unknown compounds by non-target screening [4].

A wide range of migrant compound families have been tentatively identified, including polyethylene glycols, adipates, phthalates, surfactants, antioxidants, and flame retardants, belonging to both IAS and NIAS. This highlights the complexity of migration profiles and the necessity of detailed migration studies to ensure the safety of recycled FCMs.

Moreover, this study contributes to a more comprehensive evaluation of the previously cited recycling method's efficiency by comparing the presence and concentration of migrant substances in post-consumer and recycled plastic samples. Additionally, it will provide insights into the efficiency of the recycling plant's separation process, consumer misuse of plastic packaging, and the level of compliance with packaging disposal guidelines.

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P2-063

RECYCLING OF HDPE USING BIO-BASED SOLVENTS - CLEANING EFFICIENCY COMPARISON AND RESIDUAL CONTENT ANALYSIS

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Dissolution-precipitation methods are considered a promising alternative to secondary and tertiary recycling of polymers. They combine the benefits of mechanical recycling and depolymerization, since these processes are less dependant on the chemicals present in the polymer, and the achievable quality and purity are higher [1].

Toluene and xylene are conventional solvents used to dissolve polymers, but their use poses health and environmental hazards. Therefore, cyclic monoterpenes have been proposed as alternatives in dissolution and precipitation processes to recover polymers such as high-density polyethylene (HDPE) [2] or polystyrene [3].

As a follow-up to a previously published study [4], in this work we study the cleaning efficiency of seven bio-based solvents in a recycling method based on the dissolution and precipitation of post-consumer HDPE. Additionally, the residual content of the seven bio-based solvents is determined by a new pressurized liquid extraction and gas chromatography-mass spectrometry (PLE-GC/MS) method, which has been optimized and validated.

In addition to limonene, the cleaning efficiencies of (-)- β -pinene, α -phellandrene, α -terpinene, p-cymene, γ -terpinene, and terpinolene are established via a challenge test, determining the concentration of seven model contaminants (chloroform, toluene, chlorobenzene, methyl salicylate, phenyl cyclohexane, benzophenone and methyl stearate) in post-consumer HDPE before and after being processed by the cleaning method. The determination of contaminants was carried out with a previously developed PLE-GC/MS method [4].

Furthermore, a new PLE and GC/MS method has been developed and validated to determine the residual content of the bio-based solvents in the treated HDPE. Variables such as extraction solvent, addition and content of a co-solvent, extraction temperature and time and number of cycles has been optimized combining univariate analysis, central composite design and the response surface methodology. The entire PLE-GC/MS method has been validated studying its recovery, repeatability and intermediate precision, and later tested on real samples.

The combination of both PLE-GC/MS methods will enable a comprehensive evaluation of the recycling process, assessing the removal efficiency of model contaminants from the polymer, the residual content of the bio-based solvents, and other critical process steps.

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P2-064**THE FOOTPRINT OF PESTICIDE USE IN AGRICULTURAL POLYETHYLENE**

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The agricultural industry heavily relies on plastic materials to optimize and improve crop efficiency, reducing water consumption and minimizing pesticide application rates. Among these materials, polyethylene (PE) is the most widely used polymer in applications such as greenhouse covers, mulching films, irrigation pipes, and tying tapes. These plastic products are continuously exposed to a wide range of pesticides—particularly fungicides and insecticides—that are applied to crops. Depending on their physicochemical properties, pesticides can either remain on the surface of the plastic or be absorbed into the material, accumulating over multiple agricultural cycles, especially in low-density polyethylene (LDPE). By the end of its service life, PE used in agriculture may contain pesticide concentrations even higher than those found in crops and surrounding soils [1].

Within the framework of the circular economy, recycling agricultural plastics has been proposed as a sustainable strategy to mitigate the environmental impact at the end of their life cycle. This approach helps to prevent the generation of micro- and mesoplastics, which are difficult to recover from the environment. These plastic fragments can be transported into different environmental compartments via runoff, soil erosion, or indirectly through ingestion and excretion by wildlife and livestock.

The main objective of this study is to investigate the presence of pesticide residues in end-of-life agricultural PE and assess their potential transfer to raw materials derived from recycled PE. Additionally, findings on the migration of pesticides from both new and aged PE irrigation pipes into water during irrigation will be presented, as well as the efficiency of pesticide removal during thermal treatment of PE. To achieve this, pesticide residues were analyzed in extracts from aged agricultural PE, newly manufactured products made from virgin plastic, and recycled plastic. The analysis were performed using liquid chromatography coupled to tandem mass spectrometry (LC-ESI-MS/MS), focusing on a preselected list of 40 pesticides based on usage data and their reported presence in agricultural soils. This presentation will showcase pesticide concentration levels, comparisons across different plastic types and sources, and future recommendations.

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P2-065

SUSTAINABLE EXTRACTION AND STABILITY ASSESSMENT OF ANTIOXIDANT AND ANTICHOLINERGIC PRESSURIZED NATURAL DEEP EUTECTIC SOLVENT EXTRACTS FROM CITRUS RETICULATA LEAVES

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Citrus reticulata var. Arrayana is one of the most cultivated citrus species in Colombia due to its favorable climatic conditions. In fact, mandarins account for 37.7% of the country's total citrus production [1]. However, large-scale citrus cultivation generates substantial postharvest residues, such as peels and leaves, creating environmental and economic problems. Notably, citrus leaves are rich in bioactive phenolic compounds, including flavonoids and phenolic acids, known for their antioxidant, antihypertensive, anti-allergic, anti-carcinogenic, and antibacterial properties [2]. Despite this, most research has focused on studying bioactive compounds from peels, leaving the potential of *C. reticulata* leaves largely unexplored.

To maximize the value of citrus postharvest residues, efficient extraction techniques are required. Recent studies have explored the use of natural deep eutectic solvents (NaDES), a new generation of biodegradable, non-toxic, and cost-effective solvents [3]. NaDES-based extracts have demonstrated enhanced stability under specific storage conditions compared to conventional ethanol-based extracts. Additionally, to improve the sustainability of extraction by reducing time, energy consumption, and solvent usage, NaDES have been integrated into advanced techniques such as pressurized-liquid extraction (PLE). However, research on combining PLE with NaDES remains limited.

Considering these aspects, in this study, we developed novel extraction processes to recover antioxidant and anticholinergic phenolic compounds (AC-extracts) and antioxidant-only phenolic compounds (A-extracts) from *C. reticulata* leaves using PLE with ChCl:Gly (1:2) NaDES as the extraction solvent. A Box-Behnken experimental design was employed to assess the effects of temperature, extraction time, and water percentage in NaDES on phenolic compound recovery. In this manner, two optimal extraction conditions were achieved: a) PLE-NaDES-AC (150°C, 25 min, 60% water in NaDES) for the obtention of phenolic compounds with antioxidant and anticholinergic capacities; b) PLE-NaDES-A (64°C, 27 min, 57.9% water in NaDES) for the recovery of antioxidant-only phenolic compounds.

These extracts were compared in terms of total phenolic content (TPC), antioxidant and anticholinergic activities, and stability under forced conditions against ethanolic extracts obtained under the same extraction conditions. Among all extracts, PLE-NaDES-AC exhibited the highest antioxidant and anticholinergic capacities. Additionally, HPLC-IT-MS analysis identified 29 phenolic compounds in all extracts, with PLE-NaDES-AC showing the highest peak areas of phenolic compounds identified. Notably, some phenolics, including polymethoxyflavones and phenylethanoid glycosides, were detected in *C. reticulata* leaves for the first time. Furthermore, NaDES demonstrated a strong protective effect against oxidation in stability studies compared to ethanolic extracts.

Overall, PLE-NaDES emerges as a promising, eco-friendly approach for extracting antioxidant and anticholinergic phenolic compounds from *C. reticulata* leaves while preserving bioactivity under storage conditions. However, scaling up this process presents technical challenges, particularly due to the high viscosity of NaDES, which can hinder solvent flow and extraction efficiency. Addressing these limitations may require equipment modifications and process optimization. Nevertheless, NaDES offer significant advantages over conventional solvents, including higher extraction yields and greater extract stability, making them a viable option for industrial-scale phenolic extraction.

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P2-066

INNOVATIVE SUSTAINABLE EXTRACTION OF BLOOD-BRAIN BARRIER-PERMEABLE NON-POLAR AND POLAR COMPOUNDS FROM *SALVIA OFFICINALIS* L. AND *EUCALYPTUS GLOBULUS* LABILL. FOR NEUROPROTECTIVE APPLICATIONS

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Medicinal plants have long been valued in phytotherapy for their diverse biological activities, primarily attributed to bioactive compounds such as essential oils, phenolics, minerals, and vitamins. Among these, terpenoids have gained attention for their neuroprotective properties, particularly in Alzheimer's disease (AD) [1]. These compounds inhibit acetylcholinesterase (AChE), a key enzyme in cholinergic signaling, while also targeting butyrylcholinesterase (BChE), which becomes more relevant in later AD stages [2]. Effective neuroprotective compounds must also cross the blood-brain barrier (BBB) to exert their effects. While synthetic drugs like galantamine and donepezil are FDA-approved for AD, their side effects have driven interest in plant-based alternatives, as recommended by the World Health Organization.

Among medicinal plants, *Salvia officinalis* and *Eucalyptus globulus* are rich sources of neuroactive terpenoids. Advanced extraction techniques such as supercritical CO₂ (SC-CO₂) extraction have proven effective in isolating these non-polar compounds. Compared to conventional methods like Soxhlet extraction, SC-CO₂ offers higher efficiency, shorter processing times, and improved product quality while reducing environmental impact [3]. However, SC-CO₂ extraction is less effective in recovering polar compounds, such as phenolics with antioxidant properties, which remain in the extraction residue, requiring more polar solvents for their effective retrieval.

Sequential extraction of both terpenoids and phenolics maximizes the use of raw plant material, aligning with the principles of biorefinery and sustainability. To improve the recovery of phenolic compounds from the residue of SC-CO₂, natural deep eutectic solvents (NaDES) have emerged as effective green alternatives to conventional solvents like ethanol/water [4]. The combination of NaDES with advanced extraction methods, such as pressurized liquid extraction (PLE), further improves the recovery of bioactive phenolics compared to traditional maceration. However, to date, no studies have explored the recovery of phenolic compounds from *S. officinalis* and *E. globulus* using PLE combined with NaDES (PLE-NaDES) with neuroprotective purposes [23].

Therefore, this study employs a biorefinery approach to extract BBB-permeable compounds from *E. globulus* and *S. officinalis* for neuroprotective applications. A sequential extraction process was utilized, beginning with SC-CO₂ extraction to isolate non-polar terpenoids, followed by PLE-NaDES to extract phenolic compounds from the SC-CO₂ residue. This dual-extraction strategy aimed to maximize compound recovery while maintaining sustainability.

PLE-NaDES extracts exhibited stronger antioxidant and anticholinergic activities than SC-CO₂ extracts, with *S. officinalis* showing greater bioactivity than *E. globulus*; GC-MS identified 21 and 24 terpenoids in *E. globulus* and *S. officinalis*, respectively, while HPLC-QTOF-MS detected 25 phenolic compounds in PLE-NaDES extracts, which also demonstrated superior BBB permeability, especially flavonoids in *E. globulus* and rosmarinic acid in *S. officinalis*.

This study pioneers the SC-CO₂ and PLE-NaDES sequential extraction of *S. officinalis* and *E. globulus*, yielding terpenoid- and flavonoid-rich extracts with strong neuroprotective activity and BBB permeability, offering a sustainable approach for natural neurodegenerative disease treatments.

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P2-067**WIN-WIN SITUATION: STORAGE STABLE, CO₂-NEUTRAL SYNTHETIC HEATING FUELS – RELIEF FOR THE CLIMATE AND REDUCED CLEANING REQUIREMENTS IN STORAGE TANKS****Grabler, Laurin Domenic; Vetere, Alessandro; Schrader, Wolfgang.***Max-Planck-Institut fuer Kohlenforschung, Mülheim an der Ruhr, Germany.*

Rising temperatures and increasingly extreme weather conditions caused by global warming can be traced back to the anthropogenic input of carbon dioxide into the atmosphere.^[1] To prevent further exacerbation, the input of carbon dioxide must be reduced. Various technological innovations for decarbonization are available for this purpose (e.g., CCS technologies, heat pumps, hydrogen in steel production, etc.), but these are expensive and cannot be implemented quickly. Less developed countries, in particular, are often unable to contribute to decarbonization due to these technological challenges. However, by utilizing renewable synthetic hydrocarbons, CO₂ emissions in the atmosphere can be reduced immediately without requiring changes to existing technical infrastructures.

Scalable fuel alternatives include fuels based on Fischer-Tropsch synthesis (XtL, which can be produced fully CO₂-neutral when using biomass and decarbonized electrical grids) and those based on hydrotreated vegetable oils (HVO). Their suitability as a fuel replacement in engines and reduction in greenhouse emissions is already well known.^[2,3] However, their behavior during long-term storage, particularly when stored together with fossil fuels during a future transition process, remains largely unknown.

For this study, fossil and synthetic heating oils, and blends (synthetic-fossil-ratio of 80:20 or 50:50) were stored in open containers in the dark for up to 18 months to study their stability. For analysis, they were diluted to 300 ppm in toluene/methanol (v/v 50:50) and analyzed using a 7 T LTQ-FT-ICR-MS (Thermo Fischer Scientific, Bremen, Germany). Measurements were done in both full scans and SIM mode (30 Da scan windows with 5 Da overlap) in the mass range of m/z 150–1000 using a 3s transient ($R = 400K$).

It was observed that fossil fuels formed a precipitate during storage, consisting of highly oxidized aromatic compounds. In contrast, synthetic fuels did not form such precipitates and were more resistant to potential oxidation. By increasing the paraffin content through the blending of synthetic fuels with fossil fuels, higher storage stability was achieved, and only minimal amounts of precipitate were formed. This demonstrates that synthetic e-fuels not only offer advantages for the climate but also are technically superior even to fossil fuels.

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P2-068**PFAS IN FOOD CONTACT MATERIAL: HOW FAR CAN GC-MS TAKE US?****Hauschildt, Charlotte¹; Jung, Christian¹; Haase, Hajo²; Becker, Roland¹.**¹Federal Institute for Materials Research and Testing, Berlin, Germany; ²Technische Universität Berlin, Berlin, Germany.

PFAS (per- and polyfluoroalkyl substances) represent a broad and complex class of synthetically produced chemicals with estimated 10,000 different compounds. Often referred to as forever chemicals, their defining characteristic is the complete or partial fluorination of the carbon chain. Due to their persistence, toxicity, and widespread occurrence, PFAS have been a major focus of scientific research over the past decade, particularly in the areas of identification, quantification, toxicity, and potential pathways into the environment [1,2].

In response to their harmful properties, a significant regulatory milestone was reached in 2022 when maximum levels for PFAS in certain foods were included in the commission regulation No.1881/2006 on maximum levels for certain contaminants in foodstuffs for the first time. In commission regulation 2022/2388, the European Commission defined maximum levels for two perfluorocarboxylic acids (PFOA and PFNA) and two perfluorosulfonic acids (PFOS and PFHxS) in certain foodstuff to ensure a high level of human health protection [3,4].

Effective monitoring of these contaminants requires highly sensitive analytical techniques. Currently, liquid chromatography - (tandem) mass spectrometry (LC-MS(/MS)) remains the gold standard for PFAS detection, offering a significantly extended detection range compared to gas chromatography-mass spectrometry (GC MS). However, to expand accessibility and enable more process-independent testing, GC-MS-based approaches are being actively developed and optimized [2,5]. Although several analytical methods exist for detecting fluorinated contaminants in food, advancing their reliability, sensitivity, and applicability remains a critical challenge. Further optimization of GC-MS methodologies is essential to align with or extend beyond LC-MS techniques, enhancing their effectiveness in food and packaging analysis. Ultimately, these developments will contribute to improved food safety.

This research is part of the EU project 23IND13 ScreenFood, which aims, as one of its objectives, to develop sensitive analytical methods for improved identification and quantification of various PFAS - both currently regulated and emerging - in selected food and food contact materials (FCM) [6]. In food contact material analysis, the focus is on native and recycled materials such as PET, PP, or cardboard, which are of widespread use. Various analysis techniques are to be tested and compared for the comprehensive identification and sensitive quantification of PFAS used in FCM. These will include solvent-free approaches such as TD-GC-MS or large volume injection, which could enable fast and uncomplicated analysis.

This poster will present improved GC-MS methods for the detection and quantification of various PFAS classes and first insights into the analysis of several food contact materials (e.g. paper-based FCM) with GC-MS. Furthermore, the long-term objectives of the EU project ScreenFood will be outlined.

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P2-069**FROM WASTE TO RESOURCE: SUSTAINABLE BIOACTIVE RECOVERY FROM HOP LEAVES****Lorente, Pau¹; Granados, Mercè¹; Saurina, Javier²; Sentellas, Sònia³; Núñez, Oscar³.**¹*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona, Barcelona, Spain;*²*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona / Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona., Barcelona, Spain;* ³*Department of Chemical Engineering and Analytical Chemistry, Universitat de Barcelona. / Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona / Serra Hùnter Program, Generalitat de Catalunya, Barcelona, Spain.*

Hop is widely recognized for its use in the brewing industry, particularly for its cones, which impart characteristic flavours and enhance the microbiological stability of beer, primarily due to the bioactive compounds they contain. However, the leaves and roots of hop plants are also sources of bioactive compounds. Notably, hop leaves, often considered agricultural waste, contain a variety of bioactive compounds, such as polyphenols and bitter acids. These compounds exhibit significant antioxidant, antimicrobial, and antiviral properties, sparking interest in the food, cosmetic, and pharmaceutical industries. Thus, hop leaf byproducts can be regarded as a valuable resource for bioactive recovery, transforming waste into high-value products and aligning with the principles of a circular bioeconomy.

Organic solvents, such as methanol, acetone, or ethyl acetate have been widely used for the extraction of bioactive compounds, but are not the most suitable options for food, cosmetic, or pharmaceutical applications. In this context, water and ethanol are more appropriate alternatives. This study investigates the extraction of bioactive compounds from hop leaves using water-ethanol mixtures, with simple stirring at a controlled temperature. The ethanol concentration varied from 0% to 100%, while the temperature ranged between 35 °C and 75 °C. The Folin-Ciocalteu and Ferric Reducing Antioxidant Power (FRAP) assays were employed to determine the total polyphenol content and antioxidant capacity of the extracts, respectively. Liquid chromatography with UV and MS detection was utilized to obtain the chromatographic profiles of the extracts and identify the most relevant compounds within them.

Although temperature had no significant effect, the solvent composition played a crucial role in the extraction process. Both the total polyphenol content and the antioxidant capacity of the extracts increased with higher ethanol concentrations. The chromatographic profiles obtained at low and high ethanol percentages displayed distinct patterns. The extraction efficiency of the less polar fraction improved as the ethanol percentage increased. Therefore, optimizing the solvent composition is crucial for the successful recovery of specific target compounds.

P2-070

FROM BY-PRODUCTS TO BIOACTIVES: INNOVATIVE VALORIZATION OF AGRI-FOOD WASTE

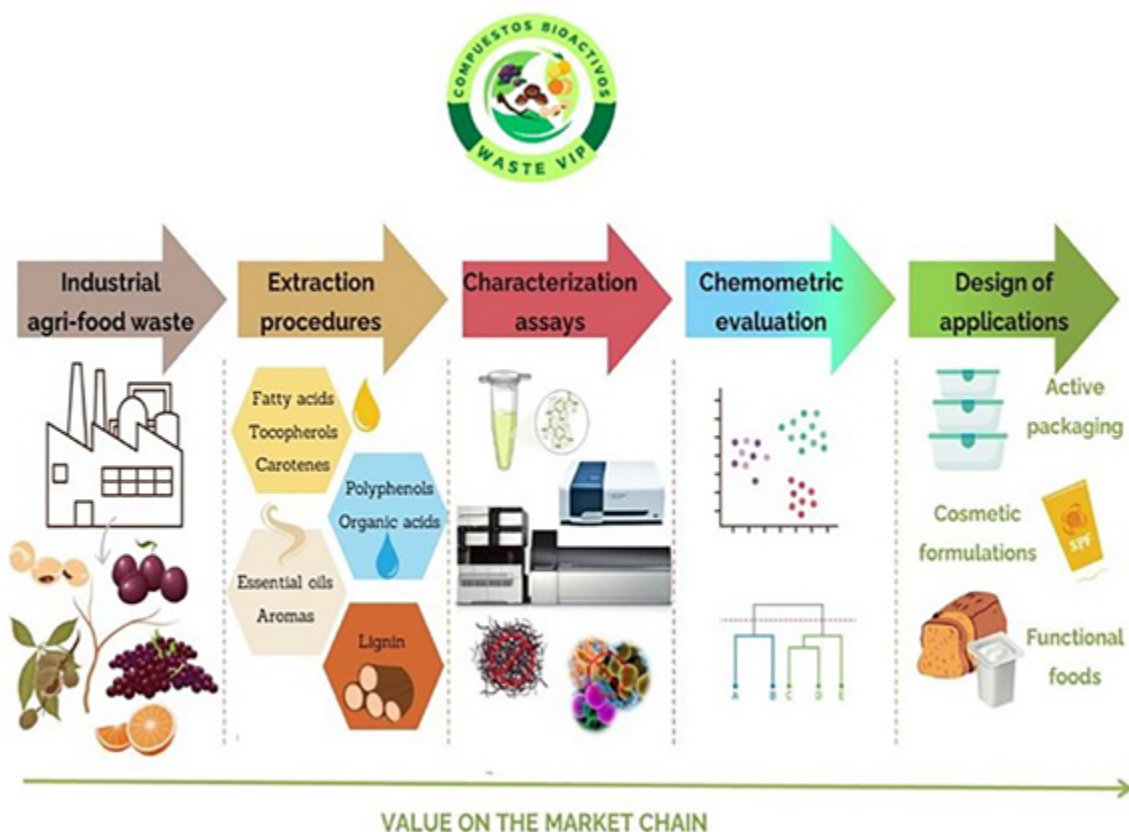
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Valorization of agri-food waste is a growing trend focused on the sustainable use of biological resources to maximize the value of by-products and waste. This approach aims to reduce waste generation, mitigate environmental impact, and optimize the use of natural resources by extracting high-value compounds from existing waste rather than relying on new raw materials. Additionally, it promotes the bioeconomy by transforming what was once considered waste into economically viable products. In particular, **simultaneous and cascade valorization** views waste as a resource that can be reintegrated into the production cycle, where the by-products of one process serve as raw materials for the next [1-3].

Our research focuses on the cascade valorization of both **high-volume residues** from the Spanish agri-food sector—such as citrus waste (seeds, peels, and leaves) and grape pomace—as well as **underexplored waste** like sloe berries, chestnut hedgehogs, vine shoots, and by-products from soy-based food manufacturing. To develop sustainable extraction methods for bioactive compounds—including polyphenols, unsaturated fatty acids, organic acids, lignin, tocopherols, and terpenes—**analytical strategies based on the principles of green chemistry** have been designed [4]. Additionally, chromatographic techniques (GC-FID, GC-MS, LC-DAD, capillary LC-DAD-MS, LC-MS/MS) have been optimized for the characterization of value-added compounds, and approaches have been developed to assess their bioactive properties, such as antioxidant, neuroprotective, anti-hypertensive, and antimicrobial activities [5].

Furthermore, the vast amount of data generated through these experimental methodologies opens the door to the application of **chemometric tools**, which help identify relationships between different bioactive properties and the compounds present in waste extracts. This approach enables a **comprehensive valorization strategy**, providing insights to enhance or redesign production processes with the goal of minimizing industrial waste disposal. It also supports the development of new products derived from agri-food waste, such as oils, essential oils, aromas, hydrophilic extracts, and lignin—free from undesirable compounds like amygdalin, trans fatty acids, and histamine. These natural extracts, with significant **health-promoting properties and bioavailability**, offer promising applications as natural additives and ingredients for cosmetics and enriched food products (Figure 1).



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P2-071**LEVELS OF BIOGENIC AMINES IN BYPRODUCTS OF SOY-BASED FOODS: COAGULANT FOR TOFU PRODUCTION AND WHEY COMPOSITION****Rodríguez-Bencomo, Juan José; Picazo-Arijón, Ismael; Rodríguez-Blázquez, Sandra; Rosales-Conrado, Noelia; Gómez-Mejía, Esther; León-González, María Eugenia.***Department of Analytical Chemistry, Faculty of Chemical Sciences, Complutense University of Madrid, Madrid, Spain.***Abstract**

Soy-based products are widely available on the market, and the soy industry is currently experiencing significant growth in both production and the generation of byproducts. In particular, tofu production (a process that involves the coagulation of soy-based beverages) generates large amounts of soy whey, which could be valorized to recover compounds such as proteins, oligosaccharides, and isoflavones [1-3]. On the other hand, the raw material used for tofu production may contain moderate levels of biogenic amines such as histamine, putrescine, cadaverine, spermidine, among others [4-5]. As a result, both tofu and the resulting soy whey may also contain these compounds.

Tofu production relies on a coagulation process involving the addition of salts or organic acids. Various coagulants have been evaluated for their effects on tofu yield, gel strength, water retention, springiness, sensory properties, and the content of proteins, fats, etc. [6]. However, since studies on biogenic amine content have primarily focused on fermented soy products, the influence of coagulant type on biogenic amine levels in tofu and its whey has scarcely been investigated. Therefore, the aim of this study was to evaluate the impact of different coagulants on tofu production, with a particular focus on their effects on the distribution of biogenic amines between the tofu and its whey.

In this study, tofu was prepared following usual protocols using magnesium chloride, calcium chloride, tartaric acid, and citric acid as coagulants. For each sample, the tofu yield was determined. The soy whey samples were analyzed for total protein and total phenolic compound content using UV-Vis spectrophotometry. Biogenic amines were derivatized with diethyl ethoxymethylenemalonate (DEEMM) and subsequently analyzed by high-performance liquid chromatography (HPLC).

The different capacity for protein coagulation and the final tofu characteristics, depending on the assayed reagent, affected the concentration of dissolved substances in the whey byproduct.

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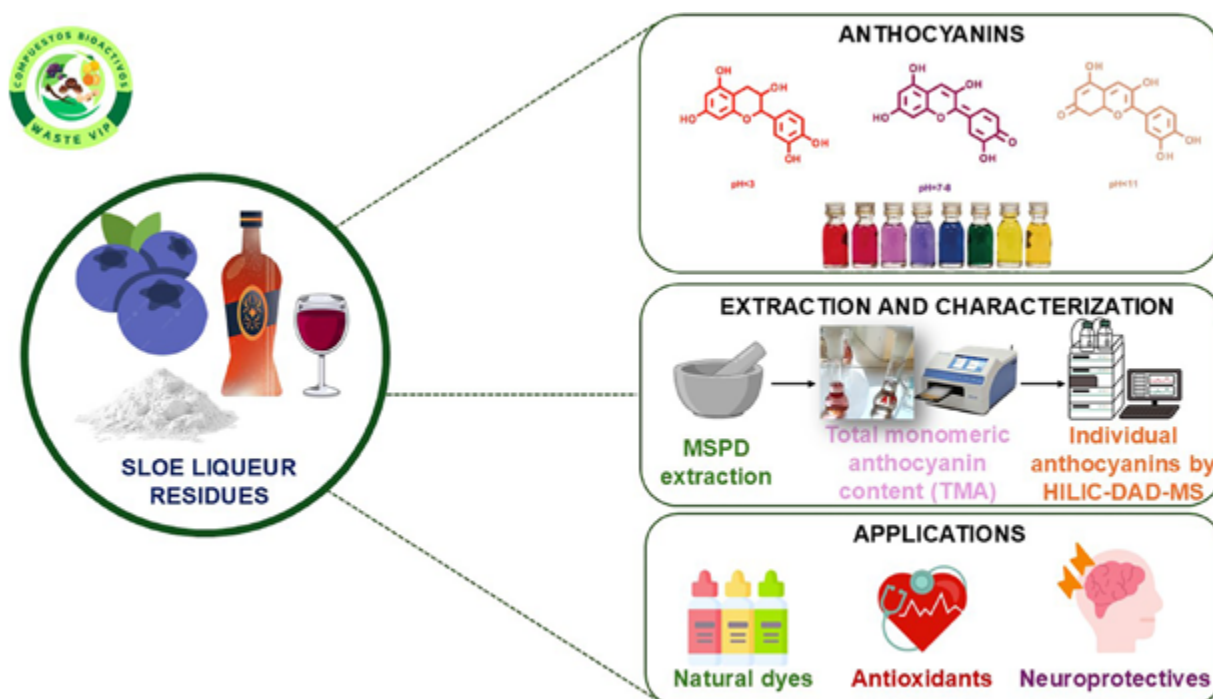
GREEN AND SUSTAINABLE RECOVERY OF BIOACTIVE ANTHOCYANINS FROM SLOE (*PRUNUS SPINOSA* L.) LIQUEUR RESIDUES

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Currently, there is a high level of interest in the search for new alternatives for the recovery of waste from the agri-food industry [1]. During the sloe (*Prunus spinosa* L.) liqueur processing, a large amount of by-products are generated. Specifically, the primary bio-residues include sloe skins and pulps post-maceration with aniseed, wash water utilized during the maceration process, and diatomaceous earth filters [1, 3]. These biowaste are well-known to be an astounding source of anthocyanins, a group of water-soluble pigments found naturally in various fruits and vegetables. Anthocyanins are pH-sensitive pigments, with colours ranging from red to blue/purple, which facilitate their use as natural dyes in various industrial sectors, such as cosmetics, food and textiles. In addition, these natural dyes have advantages over synthetic dyes such as lower toxicity, higher biocompatibility and lower cost. Given the antioxidant nature of anthocyanins, they can be used as dietary supplements to improve health, including the prevention of neurodegenerative diseases [4].

As such, their increasing demand as food and textile dyes, as well as their health-promoting properties have prompted the need for more efficient and sustainable anthocyanins extraction methods [2-3]. Among these, the matrix solid-phase dispersion (MSPD) technique has emerged as a promising approach, offering advantages in terms of simplicity, reduced solvent usage, and enhanced extraction efficiency [5]. In addition, the lack of comprehensive profiling and characterization of anthocyanins, especially using liquid chromatography coupled to mass spectrometry highlights the need for further research [6]. Therefore, this study focuses on the development of a green MSPD anthocyanin extraction procedure from sloe liqueur residues. In addition to determining the total content of monomeric (TMA) and polymeric anthocyanins by UV-Vis spectroscopy. Furthermore, the separation and quantification of the anthocyanin extracts was optimized by an innovative capillary HILIC-DAD-MS methodology. Furthermore, AGREEprep was selected as a metric tool to evaluate the greenness of the developed anthocyanin extraction method. The findings demonstrate the feasibility valorization of anthocyanins from sloe by-products generated during the generation of Pacharán liqueur, as well as its feasible potential application as natural colorants in food and textile industry, contributing to a circular economy model.



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P2-073**INNOVATIVE APPROACHES FOR SUGAR DETERMINATION IN POLYPHENOLRICH EXTRACTS FROM AGRI-FOOD WASTE**

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In recent years, society's interest in migrating towards an economic model based on the circular economy has been clearly increasing. Specifically, in the agri-food industry, the aim is to design processes that reduce the generation of by-products and, at the same time, revalorize the waste that is still generated. In this regard, the extraction of bioactive compounds allows for the reduction of waste while simultaneously leveraging their beneficial health effects by using them in various fields such as the food, pharmaceutical, or nutraceutical industries. Among bioactive compounds, polyphenols have gathered the greatest interest within the scientific community. These compounds have antioxidant, anti-inflammatory and antimicrobial properties. Therefore, extensive work has been done on their recovery and purification. However, it is still important to develop methodologies that allow for the characterization of the obtained extracts in terms of both concentration and purity. In this latter aspect, the development of appropriate methods to determine possible impurities, understood as any compound not belonging to the polyphenol family, is essential. Among these contaminants, sugars are notable, as they are always present in these agri-food residues. The determination of sugars is, therefore, especially important. However, due to their physicochemical properties, their determination using classical techniques such as liquid chromatography with UV detection is hindered since specific chromatographic columns and universal detectors must be used. Alternatively, relatively simple spectrophotometric methods for their determination have been reported based on redox reactions, in which polyphenols can interfere with the analysis providing inaccurate results. The objective of this work is to develop an analytical methodology to determine sugars in polyphenol-rich extracts. First, it has been demonstrated that spectrophotometric methods for sugar determination, such as dinitrosalicylic (DNS) or bicinchoninic acid (BCA) methods, have a strong contribution from polyphenols, giving responses similar to or even higher than those of sugars. Therefore, although these methods are useful for sugar determination when their concentration is much higher than that of polyphenols, in the opposite case, the error in their determination can be significant. As an alternative method, an LC-MS method has been developed to determine the most common sugars in these types of samples, namely glucose, fructose, rhamnose, and sucrose. The separation was carried out on a Luna Omega Sugar HILIC column, achieving optimal separation of the studied sugars in 7 minutes using isocratic elution with water/acetonitrile at a flow rate of 0.6 mL min⁻¹ and maintaining the column at 50°C. Detection was performed in negative multiple reaction monitoring (MRM) mode, monitoring transitions from the [M-H]⁻ ion. The obtained method was validated in terms of linearity, detection and quantification limits, and short- and long-term precision. The detection limits for glucose, fructose, sucrose, and rhamnose were 0.07, 0.01, 0.08, and 0.7 mg L⁻¹, respectively, and RSD values of less than 15% were obtained for precision. The developed method was applied to determine sugars in real extracts, comparing the result with that obtained using the DNS spectrophotometric method. Finally, the use of ion-mobility spectrometry-mass spectrometry (IM-MS) for the determination of target sugars was explored, yielding promising results.

P2-074

CAPILLARY ELECTROPHORETIC ANALYSIS OF CALCIUM AND MAGNESIUM ACETATE SAMPLES PRODUCED FROM OIL SHALE INDUSTRY WASTE

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Kukersite is a sedimentary rock that contains an organic fraction known as kerogen, which is widely used in shale oil production and energetics in Estonia. Power plants produce approximately 7 Mt of oil shale ash waste from kukersite per year, and most of it is deposited into piles in nature. [1, 2] Oil shale ash is rich in free lime, lime sulphates, silicates, and minerals which are valuable materials used in areas such as construction, pharmaceutical and plastic production. [3] Another potential use for oil shale ash is as a source of calcium and magnesium to produce an environmentally friendly ice-melting agent. [4]

Slippery roads are a real problem in norther countries and can pose a threat to humans and vehicles alike, which prompts the use of ice-melting agents. Sodium chloride is favored over acetate-based agents due to their high cost. In turn, salt is corrosive to cars, footwear, and animals, and it is also an environmental hazard. This is the reason why acetate-based agents are a valuable alternative to salt. [4, 5] Organic acids necessary for acetate production can be acquired from wood processing plant water. The use of oil shale ash and wood processing waters supports the idea of circular economy, which is using waste to recreate a new valuable product. [6]

In the present study, a capillary electrophoretic method with indirect ultraviolet detection was employed to analyze the prepared calcium and magnesium acetate samples. The developed method uses an imidazole buffer solution that is acidified by acetic acid, which is also responsible for creating acetate complexes with the analytes. In the developed capillary electrophoretic method, it is possible to separate calcium and magnesium in less than 5 minutes without prior sample derivatization. An internal standard lithium was used to ensure the accuracy of the results. The intraday repeatability relative standard deviation for both analytes was less than 2%, and the interday reproducibility relative standard deviation was less than 3%. The calibration curve for both analytes was linear, and the calculated R^2 value for both analytes was 0.999 on average. The method has also proven to be very sensitive. The average limit of detection for both analytes equaled 0.32 ppm and limit of quantification – 1.06 ppm.

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Oil shale ash is a waste product that is generated by shale oil production and energetics in Estonia, and it is rich in calcium and magnesium. Calcium and magnesium present in oil shale ash and wood processing plant wastewater rich in organic acids can be used for the production of environmentally friendly ice-melting agents. Calcium and magnesium acetate samples were effectively characterized via a fast, reliable, and reproducible capillary electrophoresis method.

P2-075

EVALUATION OF DIFFERENT ELECTROCHEMICAL ADVANCED OXIDATION PROCESSES FOR THE REMOVAL OF PMT COMPOUNDS FROM WASTEWATER AT PRE PILOT SCALE

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In efforts to advance a circular economy, initiatives such as the European Green Deal and the Circular Economy Action Plan play a pivotal role. These strategies promote sustainable resource management, including the efficient use and reuse of water wherever feasible. However, it is important to acknowledge that despite advanced treatment processes for water reclamation, complete removal of various organic micropollutants—classified as Contaminants of Emerging Concern (CEC)—is not always achieved [1][2]. CECs encompass a diverse array of substances, such as pharmaceuticals, industrial chemicals, pesticides, personal care products, and others. Many of these compounds are known for their persistence, mobility, and/or toxicity in aquatic ecosystems (PMT) [3–5]. Consequently, ongoing research is dedicated to developing innovative treatment methods for these pollutants, in line with sustainability and environmental stewardship goals.

To address this challenge, laboratory-scale experiments employing electro-peroxone processes—which integrate ozone with an electrochemical cell—were conducted on wastewater effluent samples. The presence of 43 preselected CECs was monitored at five reaction intervals (0, 2, 4, 6, and 8 hours) to determine optimal conditions for pollutant removal. These compounds had been previously identified and prioritised in an earlier study on wastewater effluent using a suspect screening analysis and ecological risk assessment. The monitored substances included 25 industrial chemicals (e.g., N,N-diphenylguanidine, tris(2-butoxyethyl) phosphate, benzotriazole derivatives, caprolactam), 10 pharmaceuticals (such as carbamazepine and venlafaxine, along with its metabolite O-desmethyl venlafaxine), 4 pesticides, 2 illicit drugs, and various other compounds. Analytical determination was carried out using on-line solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-QToF-MS/MS) under positive ionization mode, employing a hybrid quadrupole time-of-flight analyser.

Results revealed the presence of 32 out of the 43 selected CECs in the effluent of a conventional wastewater treatment plant used for the experiments. Concentrations ranged from 8.2–14.5 ng/L for the methadone metabolite EDDP, to 4.2–19.0 µg/L for compounds like the personal care product galaxolidone and the plasticizer dibutyl phthalate. Regarding electro-peroxone water remediation, all experimental setups demonstrated reductions in total contaminant concentrations above 27% after the initial treatment period (2 hours) and reaching values up to 80% for some families of compounds at the same time.

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P2-076

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF COPPER, TITANIUM AND IRON NANOPARTICLES BY GREEN METHOD USING INDUSTRIAL FRUIT WASTE

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Apple is one of the four most produced fruits globally. Approximately one third of apples are processed into high-value-added products industrially, and the remaining pulp from their production constitutes more than 25% of the processed fruit. It is evident that both the apple pulp and the peel contain phenolic compounds, including flavonoids and flavonoid glycosides. Materials with sizes ranging from 1 to 100 nanometres are designated as nanoparticles. There are three distinct synthesis methods – physical, chemical and green – that are employed to synthesise nanoparticles. The term "green synthesis" is employed to denote a method of producing nanoparticles by means of a process involving a natural molecule and metal salts as reducing agents. The apple waste was extracted in 70% ethanol for 24 hours, after which ultrasonic extraction and filtration were performed. The substance was then subjected to liquid-liquid partition, after which it was extracted with ethyl acetate. Approximately 3 mg of extract was dissolved in distilled water and 20 mmol of metal salt was added. The pH of the prepared mixture was adjusted to 12. The solution was then subjected to an incubation process at a temperature of 90°C for a duration of three hours. The solution that had been allowed to reach room temperature was subjected to three consecutive centrifugation cycles at 5,000 rpm, after which the precipitate was dried at 60°C [1,2]. The evaluation of antibacterial activity was performed using the broth microdilution method, in full compliance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. Subsequently, the in vitro antibiofilm activity of the nanoparticles was evaluated using the modified crystal violet binding assay, as outlined by Jardak et al. (2021) [4]. The anti-quorum sensing activity was evaluated using the reporter strain *C. violaceum* ATCC 12472. The experimental protocol was based on the methodology outlined by Batohi et al. (2021) [5]. The anti-cancer activity of the nanoparticles was evaluated by performing cell viability analysis [6]. The present study examined six distinct cell lines. In this study, the highest phenolic content was observed in the ethanol extract of industrial apple waste, and synthesis was carried out using this extract. Upon examination of the characterisation studies, it was determined that a successful synthesis had been achieved. The UV-visible region spectra are consistent with the extant literature. The Raman spectrum indicates the presence of functional groups, which have been shown to enhance stability on the surface of nanoparticles. Subsequent examination of the EDX results confirmed that the synthesised nanoparticles were devoid of any impurities. Scanning electron microscopy (SEM) images revealed that the size of the nanoparticles was less than 100 nm. In consideration of the antibacterial, antibiofilm and anti-quorum sensing activity results, it was determined that CuNP was the most effective broad-spectrum agent, while FeNP and TiNP exhibited comparatively weaker effects or no discernible effects. Cu NP demonstrated the most effective anticancer properties. In the A2780 ovarian cancer cell line, the copper (Cu) nanoparticles were the most effective, with an IC₅₀ value of 0.797±0.062µg/mL. Furthermore, Cu nanoparticles have been demonstrated to reduce cell viability in HCT-116, SW480 and OVCAR-3 cells.

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P2-077

GREEN SYNTHESIS, CHARACTERIZATION, AND ANTICANCER EVALUATION OF CERIUM AND COPPER OXIDE NANOPARTICLES SYNTHESIZED FROM *CORYLUS AVELLANA* L. HUSK EXTRACTS

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Green synthesis approaches have gained prominence as safer and more sustainable alternatives for nanoparticle production. In this study, cerium oxide (CeO₂) and copper oxide (CuO) nanoparticles were synthesized using *Corylus avellana* L. (hazelnut) husks, a phytochemical-rich agricultural waste. The aim was to investigate whether hazelnut husk extract could function as a reducing and stabilizing agent in nanoparticle synthesis and to evaluate the anticancer activity of the resulting nanomaterials [1-3]. The husks were first cleaned, air-dried, and ground into fine powder. Extraction was carried out via ultrasonic-assisted extraction (UAE) using a methanol: water mixture (80:20), selected for its efficiency in isolating both polar and moderately non-polar phytochemicals. The extract was concentrated at 45 °C under reduced pressure using a rotary evaporator and stored at +4 °C until further use. Bioactive compounds such as flavonoids and phenolic acids in the extract played a key role in nanoparticle formation, as indicated by a visible color change during the reaction. For the CeO₂ nanoparticles, 10 mg of extract was dissolved in 30 mL of the solvent mixture, and 20 mmol of cerium(III) nitrate hexahydrate was added. The pH was adjusted to 10, and the solution was incubated at 90 °C. Similarly, 12,5 mg of extract was used for CuO synthesis, combined with 20 mmol of copper chloride in 30 mL solvent. After adjusting the pH to 10, the mixture was incubated at 60 °C for 24 hours. Both types of nanoparticles were purified by centrifugation and dried at 90 °C. The synthesized nanoparticles were characterized using various analytical techniques. UV-Vis spectrophotometry showed absorption peaks at 299 nm for CeO₂ and 270 nm for CuO, confirming their formation. SEM and AFM imaging revealed predominantly spherical and quasi-spherical particles, with average diameters around 15 nm for CeO₂ and 20 nm for CuO. Elemental composition was validated through EDX analysis, which showed strong cerium, copper, and oxygen signals with no major contaminants. Raman spectroscopy provided further confirmation by detecting characteristic Ce-O and Cu-O vibrational bands [4-9]. Biological evaluation was performed using the MTT assay on HCT-116 and SW480 human colorectal cancer cell lines. After 72 hours of exposure, CuO nanoparticles showed notable dose-dependent cytotoxicity. In SW480 cells, viability was significantly reduced within the 0.8475–3.3900 µg/mL range, while HCT-116 cells showed substantial response between 1.695–3.390 µg/mL. CeO₂ nanoparticles, in contrast, did not cause a statistically significant reduction in viability at any tested concentration, suggesting low cytotoxicity and potential biocompatibility. The divergent biological outcomes are likely related to redox behavior. CuO nanoparticles are known to generate reactive oxygen species (ROS), leading to oxidative stress and apoptosis in cancer cells. On the other hand, CeO₂ nanoparticles have a dual redox function that allows them to act as antioxidants or pro-oxidants depending on the environmental conditions, possibly explaining their milder effects on cell viability. In conclusion, CuO nanoparticles synthesized through a green route using hazelnut husk extract demonstrated strong anticancer potential against colorectal cancer cells, likely through ROS-mediated mechanisms. While CeO₂ nanoparticles did not show cytotoxic effects, their stability and redox properties highlight possible uses in other biomedical contexts. Overall, this study demonstrates the viability of using agricultural waste as a raw material in the green synthesis of functional nanoparticles, offering a sustainable and cost-effective method aligned with the principles of green nanotechnology.

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P2-078**TANNIC ACID-BASED POLYMER AS A SUSTAINABLE SORBENT FOR THE DISPERSIVE SOLID-PHASE EXTRACTION OF PERSISTENT CONTAMINANTS IN WATER SAMPLES**

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The development of environmentally friendly materials for miniaturized sample preparation techniques is a growing priority in Analytical Chemistry, as the field moves toward greener and more sustainable methodologies [1]. In this context, tannic acid –a naturally occurring polyphenol– emerges as a promising and attractive building block in the development of sustainable analytical materials due to its wide availability, renewable origin, high biodegradability, and abundance of hydroxyl groups capable of forming strong interactions with a wide variety of analytes [2].

This study reports the synthesis and application of an innovative degradable polymer, designed using tannic acid as a bio-based monomer and 1,4-bis(propioloyloxymethyl)benzene as the linker, for use as a sorbent in dispersive solid-phase extraction. The polymer shows good affinity and selectivity for the simultaneous extraction of diverse classes of environmental contaminants from water samples, including organophosphate esters, polycyclic aromatic hydrocarbons, organochlorine pesticides, polychlorinated biphenyls, UV filters, and antibacterial agents. The material exhibits excellent analytical performance mainly attributed to its porous structure, diverse surface functionality, and high compatibility with aqueous matrices. These characteristics enable efficient retention and preconcentration of analytes. Extraction conditions were optimized to ensure reproducibility and maximize recovery efficiency. Analyte determination was carried out using gas chromatography coupled to mass spectrometry, yielding low detection limits. After its use for analytical purposes, the degradable nature of the polyester-like network allows monomer recovery under mild conditions, significantly reducing chemical waste, minimizing environmental impact and contributing to circular material use.

Overall, this work demonstrates the feasibility of integrating natural and biodegradable monomers into high-performance sorbents for sample preparation. The proposed material provides a sustainable, efficient, and cost-effective alternative to conventional synthetic sorbents, emphasizing the importance of green material design in enhancing the environmental sustainability of analytical methodologies.

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P2-079

HYPERSPECTRAL IMAGING COUPLED WITH MACHINE LEARNING FOR PRECISE IDENTIFICATION OF BLENDED TEXTILE FIBERS IN CIRCULAR ECONOMY APPLICATIONS

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Textile recycling remains a major bottleneck in circular economy initiatives due to the complexity of accurately identifying fiber compositions in discarded garments. The widespread use of fiber blends such as cotton-polyester or polyamide-elastane poses significant challenges for conventional sorting technologies, which are often unable to distinguish small differences in composition or low-percentage synthetic additives. In this study, we demonstrate the application of Hyperspectral Imaging (HSI) in the near infrared (NIR) region combined with advanced machine learning algorithms to accurately classify pure and blended textile fibers. This approach supports the scalable, automated sorting needed for sustainable textile recovery and recycling.

Using an HSI system operating in the 900–1700 nm range, reflectance spectra were collected from both sides of each textile sample to account for surface treatments, texture variability, and dye interference. The dataset comprised over 200 textile samples, including pure fibers (cotton, polyester, polyamide, wool) and binary blends with variable compositions (e.g., 97% cotton–3% elastane, 80% polyester–20% cotton, etc.). Data preprocessing involved Savitzky–Golay smoothing and Standard Normal Variate (SNV) correction to enhance spectral features and reduce baseline noise. Principal Component Analysis (PCA) was initially employed to explore variability across samples.

For supervised classification, we trained and tested Support Vector Machines (SVM), Random Forests (RF), and k-Nearest Neighbors (k-NN) using both Leave-One-Out Cross-Validation (LOOCV) and external validation protocols. Results showed that RF achieved the highest overall accuracy across all fiber blend types, reaching high percentage (~90%) in LOOCV versus external validation, particularly excelling in differentiating low-percentage elastane blends a task where SVM struggled. Regression models (SVR, RF regression, k-NN regression) were further applied to estimate fiber ratios in blended textiles, with RF regression achieving R^2 values above 0.90 and low Root Mean Square Error of Prediction (RMSEP < 1), even for complex mixtures.

Our findings confirm that HSI, when combined with ensemble learning models, offers a reliable, non-destructive method for identifying fiber content with high resolution. This enables the development of intelligent sorting lines capable of handling real-world post-consumer waste, a critical step toward closing the loop in textile value chains. Previous studies have demonstrated HSI's effectiveness in agricultural and food product sorting [1,2], but its adoption in textile recycling remains limited. This study bridges that gap, presenting a scalable solution with significant implications for material recovery facilities and the textile industry's shift toward circularity [3,4].

In conclusion, HSI-based classification models offer a promising toolset for textile recycling applications by enabling rapid, accurate, and automated identification of fiber blends. By integrating these technologies into industrial sorting workflows, we can significantly reduce material loss, improve purity of recovered fibers, and support sustainable manufacturing practices. These advancements contribute to achieving EU Green Deal targets and reducing the environmental footprint of the textile sector.

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P2-080**QUALI- AND QUANTITATIVE DETERMINATION OF THE ENERGY POTENTIAL OF BIOMETHANE FROM AGRICULTURAL PRODUCTS**

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An economy based on renewable energy sources is the hallmark of a concrete and effective energy transition. Climate policy and commitments made in response to the energy crisis have led to a search for alternative ways to produce energy. In Europe, 950 million tons of biomass are produced each year, including agricultural, animal, industrial, and food wastes, which are excellent substrates for the production of clean and sustainable energy within circular economy models [1]. These data highlight how biomass waste can cover up to 65% of total fossil fuel consumption in Europe. Biogas and biomethane are promising forms of renewable energy and sustainable solutions to the challenges posed by fossil fuel depletion, climate change, and greenhouse gas emissions [2]. Anaerobic digestion (AD) is an efficient method for converting waste biomass into biomethane, offering a viable solution for treating waste from various sources. AD is the biochemical process of decomposing complex organic substances into simpler chemical components under anaerobic conditions [3]. As the organic material in the substrate is degraded through a series of complex microbiological processes, biogas is produced continuously during digestion until no more biodegradable material remains [4]. Proper management of different agricultural biomass, residues, and by-products can reduce their negative impact on the environment. This study aimed to examine the methane production potential of some experimental substrates (n=5), namely samples of agricultural waste (sunflower waste, animal manure, straw, corn husks and corn silage) using the automatic system for measuring methane potential (BMP) (AMPTS® II - Automatic Methane Potential Test System). The AMPTS aims to assess the biodegradability of organic substrates under mesophilic conditions (39 °C) by monitoring methane production in real time. This test provides a preliminary indication of the biodegradability of the substrate and its potential for methane production through anaerobic degradation. Each reactor has a volume of 600 mL and is connected to a mechanical stirrer and an alkaline solution unit for the chemical removal of CO₂ and H₂S. The samples were placed in a water bath according to AMPTS® II standard operating procedures. The total volume of substrate and inoculum in the reactors, always equal to 400 mL, produced a gas volume of 200 mL. The digestion time was set at 30 days, corresponding to a commonly used average interval to ensure adequate anaerobic degradation in mesophilic tests. Anaerobic batch fermentation tests, performed in duplicate, showed that the “corn silage” (4992 NmL) and “corn husk” (4500 NmL) samples obtained the highest total biomethane yields among those analysed. These results indicate a high methanogenic potential of the agricultural biomass studied, confirming Italy's significant capacity to exploit these resources for sustainable biogas production and as a support to national decarbonisation targets. Furthermore, the possible application of the substrates analysed under real operating conditions suggests the practical feasibility of their integration into industrial-scale anaerobic digestion processes, thus supporting the evolution of a more sustainable energy system.

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P2-081**EXPLORING THE BIOACTIVE PROPERTIES OF THE CANARY ISLANDS BANANA AND ALOE VERA BY-PRODUCTS: PRELIMINARY RESULTS**

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Across Europe, approximately 88 million tonnes of food are discarded annually, incurring economic losses estimated at around 143 billion euros. Fruits and vegetables represent the largest share of this waste [1]. In this context, reducing food loss and waste emerges as a key approach to improving food system efficiency, lowering production expenses, and promoting environmental sustainability. The European Union has prioritized this objective by encouraging the repurposing of food by-products and the development of innovative applications for them [2]. Within this framework, the valorization of plant-based by-products in the food and nutraceutical industries offers a promising, sustainable solution. In the case of the Canary Islands, despite financial support from the European Union in recent years, different agricultural sectors continue to face substantial challenges in competing with international producers. As a result, identifying strategies to reduce production costs and enhance the export potential of Canarian products is essential to ensure the sustainability of the industry. Thus, this approach not only addresses the environmental burden of agricultural waste but also contributes to cost reduction and strengthens the agricultural sector in the Canary Islands.

With this aim, in this work, the bioactive properties of different Canarian by-products have been evaluated. The results obtained about the nutritional and phytochemical composition of these matrices provided information to extend the uses of some by-products and promote new practices in line with new trends towards a more sustainable and circular system.

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P2-082**RAMAN SPECTRAL SIMILARITY METRICS: KEY CHALLENGES AND CONSIDERATIONS****Mostafapour, Sara¹; Mokari, Azadeh²; Guo, Shuxia²; Popp, Jürgen²; Bocklitz, Thomas².**

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Raman spectroscopy is valued for its rapid, reliable, and non-destructive analytical capabilities, making it widely used in industry and academia for chemical analysis and material identification. In addition, Raman spectroscopy provides fingerprint information that can be used for molecular characterization. Compounds can be identified by comparing the measured spectrum with databases to determine the probable compounds contained in the sample under investigation. Similarity metrics such as Pearson's correlation coefficient, cosine distance, Euclidean distance, etc. are used for comparing measured spectra and spectra in a database [1]. These techniques are effective under ideal conditions. However, real-world spectral measurements are often accompanied by artifacts and/or variability arising from instruments, samples, and experimental conditions that can distort spectral features. These artifacts compromise the reliability of similarity assessments [2]. Therefore, this highlights the importance of understanding the robustness and reliability of different similarity metrics encountering spectral artifacts and variations.

In this study, we investigate various spectral similarity metrics to understand their response to different types and strengths of artifacts. We added common spectral artifacts, including Gaussian noise, baseline profiles, and wavenumber shifts to a synthetic Raman spectral data set to simulate real-world variability. We then evaluated their impact on similarity metrics such as correlation distance, cosine distance, root mean square error (RMSE), angle value, Euclidean distance, Manhattan distance, structural similarity index measurement (SSIM), and Spearman's rank correlation coefficient (Rho).

The results show that RMSE, angle value, and Euclidean distance are less affected by Gaussian noise, making them more reliable in noisy conditions. The influence of baseline profiles depends largely on the normalization method used. Wavenumber shifts, however, pose a significant challenge, with their impact varying based on both shift magnitude and spectral band patterns. These results emphasize the need for careful preprocessing to ensure accurate spectral similarity assessments.

Keyword: Raman spectroscopy, Database indexing, Similarity metrics

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P2-083

ARTIFICIAL INTELLIGENCE FOR MODELING STRUCTURE-ENANTIORESOLUTION RELATIONSHIPS IN HPLC USING CELLULOSE-BASED CHIRAL STATIONARY PHASES

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Chirality is a fundamental property of molecular building blocks in biological systems. Given the high degree of stereospecificity exhibited by biological processes, the effects on living organisms of enantiomers can be distinct. Consequently, there is a pressing need to develop robust analytical methodologies for the identification and quantification of chiral compounds. At present, liquid chromatography with chiral stationary phases (CSPs) is the preferred technique. However, the identification of the optimal mobile phase (MP) for a given CSP remains a costly, time-consuming trial-and-error process.

To approach this problem, an effective enantioseparation (*EES*) parameter was introduced, providing enantiomeric resolution penalized in the case of excessive analysis times. Artificial neural networks (ANNs; for regression: $T = f(X)$) facilitate modeling molecular structure-*EES* relationships for the prediction of *EES*-MP profiles. More than 70 ANNs were trained on 76 structurally diverse chiral compounds, using 62 molecular descriptors, and chromatographic data from binary mobile phases (MPs) of acetonitrile (ACN) and ammonium bicarbonate (5 mM, pH 8), with ACN ratios ranging from 98% to 30% (v/v), and tris(3,5-dimethylphenylcarbamate) cellulose CSP (*T*-matrix). A chaotic neural network algorithm with competitive learning (CCLNAA) was used to optimize the internal architecture of the ANNs and the feature selection of the descriptors employed as *X*-matrix.

The CCLNNA algorithm employs a customized function to find the optimal values of the solution vector. The optimization function is a combination of two personalized quality indicators for R-squared but mitigating overfitting and residual impact in the validation plot (experimental (*T*) vs. predicted (*Y*) value).

An artificial neural network (ANN) consensus model, comprising the top five ANNs in terms of best predictive capacity, was utilized for predicting the *EES* values across the nine MPs studied (*EES*-MP profiles), varying in ACN content for the 76 learning compounds and then 2 test external compounds. The consensus *EES* value was obtained as the median (*Median(Y)*) of the individual predictions from the 5 ANNs. The implementation of an ANN-consensus model proved effective in the development of a predictive system capable of predicting *EES*-MP profiles and indirectly selecting a suitable MP in the case of favorable enantioresolution.

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P2-084**AUTOMATING PEAK TRACKING IN LIQUID CHROMATOGRAPHY USING GLOBAL RETENTION MODELS****Peiró Vila, Pau¹; Torres Lapasió, José Ramón¹; García Álvarez-Coque, María Celia¹; W. J. Pirok, Bob²; S. Bos, Tijmen².**

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Liquid chromatography (LC) is widely used across various fields, but inefficiencies persist due to limited expertise and time-consuming method development. Optimising mobile-phase composition for high resolution remains a challenge, particularly with trial-and-error approaches. Retention modelling offers a systematic alternative by correlating analyte retention with mobile-phase composition. Conventional models often require extensive data fitting across multiple experiments, which can be difficult for complex samples where injecting standards is unfeasible. Additionally, peak tracking with UV-DAD detectors is complicated by peak overlap and coelution. Our research group has developed global retention models to characterise solutes as a group rather than individually. By extracting common system parameters related to the column and solvent, we can estimate the retention for other compounds. A restricted global model is initially constructed using retention data from 10 to 16 peaks, which can be extended to predict additional peaks, creating an extended global model. To address the time-consuming and error-prone nature of manual peak tracking, we automated the matching process, focusing on the main peaks in the chromatograms.

We developed a set of MATLAB functions to process chromatograms from methanol-water lemon balm extracts, using six multi-linear gradients recorded at eight wavelengths (210-280 nm). Baseline correction was performed using the arPLS algorithm. Peak properties, such as position and area, were determined modifying the findpeaks function, with areas calculated via the trapezoidal method. Peaks accounting for over 2% of the total chromatogram were retained, and 15 main peaks from the reference chromatogram (G1) were used for matching with other chromatograms. Chromatogram processing generated a structure containing peak properties, including amplitude, width, area, prominence, and percentage relative to the total chromatogram. To improve peak comparison, we used various distance metrics and normalisation techniques. Despite gradient variability, a time window restriction ($\pm 15\%$) was introduced for candidate comparison. For more accurate peak fitting, we used an exponentially modified Gaussian hybrid function (EGM), which reduced interference from coeluting peaks. We then applied correlation coefficients to compare fitted curves, moving away from property-based comparisons.

However, combining these metrics did not consistently yield accurate assignments, particularly with peak clustering. Future work will focus on refining global retention models to enhance peak matching accuracy, including optimising the search window for candidate peaks.

P2-085**APPLICATION OF DEEP MACHINE LEARNING FOR THE CLASSIFICATION OF 10 TYPES OF PLASTICS BASED ON RAMAN SPECTRA****Wójcik, Szymon; Stoch, Paweł; Król, Magdalena.***AGH University of Krakow, Kraków, Poland.*

The classification of plastics is essential for several reasons, particularly in the context of recycling practices, environmental protection efforts, and the global transition toward a circular economy. A comprehensive understanding of the physicochemical properties of different polymeric materials enables the implementation of more efficient waste management strategies that contribute to sustainable development. Effective plastic classification enhances the recycling process, which is critical for mitigating plastic waste accumulation. The International Organization for Standardization (ISO) has established a framework categorizing plastics into four primary recycling techniques: reuse, mechanical recycling, chemical recycling, and energy recovery.

Raman spectroscopy represents a powerful analytical tool for plastic classification, offering numerous advantages that enhance material identification and characterization. Its non-destructive nature, high sensitivity, and ability to reveal detailed molecular information make it particularly effective in the analysis of plastics across various applications, such as environmental monitoring and recycling processes. This capability is underscored by studies demonstrating how Raman spectra uniquely characterize different polymers, including their structural properties and chemical composition. Notably, the distinct spectral features arising from fundamental molecular vibrations enable precise differentiation of plastic types, facilitating efficient sorting and recycling within waste management systems.

The aim of the studies was to develop a potentially automatic procedure of different plastics classification based on the Raman spectroscopy. It was assumed that the proposed classification model should be as general as possible and work correctly even if different experimental conditions were applied. The plastics originated from various sources and were the subject of Raman spectroscopy measurements using fast portable devices. In the studies ten different plastic types were utilized, specifically: PET (polyethylene terephthalate), HDPE (high-density polyethylene), PVC (polyvinyl chloride), LDPE (low-density polyethylene), PP (polypropylene), PS (polystyrene), ABS (acrylonitrile-butadiene-styrene), PC (polycarbonate), PLA (polylactide), and PTFE (polytetrafluoroethylene). These samples were sourced from everyday local environments, including bottles, packaging materials, device housing, etc. In total, a dataset of 3,000 Raman spectra was acquired under varying laser power conditions.

The preprocessing workflow involved several key steps. The acquired Raman raw spectra underwent various preprocessing procedures, including despiking, denoising, baseline correction, and normalization. To reduce the complexity of the problem, principal component analysis (PCA) was applied, retaining principal components that accounted for 95% of the system's variance. This resulted in 74 principal components, which were used as the feature vector. Several machine learning and neural network models were tested, and the most effective model was identified as the Wide & Deep deep learning architecture. This model represents a hybrid machine learning framework designed to combine the strengths of both linear models and deep neural networks (DNNs) to enhance predictive performance. Its application significantly improved model generalization, achieving a classification accuracy of approximately 99% on external plastic samples measured under different experimental conditions.

The proposed integration of an advanced, versatile deep learning model with Raman spectroscopy yields promising results. The development of a comprehensive, previously undocumented Raman spectral database for plastics provides a foundation for further research and expansion to include additional polymeric materials. Moreover, this holistic approach facilitates the adaptation of the technique for real-time recycling line applications, particularly for samples of uncertain origin, enhancing the efficiency and accuracy of plastic waste sorting and classification.

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The classification of plastics is crucial for effective recycling and environmental protection, supporting the transition to a circular economy. Raman spectroscopy, with its non-destructive nature and high sensitivity, represents a powerful tool for polymer identification. In this study, an automated procedure for classifying ten types of plastics (PET, HDPE, PVC, LDPE, PP, PS, ABS, PC, PLA, PTFE) was developed based on Raman spectroscopy data collected using portable devices under varying experimental conditions. A dataset comprising 3,000 Raman spectra was compiled. Data preprocessing included despiking, denoising, baseline correction, and normalization, followed by principal component analysis (PCA) for dimensionality reduction. The Wide & Deep deep learning model achieved a classification accuracy of approximately 99% on external samples, demonstrating high generalization. A Raman spectral database for plastics was developed, providing a foundation for further research and applications in real-time waste sorting systems.

P2-086**HANDHELD SPECTROSCOPY FOR QUALITY ASSESSMENT IN CHEMICAL INDUSTRY**

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In industry, it is important to do a quick quality assessment to steer the process in terms of cost and sustainability, however, routine measurements in the lab are time and effort consuming. Handheld spectroscopy offers a promising solution for quick, non-invasive quality assessment in the chemical industry. Here we explore the use of handheld spectroscopic devices to predict physical properties of methylene diphenyl isocyanate (MDI), indicators of product quality that influence its performance and suitability for industrial applications. Various spectrometers were evaluated under different experimental conditions to determine their effectiveness. One of the NIR spectrometers emerged as the most suitable device for both colour and viscosity predictions. The optimal conditions for these measurements were found to depend on the modelling results as well as the operator input, with the recommended protocol being to scan in a dark environment using a round jar. Future work will focus on validating this protocol in real-world conditions, developing robust predictive models for field use, and training operators to ensure accurate implementation.

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P2-087**CHEMOMETRIC RESOLUTION OF IM-MS DATA: ROIMCR WORKFLOW**

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Ion mobility (IM) adds a powerful dimension to liquid chromatography coupled to mass spectrometry (LC-MS) analysis, improving selectivity and identification of structurally similar compounds. However, the addition of the new analysis dimension (IM) and the large datasets implies a significant challenge for data processing. Therefore, most investigations focus primarily on the use of the collision cross section (CCS) value as a complementary parameter in mass spectrometry untargeted identification, rather than exploring the huge multidimensional information available. Multidimensional and multivariate data processing tools are required to process IM-MS data, integrate IM data with MS/MS fragmentation, and resolve the different chemical species. In this context, the Regions of Interest Multivariate Curve Resolution – Alternating Least Squares (ROIMCR-ALS) approach is a combined chemometrics method based on one hand, on the ROI compression and filtering of MS data (Gorrochategui et al., 2019), and on the other hand on the MCR-ALS bilinear factor decomposition (De Juan et al., 2014) of the previously obtained ROI MS data. This combined method represents an effective strategy for effective feature extraction, and resolution of the spectra and elution (overlapping) profiles of the constituents present in complex samples without losing relevant chemical information and maintaining instrumental mass accuracy. In this work, we provide a step-by-step overview of the data workflow, including importing and structuring LC-IM-MS data (MS¹ and MS²) in MATLAB, defining the regions of interest (ROIs) across multiple dimensions, constructing MS data matrices with retention, m/z ROI and mobility values, and applying multivariate curve resolution – alternating least squares (MCR-ALS) for component separation in large IM-MS data sets. In addition, the estimation of CCS values will be shown using instrumental information obtained in the analysis of previously calibrated samples. A case of study involving extractable and leachable of plastic additives is shown. This technical workflow offers a new strategy to analyse these IM-MS multidimensional complex data sets.

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P2-088**IMPROVING BENCHTOP NMR PERFORMANCE THROUGH CHEMOMETRIC OR AI-BASED APPROACHES****Pujol, Camille¹; Jéré, Quentin²; Gilard, Véronique¹; Jolibois, Franck²; Poteau, Romuald¹; Balayssac, Stéphane¹.**

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Benchtop nuclear magnetic resonance (NMR) spectrometers are increasingly used across various industries due to their affordability, portability, and accessibility compared to high-field NMR systems. They enable rapid, non-destructive analysis of diverse samples, making them suitable for food quality control, pharmaceutical analysis, and forensic applications. However, these systems often face limitations in sensitivity and resolution, which can complicate the analysis of complex samples. To overcome these challenges, chemometric techniques have been widely applied, enhancing the accuracy and reliability of benchtop NMR analyses. In parallel, AI-based methods such as Artificial Neural Networks are emerging as powerful tools for improved pattern recognition and data interpretation in complex spectral datasets.

Chemometrics, including methods such as Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and PLS regression, significantly enhance the interpretative power of NMR data. These techniques enable the identification, classification, and quantification of compounds in complex matrices, making them highly effective for a variety of analytical tasks. Similarly, Artificial Neural Networks provide a flexible and robust framework for modeling non-linear relationships in spectral data, further extending the scope of benchtop NMR applications, including accurate and efficient quantification of mixture components.

This poster presents two applications using proton NMR data from a 60 MHz benchtop spectrometer, processed through complementary approaches:

- The first case illustrates the use of a chemometric workflow for screening honey-based food supplements adulterated with pharmaceutical compounds. The workflow employs PLS-DA models to classify samples as adulterated or non-adulterated and to identify the presence of specific adulterants. Additionally, PLS regression models enable quantification of sildenafil or tadalafil, allowing efficient and reliable detection through NMR data.
- The second case involves a rapid Artificial Neural Network-based method to evaluate component proportions in mixtures. The application focuses on e-liquids (liquid solutions used in electronic cigarettes), whose matrix primarily consists of glycerol and propylene glycol. This approach enables fast and accurate determination of mixture composition from spectral features.

These examples highlight the potential of integrating benchtop NMR with chemometric and AI-based techniques to tackle complex analytical challenges, enhancing result reliability and opening new opportunities in quality control and regulatory compliance.

P2-089**STUDY OF ZEBRAFISH EARLY EMBRYONIC DEVELOPMENT USING RAMAN AND FLUORESCENCE IMAGING AND CHEMOMETRICS**

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Hyperspectral images (HSI) are analytical measurements that provide spatial and spectral information. HSI provide complex data that often require chemometric unmixing analysis tools, such as Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS), to facilitate their interpretation.

Hyperspectral Raman and fluorescence images followed by MCR-ALS application yield spatially and spectrally resolved components, related to distinct fingerprints and their spatial location within complex, heterogeneous biological samples^{1,2}. This is the case for early-stage zebrafish embryos that serve as an invaluable model for studying complex biological processes due to their optical transparency, genetic similarity with humans and rapid development. During gastrulation, a morphogenetic movement known as epiboly occurs, involving the coordinated expansion of an epithelial-like monolayer of cells (EVL), the multilayered deep cells (DCs) and the yolk syncytial layer (YSL) to engulf the yolk and close the blastopore^{3,4}. Confocal Raman spectroscopic imaging (cRSI) and confocal fluorescence microscopy enable the capture of intricate biomolecular dynamics underlying epiboly without compromising embryo structure integrity.

Prior to HSI acquisition, embryos were fixed with paraformaldehyde (PFA), had their chorion removed, labeled with fluorescent dyes and were mounted in low-melting-point agarose at 25% and 75% epiboly progression, matching the different epiboly stages. MCR-ALS results showed three distinct biological and chemical components: lipids, protein regions, and carotene and allowed the visualization of biomolecular distributions in three dimensions and characterization of anatomical features at cellular level. These insights enhanced the understanding of zebrafish epiboly and its broader implications for cellular and molecular morphogenesis, thereby providing a foundation for comparative biomolecular analysis across developmental contexts.

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P2-090**APPLICATION OF DIGITAL IMAGING IN NEPHELOMETRIC DETECTION**

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Digital imaging systems are increasingly recognised as cost-effective and versatile alternatives to traditional photometric detectors in analytical chemistry. These systems typically integrate an inexpensive webcam, LED lighting, and open-source image analysis software, enabling the recording and quantitative assessment of colour or light-scattering changes that occur during chemical reactions. Unlike conventional spectrophotometers, which measure light intensity at a single point, digital imaging enables the analysis of whole images and the extraction of RGB or grayscale intensity values from specified regions of interest within the sample.

Numerous approaches to applying digital imaging in chemical analysis are documented in the literature. The present study focuses on its application in nephelometric detection—a technique that measures light scattered by particles suspended in solution. A setup consisting of a webcam, a 3D-printed cuvette holder, and appropriately selected LED lighting was employed. Image acquisition and processing were performed using ImageJ software, with particular attention given to the selection of RGB colour channels and critical instrumental parameters such as measurement time, LED type, and mixing conditions.

Two nephelometric methods were developed and evaluated: (1) the immunonephelometric determination of cystatin C, a biomarker of chronic kidney disease, and (2) the determination of chloride ions via precipitation with silver nitrate. Validation of the proposed methods included assessment of linearity, detection and quantification limits, sensitivity, precision, and accuracy. The results confirmed that digital imaging provides sensitive and reproducible measurement of scattered light, representing a portable, low-cost, and effective alternative for clinical and environmental applications.

The research was conducted with the financial support of the Anthropocene Priority Research Area as part of the Strategic Programme Excellence Initiative at the Jagiellonian University.

P2-091**METHODS VALIDATION APP: A FREWARE GRAPHICAL USER INTERFACE FOR METHOD VALIDATION**

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Methods Validation App (MVA) is a new desktop application developed to address the growing demand for efficient and reliable method validation in regulated laboratories. Developed in Python, it integrates powerful libraries for statistical analysis with an intuitive, user-friendly interface that simplifies complex validation tasks.

MVA streamlines the validation process by implementing a protocol that maximizes the number of validation parameters obtained from a minimal set of experiments [1, 2]. By automating key steps such as calibration, limit of detection, precision, and accuracy, it reduces the risk of human error, ensures consistency, and accelerates evaluations.

Furthermore, MVA supports laboratories in performing efficient, standardized validations, meeting the requirements of major regulatory guidelines [3]. Its versatility makes it especially valuable in laboratories where validated methods are essential, including pharmaceuticals, environmental testing, food safety, and toxicology.

In summary, MVA modernizes method validation by combining advanced statistical tools with an accessible interface boosting productivity, enhancing result reliability, and expanding access to robust validation practices across a wide user base.

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P2-092**FIELD-READY APPROACH FOR SIMULTANEOUS QUANTIFICATION OF SILOXANES AND CONDENSABLE SULFUR SPECIES IN BIOGAS****Agarwal, Ayush¹; Torrent, Laura²; Indlekofer, Julian³; Bouchet, Sylvain⁴; P. Culleton, Lucy⁵; Biollaz, Serge³; Ludwig, Christian¹.**

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Broader deployment of biogas is often constrained by trace impurities such as siloxanes and sulfur compounds. Even at low concentrations, these contaminants can cause catalyst deactivation, corrosion, and reduced operational efficiency. The increasing deployment of biogas as a renewable energy source across Europe, particularly in decentralized small- and medium-scale facilities, underscores the need for reliable impurity monitoring techniques. Regulatory frameworks such as EN 16723 impose strict limits on sulfur and silicon content, yet many facilities lack access to advanced analytical instrumentation or standardized methodologies. This gap introduces uncertainty in compliance and remains a barrier to the wider adoption of biomethane. Robust quantification methods are therefore essential to ensure gas quality, protect infrastructure, and support the long-term sustainability of biogas systems.

This study presents a practical analytical approach for the simultaneous quantification of siloxanes and condensable sulfur species in biogas. The method combines a portable liquid quench sampling system (LQ) with gas chromatography hyphenated to inductively coupled plasma mass spectrometry (GC-ICP-MS), enabling accurate capture and analysis of trace contaminants. By utilizing liquid-phase calibration instead of conventional gaseous standards, the approach simplifies quantification without compromising analytical precision or accuracy. In contrast to traditional sampling techniques that rely on adsorbents or whole-gas collection, the LQ system preconcentrates analytes directly into a solvent matrix, minimizing handling errors and preserving sample integrity. The element-specific detection capabilities of the hyphenated system further enhance selectivity, allowing for quantification at sub-milligram-per-cubic-meter levels, even in complex matrices.

The method was validated through inter-laboratory comparison and applied across multiple biogas plants in Switzerland, demonstrating high reproducibility and robustness. [1] Storage stability tests conducted over four weeks confirmed minimal loss of target compounds, making the method suitable for remote sampling with subsequent centralized analysis. Field deployments included biogas plants processing diverse feedstocks such as sewage sludge, agricultural residues, and wood-derived biomass. Impurity profiles were found to vary significantly with feedstock composition and process configuration: wastewater-derived biogas showed elevated levels of cyclic siloxanes, while sulfur species were predominant in gas from wood gasification.

By integrating simplified sampling with element-specific detection, this method provides a practical and scalable solution for facilities lacking advanced analytical infrastructure. It enables routine monitoring of critical impurities and supports the optimization of gas cleaning operations. Beyond operational improvements, the approach advances standardized trace-level analytics for renewable gases, ultimately enhancing the reliability, safety, and regulatory compliance of biomethane integration into modern energy systems.

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P2-093**NEW REVOLUTIONARY PROCESS GC WITH CAPILLARY CHROMATOGRAPHY****Geiger, Jochen¹; Wasson, John².**¹Representative, Ratingen, Germany; ²Representative, 101 Rome, Fort Collins, United States.

A new era of Process Chromatography has arrived with introduction of the ECLIPSE product family. Process Chromatography is nothing new to the market also different detection methods are not new. But what is new? So far process GC's are widely used in the Chemical and Petrochemical industry as a process control tool. While more complex systems such as GC - MS and other more complex Capillary systems have been used in Laboratory environments only.

By introducing the Wasson-ECE's Eclipse product family it becomes an option to duplicate your laboratory results as a 24/7 method into the process. The target is to have a full quality monitor eye into your process and use the data in addition to the existing data from the "standard" Process GC.

Chemical processing plants around the world have complex streams that require complex analyses. Another challenge is obtaining real-time, high-quality data about the streams. It is important to the plant's profitability to know the quality of the product that is flowing through their process unit at any given moment. Process gas chromatographs (PGCs) can provide many plants with some of this critical on-line data, but too often plants must also rely on laboratory GC analysis as backups or quality checks to the PGCs. This is because the laboratory GC analysis provides data on more components at higher resolution and higher sensitivity. In other cases, the information that a plant needs can only be achieved by laboratory GC analysis since limitations in technology prevent the analysis from being performed on a PGC at all. What's needed is a PGC that can provide repeatable capillary chromatography with pressure programming and temperature-programmable ovens. It also needs to be built to ATEX or Class I, Div. 2 specifications so that it can be installed in hazardous rated areas. Wasson-ECE's Eclipse (FID/TCD/PDHID), Neutrino (MSD), and E-VUV (VUV detector) PGCs provide such a solution. These PGCs use convection ovens with precise controls as well as electronic pressure and flow programming to drive the performance and resolving power of capillary column chromatography. The precision of the temperature and electronic pressure controls was proven to demonstrate highly reproducible retention times and peak areas, results rivaling those obtained on laboratory GCs. Furthermore, an extremely broad range of analytes can be detected because of the detectors that these PGCs offer, most notably the MSD. The Eclipse, Neutrino, and E-VUV PGCs provide single analyzer solutions that deliver results for a diverse range of analytes which would typically require several traditional PGCs and the shelter space to house them.

Jochen Geiger was in charge of Sales ; Service and Business Development with an US based global Process Instrument manufacturer. At the beginning of 2025, after almost 30 years as an employee, a new chapter in his professional career has started. Jochen set up his own consulting agency. In this new role he continues to support international Process Instruments manufacturers and Industrial users.

Jochen is a specialist in Process Analytical Applications at Refineries, Oil & Gas Production facilities and Chemical plants. His main focus is on optimizing Instrumentation to meet today's requirements of process optimization and energy savings. He is also a specialist of process control instrumentation.

Jochen is providing technical training courses for those industries. He is frequently invited as guest speaker at various International Seminars and Meetings such as SulGas (member of the advisory team) and MESCon. Mr. Geiger has published multiple technical articles in industrial magazines.

Jochen holds a Master in Electronics and is Process Analyzer Specialist.

This paper in relation and on behalf of WASSON -ECE (www.wasson-ece.com).

P2-094

BIOSENSOR TO CONTINUOUSLY MONITOR NUCLEIC ACIDS IN BIOMANUFACTURING PROCESSES

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Bioprocesses based on living cells are used to manufacture, for example, biopharmaceuticals, viral vectors, and cell-based therapeutics. Process Analytical Technology (PAT) is essential for monitoring and controlling the different stages of the bioprocess, from upstream cell culture and downstream extraction and purification to final product formulation¹. Each of these steps involves multiple unit operations, where monitoring key attributes is crucial for process optimization and minimizing batch failures. While sensors exist to continuously monitor parameters like temperature, glucose concentrations, pH, and dissolved oxygen, sensors are not yet available for the real-time monitoring of substances at low concentrations (micromolar and below), such as specific proteins and nucleic acids.

Here, we present a sensing technology for the continuous monitoring of nucleic acids using Biosensing by Particle Motion (BPM)²⁻⁵. The sensor comprises hundreds to thousands of biofunctionalized micrometer-sized particles that undergo reversible single-molecule interactions with a biofunctionalized surface. The reversible interactions lead to a switching behavior that is continuously optically detected and dynamically reflects the concentration of the target molecules in solution.

We describe molecular designs of BPM sensors for the monitoring of double-stranded DNA (dsDNA)⁶ as well as single-stranded DNA (ssDNA). The dsDNA sensor is based on antibodies that bind sequence-independently to dsDNA fragments (Figure 1A). The ssDNA sensor is based on oligonucleotides that bind to specific ssDNA sequences (Figure 1B). We will explain the sensor designs, present their operating principles, and show that both sensors are reversible and can sense the targeted analytes continuously. Finally, we will discuss the perspective of how continuous real-time nucleic-acid sensing can be applied in the manufacturing of biopharmaceuticals, viral vectors, and cell-based therapeutics.

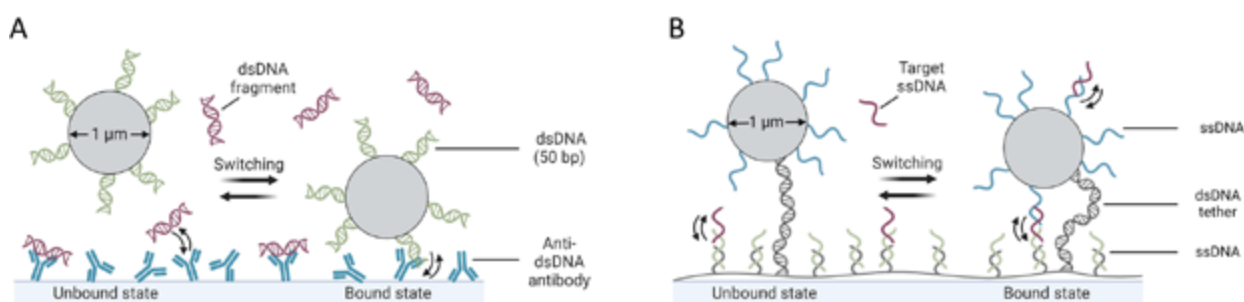


Figure 1 Sketch of the BPM biosensors. (A) Molecular design of the sensor to monitor dsDNA fragments in a competition sensing format. The particles are moving freely in solution. The particles are functionalized with dsDNA fragments, and the sensing surface is functionalized with anti-dsDNA antibodies that can bind to the dsDNA fragments in solution. (B) Molecular design of the sensor to monitor ssDNA strands in a sandwich sensing format. Both the particles and the sensing surface are functionalized with ssDNA strands that can bind to the ssDNA target. The particles are attached to the surface via a dsDNA tether.

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P2-095**COMPARATIVE SPECTRAL IMAGING FOR IDENTIFICATION AND CLASSIFICATION OF AIR-LIQUID INTERFACE BIOFILMS ON FOOD PROCESSING SURFACES****Caponigro, Vicky¹; Ferone, Mariateresa²; Marini, Federico³; Scannell, Amalia²; Gowen, Aoife².**¹University of Salerno, Salerno, Italy; ²University College Dublin, Dublin, Republic of Ireland; ³University of Rome La Sapienza, Rome, Italy.

Biofilms forming at air-liquid interfaces [1] pose a significant challenge in food processing due to their resilience and difficulty in detection [2;3]. This study explores the potential of spectral imaging techniques—Fourier Transform Infrared (FT-IR), Raman, Short-Wave Infrared (SWIR), and Visible-Near Infrared (VNIR)—for characterizing biofilm formation on food processing surfaces. Five bacterial species (*E. coli*, *B. subtilis*, *C. sakazakii*, *P. fluorescens*, and *L. plantarum*) were cultivated on stainless steel (AISI 304 and 316) and aluminum substrates under milk and broth conditions to assess biofilm growth and spectral variability.

Biofilm formation was confirmed through viable cell counts and crystal violet staining, revealing that *C. sakazakii* exhibited the highest biofilm biomass, particularly on stainless-steel surfaces in milk media. Principal Component Analysis (PCA) demonstrated clear spectral differentiation in milk-incubated biofilms, whereas broth-incubated samples showed greater consistency. Then, the mean spectrum for each modality was employed to construct classification models at various levels: presence or absence of biofilm, classification of biofilms based on Gram-positive (*E. coli* and *L. plantarum*) versus Gram-negative (*B. subtilis*, *P. fluorescens*, and *C. sakazakii*) bacteria, and classification of biofilms formed by each of the five tested bacterial strains. The results show that SWIR and VNIR outperformed other techniques in biofilm detection and Gram classification, particularly when sub-regional analysis and data fusion approaches were employed.

Our findings underscore the importance of substrate selection in microbial contamination control and highlight the potential of spectral imaging for real-time, non-invasive biofilm detection. Future work should focus on enhancing classification models through machine learning to refine spectral-based monitoring systems for food safety applications.

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P2-096**NEAR-INFRARED HYPERSPECTRAL AND COLOR-RGB IMAGE-BASED APPROACHES FOR HETEROGENEITY ASSESSMENT IN COCOA POWDER BLENDING PROCESSES****Compte, Josep; De Juan, Anna; Rocha De Oliveira, Rodrigo.***Universitat de Barcelona, Barcelona, Spain.*

Effective monitoring of powder blending processes is crucial for ensuring product quality in food manufacturing. This work presents a novel approach combining near-infrared hyperspectral imaging (NIR-HSI) and color-RGB imaging with variographic analysis to provide fast, non-destructive heterogeneity assessment during cocoa powder blending processes.

Cocoa-starch-sugar mixtures with varying compositions were prepared using a rotating mixing device. NIR-HSI data in the 930-1750 nm range and RGB images were simultaneously acquired throughout the blending process. Approximately 30 calibration samples were used for method development, with six complete blending batches analyzed.

The key methodological innovation lies in applying variographic analysis to both NIR-derived component distribution maps and RGB images for quantitative heterogeneity assessment. Multivariate Curve Resolution (MCR)¹ was employed to unmix the complex NIR-HSI data of calibration samples, yielding pure spectral profiles and spatial distribution maps for each component (cocoa, starch, sugar). The pure spectra obtained were used to recover maps from the blending images and, due to the existence of calibration samples, blending maps could be obtained in real concentration units. Two complementary indices were developed from the results of variographic analysis: a Global Heterogeneity Index (GHI) reflecting constitutional heterogeneity, and a Distributional Uniformity Index (DUI) quantifying distributional heterogeneity^{2,3}.

GHI and DUI indices successfully distinguished mixing phases and quantified heterogeneity evolution during blending. NIR-HSI provided chemical specificity, allowing clear discrimination between the three components and detailed assessment of their individual distribution patterns. RGB imaging, while lacking chemical specificity, offered complementary information for global visual assessment and rapid heterogeneity screening.

The comparative analysis revealed that NIR-HSI excels in providing chemically-specific heterogeneity information, enabling the component-wise description of the mixing behavior. RGB imaging, instead, proved valuable for rapid, cost-effective screening when colored mixture constituents exist, such as cocoa. The combination of both techniques offers a comprehensive analytical framework adaptable to different analytical requirements.

The proposed methodology demonstrates significant practical advantages: rapid data acquisition, non-destructive analysis, easy implementation and objective quantification of mixing quality. The integration of chemometric unmixing with variographic analysis represents a robust analytical strategy for powder blending assessment, providing the foundation for improved quality control in food manufacturing.

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P2-097**KEEP IT SIMPLE AND SMART– WHY LESS IS OFTEN MORE IN PAT****Kadijevic, Dusko.***Consulting DK, Salach, Germany.*

Process Analytical Technology (PAT) is often associated with advanced instrumentation and complex data models. However, in many industrial environments, simpler, more robust solutions can deliver greater value—especially when chosen and implemented strategically. This contribution follows the principle of 'Keep It Simple and Smart' and presents realworld PAT applications where straightforward analytical tools outperformed complex systems.

One example involves monitoring Dinitrotoluene (DNT) in a nitration process. Originally measured offline using gas chromatography (GC), the analysis was slow, costly, and detached from real-time decision-making. By implementing an inline photometric method, the process gained faster response times, better integration into operations, and reduced analytical overhead—without compromising reliability.

Another case focuses on an exhaust gas scrubber column, where acid/base neutralization was traditionally controlled via manual titration. This was replaced by an inline ultrasonic velocity measurement, which provided a direct, reagent-free correlation to acid concentration. The real-time feedback enabled more precise control of the neutralization process, improved safety, and eliminated the need for continuous sampling and laboratory analysis.

These examples show that successful PAT is not necessarily high-tech. The real value lies in choosing the right level of complexity, tailored to the process, the people, and the operational context. In small and medium-sized enterprises (SMEs), the most effective PAT tools are often those that are easy to maintain, fast to implement, and well understood by shop floor personnel.

This talk will highlight the potential of 'simple' PAT through industrial case studies and open a discussion on how embracing simplicity can drive faster adoption, better results, and lower costs in analytical process control.

P2-098

PAPER-BASED (BIO)SENSORS FOR MULTIANALYTE DETECTION IN A NOVEL ORIGAMI ORGAN-ON-A-CHIP DEVICE: PHOENIX-OoC

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Organ-on-a-chip (OoC) technology has emerged as a groundbreaking approach in the biomedical research field, offering significant improvements over traditional *in vitro* and *in vivo* models. OoC devices mimic the intricate physiological and mechanical properties of human organs and tissues on a micro scale, reproducing dynamic biological processes like tissue-tissue interactions, fluid dynamics, and mechanical forces. Mimicry of these phenomena is crucial for replicating proper organ function to provide a more accurate and reliable platform for studying human biology and disease. [1] OoC devices consist of microfluidic systems that integrate living cells cultured in a 3D environment, mimicking architecture and functions of specific human organs, and may suffer from several problems, such as formation of air bubbles affecting the functionality of the device and lack of sensing areas integrated in the device for real-time monitoring of the system. Within this framework, we propose a revolutionary paper-based platform using paper in origami configuration for developing an OoC device. PHOENIX-OoC aims to exploit paper's unique features in origami configuration for i) cell co-cultures with the aim to better simulate different organ tissues, (ii) (bio)sensors development and integration with the aim of on-site/continuous monitoring of cells status/response to stimuli, and (iii) microfluidics, with the goal of performing accurate pharmacological studies. Sustainability is one of the most important concepts today, as it can drive activities in several areas, namely environmental, social, and economic. In analytical chemistry, the development of sustainable devices has been boosted by the introduction of paper-based microfluidic analytical devices (μ PADs), whose benefits are not limited to the concept of sustainability. Indeed, paper as a functional material gives μ PADs unprecedented properties that can disrupt the OoC field. In detail, PHOENIX-OoC device includes a paper-based scaffold consisting of a cage printed using a 3D technique, containing the cell pads in origami configurations preloaded with the different cell lines, the electrochemical sensors modified for the detection of several analytes, and the adsorbent pad as waste. In the first part of the project, electrochemical sensors for pH, nitrate, and glucose were developed and characterized in standard solutions. The sensors were screen printed onto a filter paper support using wax to create a hydrophobic sensing area. For different analytes detection, the working electrode surface was differently modified: i) with nanomaterial and ion-selective membranes for nitrate detection, ii) with a layer sensitive to pH variations for H⁺ detection, and iii) by drop casting with nanoparticle dispersion and glucose oxidase enzyme for glucose monitoring.

Calibration curves were obtained by potentiometric measurements in standard solutions with regression equations $y = -(0.048 \pm 0.002) x + (0.359 \pm 0.004)$; $R^2 = 0.981$,

$y = -(0.083 \pm 0.001) x + (0.763 \pm 0.007)$; $R^2 = 0.998$, for nitrate and pH respectively, and by amperometric measurements for glucose detection, obtaining a linear regression described by the following equation $y = (-0.12 \pm 0.01) x - (0.7 \pm 0.1)$; $R^2 = 0.960$.

The development and application of OoC systems holds great promise for improving drug discovery, reducing animal testing, and advancing our understanding of human physiology and pathology. As this technology continues to evolve, it is expected to play a critical role in the future of medical research and personalized healthcare.

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P2-099

INFLUENCE OF ELECTROPOLYMERIZATION CONDITIONS ON THE CHEMIRESENSITIVE RESPONSE OF POLY(3,4-ETHYLENEDIOXYTHIOPHENE) TOWARDS AMMONIA

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Conducting polymers have been studied as conductometric gas sensors (chemiresistors) since the 1980s [1]. Typically, the conductivity of p-doped conducting polymers decrease in contact with electron-donating gases such as ammonia, which can be related to a shift in the redox state of the conducting polymer from p-doped towards the undoped state. However, gas sensors that are based on a thin film of conducting polymer, bridging the gaps of interdigitated electrodes, tend to show a slow response that is only partly reversible at higher concentrations of the analyte. Recently, focus has been shifted from using thin films of conducting polymers towards utilizing the so-called percolation region to obtain more sensitive chemiresistors [2].

In this work we show that the direction of conductivity change of the conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) upon exposure to ammonia depends on the electropolymerization parameters. This can be related to two types of percolation, i.e. “classical percolation” and “explosive percolation” as illustrated schematically in Figure 1. The PEDOT-based chemiresistor that operates in the “explosive percolation” region shows a decrease in resistance upon exposure to ammonia, which indicates n-doping of PEDOT. Interestingly, such a PEDOT-based gas sensor shows an ultra-fast (below 5 s) response to ammonia even at high gas concentrations (28 % ammonia) at room temperature. The response is fully reversible with short recovery times (below 20 s). These favourable gas-sensing characteristics were achieved via meticulous optimization of the electropolymerization parameters for PEDOT to effectively utilize the so-called “explosive percolation” region, in contrast to “classical percolation” (Figure 1). This work opens the possibility for rapid detection of ammonia and the concept can possibly be extended towards other gases as well.

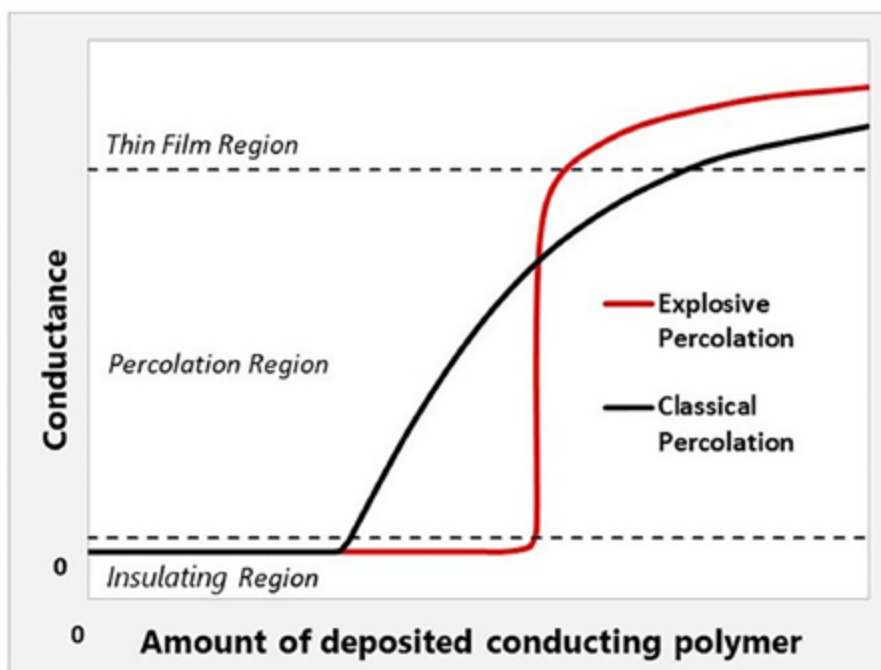


Figure 1. Schematic illustration of the conductance vs. the amount of deposited conducting polymer for classical and explosive percolation.

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P2-100

STUDY OF THE ZnO@NiCo-GLYCEROLATE COMPOSITE IN THE DEVELOPMENT OF A NON-ENZYMATIC ELECTROCHEMICAL SENSOR FOR THE QUANTIFICATION OF GLUCOSE

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In this work, was developed a glycerolate-bimetallic (NiCo-Gly), known as coordination compounds with metallic precursors, combined with zinc oxide nanorods (ZnO NRs) to form the ZnO@NiCo-Gly composite. The significant improvement in the electrochemical response upon introduction of bimetallic glycerate highlights the potential of these compounds for glucose (Glu) detection, enabling the development of an effective non-enzymatic electrochemical sensor for glucose quantification.

The synthesis of ZnO NRs was carried out in chemical precipitation step at 90 °C for 1 h, in a basic medium. Meanwhile, the bimetallic NiCo-Glycerolate compound was growth prepared on the ZnO NRs by a 6h solvothermal step at 180°C, to finally obtain a core-shell structure [1, 2]. In this study, the synthesis of ZnO@NiCo-Gly was carried out by varying the proportions of Ni and Co in the formation of glycerolate, and the percentage of NiCo-Gly introduced into the composite was even evaluated. The physicochemical characterizations of ZnO@NiCo-Gly were performed by X-ray diffraction, FTIR spectroscopy, UV-vis spectrophotometry and SEM-EDS microscopy.

For non-enzymatic detection of Glu, a glassy carbon electrode (GCE) was modified by drop casting using a ZnO@NiCo-Gly suspension. The ZnO@NiCo-Gly/GCE electrode showed an interesting performance for Glu quantification by chronoamperometry, demonstrating good response linearity in the concentration range of 20 $\mu\text{mol L}^{-1}$ to 120 $\mu\text{mol L}^{-1}$.

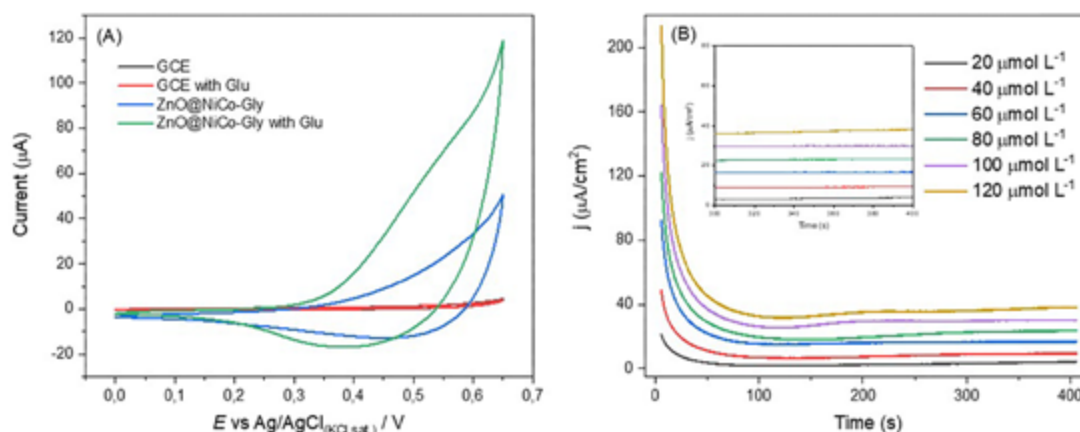


Figure 1: (A) Cyclic voltammograms (CV) obtained from a solution containing 700 $\mu\text{mol L}^{-1}$ glucose (Glu) in 0.1 mol L^{-1} NaOH, at a scan rate of 100 mV s^{-1} , using a glassy carbon electrode (GCE) modified with ZnO@NiCo-Gly in the absence and presence of Glu. (B) Chronoamperometric responses of different concentrations of Glu at the ZnO@NiCo-Gly/GCE.

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P2-101**SMART CAFFEINE SENSING: AN e-MIP-BASED SCREEN-PRINTED VOLTAMMETRIC CELL FOR BEVERAGE QUALITY CONTROL****Cutaia, Alessandra; Aldrovandi, Silvia; Merli, Daniele; Alberti, Giancarla.***University of Pavia, Pavia, Italy.*

Caffeine is a stimulant and the most widely consumed psychoactive substance in the world. It is a naturally occurring alkaloid that belongs to the xanthine class of compounds. Caffeine is primarily found in coffee and tea but is also a common additive in many soft drinks¹.

The need for rapid screening of caffeine as an alternative to conventional analytical techniques, enabling both the detection of caffeine and the verification of its labeled content, is mandatory for quality control in food and pharmaceutical industries. In this context lies the present work, proposing a sensitive and selective electrochemical sensor for caffeine determination using screen-printed voltammetric cells with a carbon-ink working electrode modified with an electrosynthesized molecularly imprinted polymer (e-MIP).

Screen-printed electrodes (SPEs) offer numerous advantages, including high versatility in electrode design, compatibility with a wide range of materials, low production cost, and good reproducibility. In addition, they require only small sample volumes, simplify sample handling and transport, and allow on-site analysis using portable instrumentation².

The modification of the working electrode surface of screen-printed voltammetric cells with chemical or biological receptors improves sensitivity and selectivity of the sensor.

In this work, the receptor selected was an e-MIP, using pyrrole as the monomer and caffeine as the template.

A significant advantage of e-MIPs over bulk MIPs is that electropolymerization enables precise control over polymer growth directly on the electrode surface, allowing a fine-tuning of the film thickness by adjusting experimental parameters and also reducing solvent consumption³.

The electropolymerization was conducted using cyclic voltammetry (CV); the number of CV scans for the polymerization and the ratio of pyrrole/template were optimized through the Design of Experiment (DoE). Caffeine molecules are effectively embedded within the polymeric matrix, creating highly selective recognition cavities.

Differential pulse voltammetry (DPV) was used as the measurement technique. Caffeine exhibited a highly reproducible and well-defined irreversible oxidation peak at a very positive potential (+1V).

Comparison with bare and non-imprinted polypyrrole-modified electrodes clearly demonstrated the superior sensitivity and reproducibility of the e-MIP-based sensor; moreover, interference tests confirmed its highest selectivity.

The method was successfully applied to various soft drink samples with known caffeine content. The recovery percentages, consistently ranging between 90% and 110%, confirm the potential of this technique as a reliable and efficient screening tool for food analysis.

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P2-102

DIFFERENT SYNTHESIS ROUTES IN YTTERBIUM OXIDE (Yb_2O_3): STRUCTURAL, MORPHOLOGICAL, AND ELECTROCHEMICAL CHARACTERIZATION

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Rare earth elements (REEs) are characterized by unique 4f-electron structures, long excited state lifetimes, large band gap energies, atomic magnetic moments, high resistivity, strong spin-orbit coupling, and long excited state lifetimes. Due to their unique chemical and physical properties, REEs and their oxides have wide applications in electronics, magnetic, optic, (photo)catalysis, and bioimaging science. Ytterbium (Yb) belongs to a group of REEs. Ytterbium-oxide (Yb_2O_3) represents essential C-type cubic refractory sesquioxide, and it is characterized by high band gap energy, high dielectric constant, and thermal and chemical stabilities. In electrochemical sensing, Yb_2O_3 shows high electrical conductivity, surface area, biocompatibility, and potential in degradation studies [1,2]. This research deals with the different synthesis methods of Yb_2O_3 and their structural, morphological, and electrochemical characterization. *Pechini* [3], co-precipitation [4], and hydrothermal [5] methods synthesized Yb_2O_3 material. The obtained white powder was structurally and morphologically characterized by X-ray Diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR), and Scanning Electron Microscopic (SEM) methods. The XRD spectral patterns of the synthesized Yb_2O_3 showed that the *Pechini* and hydrothermal methods yielded a higher crystallinity of synthesized material than the co-precipitation method. For electrochemical characterization, the glassy carbon paste (GCP) was modified by synthesized Yb_2O_3 material (thus prepared GCP/ Yb_2O_3 working electrode). Working electrode's electrocatalytic and charge transfer resistance performances were examined using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in 0.1 mol/L KCl containing 5 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. Comparing synthesized procedures, the CV results showed that GCP modified with Yb_2O_3 , which is synthesized by the *Pechini* method, provided the highest intensity of the redox peaks of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ probe and favorable system reversibility, compared to co-precipitation and hydrothermal methods. A similar trend was observed with EIS measurements, where GCP modified with Yb_2O_3 synthesized by the *Pechini* method showed the lowest charge transfer resistance performances compared to other synthesis methods. The CV results indicate the direct influence of modifiers on the electrocatalytic activity on the electrode surface and the good choice of the Yb_2O_3 (synthesized by the *Pechini* method) as a promising material/modifier for further electrochemical sensing.

Acknowledgments

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P2-103

DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR BASED ON MOLECULARLY IMPRINTED POLYMER USING FUNCTIONALIZED GOLD NANOPARTICLES FOR CAFFEINE QUANTIFICATION

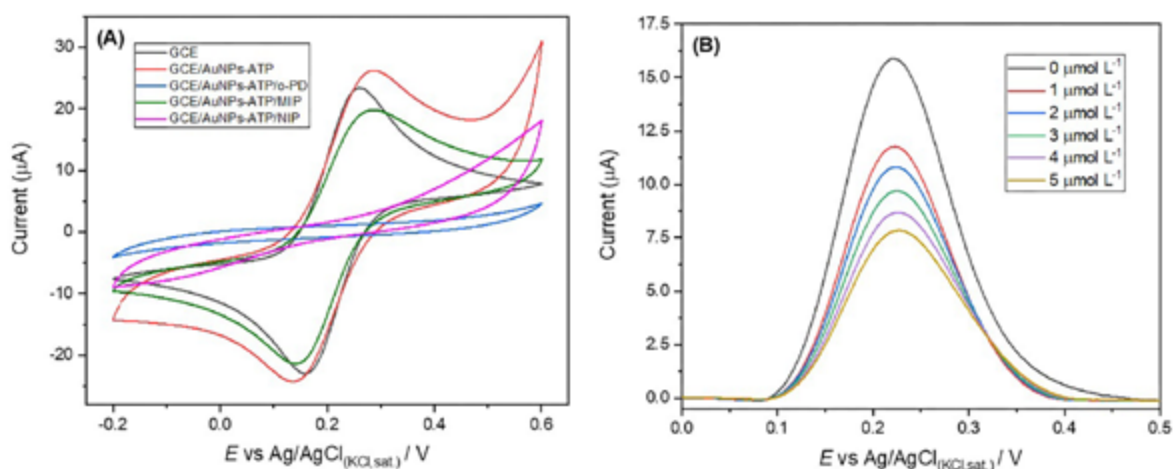
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University of São Paulo, São Paulo, Brazil.

An electrochemical sensor based on molecularly imprinted polymer (MIP) for detection of caffeine is presented. This MIP is formed via co-electropolymerization of p-aminothiophenol (pATP) and o-phenylenediamine (oPD) in the presence of caffeine as a template molecule. Gold nanoparticles (AuNPs) functionalized with p-aminothiophenol (pATP) were prepared by reduction reaction using sodium borohydride (NaBH_4) [1]. The material was characterized by FTIR spectroscopy and UV-vis spectrophotometry to evidence the characteristic band of gold nanoparticles.

To prepare an electrochemical sensor, glassy carbon electrode was modified by drop casting of a suspension of gold nanoparticles functionalized (AuNPs-pATP) followed by incubation in 1 mmol L^{-1} of caffeine in PBS 7.0 for 2 hours and then co-electropolymerization of pATP from AuNPs functionalized and oPD by cyclic voltammetry [2]. The selective cavities were formed by the extraction of caffeine from the MIP structure formed on the electrode surface. This extraction was performed by washing the electrode in NaOH solution. All electrode modification steps were tested by cyclic voltammetry in the presence of potassium hexacyanoferrate (II)/hexacyanoferrate (III) as a redox probe.

Detection of caffeine was conducted by SWV with the molecularly imprinted electrochemical sensor consisting of GCE/AuNPs-PoPD/MI-PoPD as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum plate as the counter electrode. The electrochemical sensor was immersed into 1.0 mL of caffeine solution of different concentrations for the rebinding of the template molecule into the imprinted cavities. This sensor was able to detect concentrations below $5 \mu\text{mol L}^{-1}$. Caffeine detection parameters such as electropolymerization cycles, template extraction time, and template rebinding time were also optimized in this work. The molecularly imprinted caffeine sensor demonstrated high stability, selectivity, and reproducibility.



(A) Cyclic voltammograms of different steps of electrode modification and (B) Square wave voltammograms of GCE/AuNPs-ATP/MIP after the rebinding of caffeine at different concentrations, in 50 mmol L^{-1} PBS 7.0 containing $5 \text{ mmol L}^{-1} [\text{Fe}(\text{CN})_6]^{3-/4-}$.

Acknowledgements: This research was supported by the Foundation for the Coordination of Improvement of Higher Education Personnel (CAPES) number: 88887966167/2024-00

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P2-104

2D DISPOSABLE STOCHASTIC SENSOR FOR THE ENANTIOANALYSIS OF LYSINE IN LUNG CANCER

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Amino acid metabolism is essential for biological functioning, affecting protein synthesis, energy production, and signaling pathways. Changes in amino acid metabolism have been associated with numerous disorders, including cancer. Lysine, an essential amino acid, is significant for its role in post-translational changes and its influence on tumor growth. Precise monitoring of lysine concentrations in biological samples is crucial for establishing its function in lung cancer and for formulating possible treatment approaches. Conventional methods of analysis frequently require lengthy sample preparation and may be unsuitable for fast or point-of-care testing. Stochastic sensors have emerged as a viable alternative, providing quick and sensitive detection of analytes directly within complex matrices such as whole blood samples. For the assay of L- and D-lysine in whole blood samples from patients diagnosed with lung cancer, a 2D disposable stochastic sensor, modified with α -cyclodextrin (α -CD), was used. The samples were analyzed with the proposed 2D disposable stochastic sensor employing the stochastic method. The respective presence of L- and D-lysine in the samples was identified using their distinct signatures and t_{off} values. The sensor's enantioselectivity showed its capability to distinguish between the two enantiomers of lysine. The proposed sensor is an ideal option due to its high sensitivity, low limits of quantification, and wide working concentration ranges.

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P2-105**CARBON BLACK AND PEDOT:PSS IN A NEW SYNERGISTIC SOLID CONTACT FOR RELIABLE PRINTED POTENTIOMETRIC SENSORS****Gosti, Christian¹; Mousavi, Zekra²; Fiore, Luca³; Mazzaracchio, Vincenzo¹; Arduini, Fabiana³; Bobacka, Johan².**

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Ion-selective electrodes (ISEs) require reliable solid contacts to ensure stable and reproducible potentiometric response.¹⁻³ Nevertheless, the majority of early devices reported in literature revealed commercially unfavorable features for large-scale production, such as time-consuming electrode functionalization, hampering maintenance conditions, and undesired manufacture.⁴ Aiming to integrate analytical reliability with mass-producing fabrication, we introduced an innovative solid-contact calcium-selective screen-printed electrode leveraging on the combination of Carbon Black (CB) and poly(3,4-ethylene dioxythiophene) doped with poly(sodium 4-styrenesulphonate) (PEDOT:PSS) as ion-to-electron transducers. Drop-casting was used to ensure a straightforward deposition of the CB solid-contact layer (6 μ l), while PEDOT:PSS was rapidly electropolymerized (71 s) onto the working electrode. Subsequently, the ion-selective membrane and the polyvinyl butyral-based reference membrane were drop-cast onto the working and reference electrode, respectively. A comprehensive investigation of the solid-contact layers was conducted through Electrochemical Impedance Spectroscopy to assess the optimal thickness highlighting the most efficient ion-to-electron transduction. Based on the optimization studies, various solid-contact Ca^{2+} -ISEs were designed and investigated by monitoring the potentiometric performances versus a conventional reference electrode. Among the solid-contact configurations, CB/PEDOT:PSS-modified screen-printed electrodes demonstrated a low-frequency capacitance of 55 μ F, and an outstanding standard potential inter-electrode reproducibility (± 1 mV). The combination of both types of ion-to-electron transducers provided calcium detection in the linear range 10^{-1} M to 10^{-8} M with a Nernstian sensitivity (28.3 ± 0.3 mV/decade), ensured over 28 days under dry storage. Furthermore, the lack of water layer affecting the potential stability was demonstrated along with sensor's selectivity in presence of feasible interfering ions. The fully printed platform was assembled to achieve a ready-to-use potentiometric device, consisting of CB/PEDOT:PSS/ISM configuration and PVB-based reference membrane on the working and the reference electrode, respectively. The final sensor revealed a Nernstian sensitivity (29.0 ± 0.5 mV/decade) and a stable signal up to 72 hours of continuous measurements (drift = -0.2 mV/h), emerging as an affordable and effective sensing tool for calcium detection.

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P2-106

A DISPOSABLE SCREEN-PRINTED ELECTRODE FOR GLUTAMINE ENANTIOANALYSIS IN BIOLOGICAL SAMPLES

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A large percentage of cancers consume glutamine at an alarming rate, according to a number of *in vivo* and *in vitro* investigations of cancer glutamine metabolism. Furthermore, there is mounting evidence that the tumor's microenvironment, epigenetic alterations, tumor genesis, oncogene and tumor suppressor status, and the tumor itself all play a role in the regulation of glutamine metabolism.

Accurate disease detection relies on enantioanalysis of chiral compounds like amino acids, which is especially true in the specialized area of cancer diagnosis. Using a disposable stochastic sensor, biological samples were analyzed in order to determine the enantiomers of glutamine.

To develop a disposable stochastic sensor, a drop of maltodextrin solution (10^{-3} mol L⁻¹) was applied to the active side of a gold-based disposable screen-printed sensor, which was then left to dry for 24 hours before being used. The glutamine solutions were prepared using phosphate buffer solution (PBS) that had a pH of 7.50. L- and D-glutamine solutions with concentrations ranging from 10^{-3} to 10^{-21} mol L⁻¹ were prepared using the successive dilution approach. Tissue, saliva, urine, and whole blood samples were utilized to characterize and validate enantioanalysis studies with the use of the disposable stochastic sensor.

A disposable stochastic sensor was used to screen the samples using the stochastic approach. The signatures (t_{off} values) of L-glutamine and D-glutamine were used to identify the two compounds in the samples. The sensor's enantioselectivity proved that it could distinguish between the two glutamine enantiomers. The suggested sensor is an excellent choice due to its wide working concentration ranges, low quantification limits, and high sensitivities.

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P2-107

REVERSED AMPEROMETRIC SETUP FOR ENHANCED SENSITIVITY IN ION-SELECTIVE ELECTRODE MEASUREMENTS

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Typical ion-selective electrodes (ISEs) used in potentiometric mode suffer from low sensitivity, as governed by the Nernst (Nikolsky-Eisenman) equation. This limits the resolution of potentiometric analysis and poses challenges in detecting relatively small changes in analyte ions concentrations.

A novel method has been proposed, applying a 'reversed' amperometric setup, in which an Ag|AgCl electrode of low polarizability serves as the working electrode [1,2], while an ion-selective electrode functions as the reference electrode. Notably, this approach does not require the addition of any reagents to the sample.

Changes in the concentration of primary ions cause a shift in the potential of the ISE (used as the reference electrode), which in turn induces a corresponding shift in the Ag|AgCl electrode potential under amperometric (potentiostatic) control. Due to the steep current–potential dependence of the silver/silver chloride electrode, even small changes in the ISE potential result in significant variations in the measured current. This leads to a strong amplification of the analytical signal corresponding to the target ions.

An additional advantage of this approach is the linear relationship observed between the measured current and the analyte concentration. This was demonstrated using model potassium- and calcium-selective ISEs. In these cases, the current recorded at the Ag|AgCl electrode showed a linear dependence on the concentration of Ca^{2+} or K^{+} , with sensitivities of approximately $27 \mu\text{A} \cdot \text{mmol}^{-1} \cdot \text{dm}^3$ and $9 \mu\text{A} \cdot \text{mmol}^{-1} \cdot \text{dm}^3$, respectively (Figure A, B).

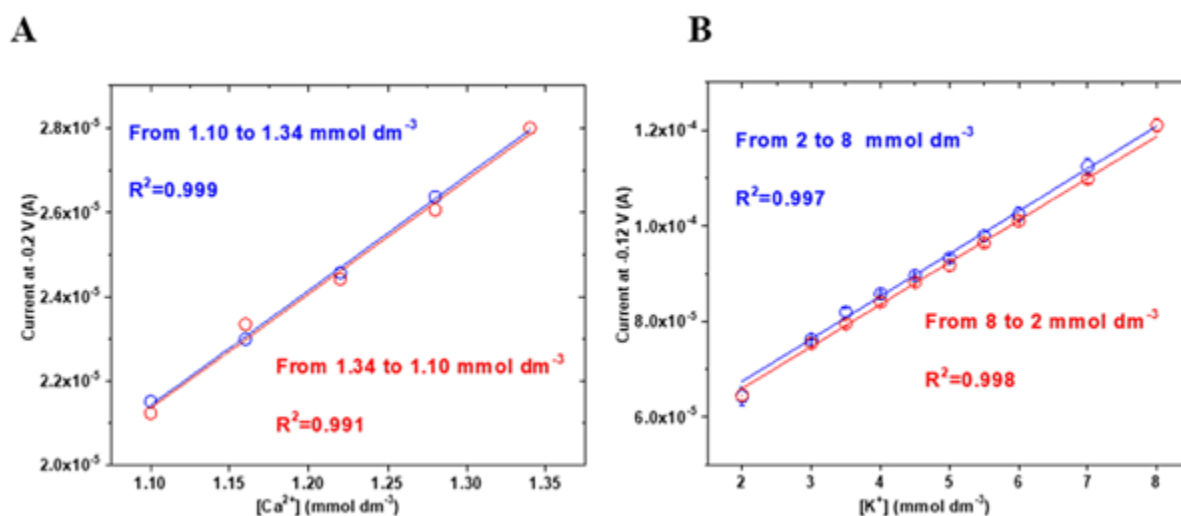


Figure Current recorded at Ag|AgCl electrode in various concentration of Ca^{2+} (A) or K^{+} (B) ion in the presence of $10^{-2} \text{ mol dm}^{-3}$ NaCl.

These findings demonstrate that the proposed reversed amperometric approach offers a promising and highly sensitive alternative to conventional potentiometric ISE measurements, addressing one of the key limitations in ion-selective sensing.

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P2-108**SCALABLE DEVELOPMENT OF FLEXIBLE AMPEROMETRIC BIOSENSORS BY INKJET PRINTING AND INTENSE PULSED LIGHT****Kassal, Petar; Zubak, Marko; Boček, Željka.***University of Zagreb Faculty of Chemical Engineering and Technology, Zagreb, Croatia.*

Alternate scalable routes for fabrication of disposable electrochemical sensors are needed to meet the ever growing need for quick and simple point-of-need biomedical and environmental analyses. Inkjet printing presents a green, versatile, and cost-effective way to fabricate such devices, yet it remains underdeveloped compared to screen printing. We present here the development and optimization of flexible low-cost inkjet printed graphene electrodes. After inkjet printing, the electrodes are processed by intense pulsed light (IPL) – an energy efficient route compared to conventional thermal processing. The IPL treatment lasts only 3 ms, yet these electrodes outperform thermally treated inkjet-printed electrodes and commercial screen-printed carbon electrodes in terms of electrochemical and electroanalytical performance. IPL processing increases the electroactive surface area of the electrode and facilitates electron transfer. The analytical parameters of the determination of the antibiotic azithromycin, in batch and flow modes, were also improved by IPL processing [1].

The possibility of using the printed electrodes for biosensing was demonstrated next. The electrode surface was modified by inkjet printing of a Prussian Blue nanoparticle suspension, which is an electrocatalyst for hydrogen peroxide. This enabled sensitive amperometric determination of the enzymatically produced hydrogen peroxide at low potentials. In the last step, lactate oxidase was immobilized, and lactate determined in a broad linear range covering sweat lactate concentrations [2]. Systems like this will promote the development of a variety of low-cost flexible (bio)chemical sensors, particularly attractive for non-invasive wearable biofluid analysis.

This work was supported by the Croatian Science Foundation (projects UIP-2020-02-9139 and DOK-2021-02-2362).

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P2-110

ENHANCED ELECTROCHEMICAL SENSING PLATFORM BASED ON 2D-MXenes AND DIAMOND NANOPARTICLES. APPLICATION FOR TANSBINOL DETERMINATION

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The application of low-dimensional materials in the development of nanostructured electrochemical sensors has emerged as an excellent strategy to enhance the performance of these devices in terms of electrocatalytic activity, sensitivity, and selectivity for detecting target compounds across various fields.

All 2D nanomaterials share certain common features derived from their *surface-dominant character*, such as a large specific surface area and, consequently, a high surface area-to-volume ratio. Among these materials, MXenes—defined by the general formula $M_nX_nT_x$, where M represents an early transition metal, X can be carbon (C) and/or nitrogen (N), and T denotes surface terminations such as OH, O, or F—stand out. They combine the metallic conductivity of the transition metal with the hydrophilic nature of their functionalized surface, making them exceptional candidates for electrochemical sensor applications. Additionally, MXenes offer further advantages, including excellent biocompatibility, as well as resistance to passivation and fouling, thereby improving device stability. These attributes position MXenes as an outstanding choice for the design and development of electrochemical (bio) sensors with enhanced properties suitable for both batch and flow methodologies.

This work presents the development of an electrochemical sensor that leverages the advantages of the use of 2D-MXene, specifically Ti_3C_2 , in combination with OD-nanoparticles (DNp). The synergistic effect observed from the integration of these two nanomaterials led to a sensor with improved response towards tanshinol, enabling the development of an analytical method with enhanced sensitivity for the determination of this analyte. Tanshinol is a water-soluble compound derived from the roots of *Salvia miltiorrhiza* that exhibits potent antioxidant and anti-inflammatory properties. Its analysis has gained significant interest due to its potential therapeutic applications, particularly in cardiovascular and neuroprotective treatments.

Furthermore, this study evaluates the influence of different MXene synthesis procedures on sensor performance, including fluoride etching method^{1,2}, *in situ* fluoride generated etching procedure³, and an electrochemically fluoride-free approach⁴. All developed sensing platforms were electrochemically and morphologically characterized using cyclic voltammetry (CV), scanning electron microscopy (SEM), and atomic force microscopy (AFM).

After optimizing the sensor fabrication and fine-tuning the chemical and instrumental parameters affecting the differential pulse voltammetric response toward tanshinol, the proposed method allows the detection of the target at 0.88 μ M level. Both, the relative error $Er(\%)_{n=3}$ and relative standard deviation (RSD%)_{n=3}, were evaluated across different levels within the linear concentration range. Values of 8% and 6%, respectively, were obtained in average of the different concentrations assayed.

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P2-111**ELECTROCHEMICAL DEVICES UTILIZING CHITOSAN AND GOLD NANOPARTICLES FILMS FOR SIGNAL AMPLIFICATION**

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Over the years, the importance of the study and monitoring of markers of clinical interest has become clear due to the impact they have on the evolution of some diseases. Despite the research on these topics, there are some challenges to overcome, such as the specificity of recognition, miniaturisation, rapid detection or problems with multiplexed systems [1]. In this context, nanomaterials show interesting features in recognition chemistry for the fabrication of sensors and biosensors. In this work, we present the development of a methodology for modifying screen-printed electrodes to amplify the signal of redox species.

Chitosan is an easy-to-obtain material with a polysaccharide nature. This structure, derived from chitin, provides a wide spectrum of applications such as the development of membranes, gels or films using a variety of methods. In recent years, its use in the development of devices for clinical use has increased thanks to the biocompatibility of the polymer. On the other hand, the use of gold nanoparticles is very useful in the development of electrochemical devices thanks to the amplification characteristics they confer in the detection of analytes with redox properties [2].

Screen-printed electrodes are a suitable method for the preparation of miniaturised devices of analytical interest and offer a large number of advantages. These devices can be easily modified by different techniques, thus increasing their selectivity for the desired procedure. In this way, the modification of these screen-printed electrodes allows the amplification of the electrochemical signal between two and three times due to the gold nanoparticles, improving properties of the determination such as sensitivity.

For the development of the sensors, screen-printed electrodes have been prepared in the laboratory, which have subsequently been modified with chitosan and gold nanoparticles using different techniques in order to select the optimum methodology. The results obtained allow the development of electrochemical sensors based on chitosan and gold nanoparticles with potential application to the determination of uric acid and silver.

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ELECTROCHEMICAL DETECTION OF CODEINE IN COMPLEX MATRICES USING CARBON NANODOT-MODIFIED SCREEN-PRINTED ELECTRODES

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Abstract

Rapid and accurate detection of drugs like codeine in complex matrices is essential for forensic and clinical toxicology. While electroanalytical methods show promise, a significant gap remains in the integration of effective sample preparation techniques with electrochemical sensing.

We present a novel approach for the electrochemical detection of codeine in complex matrices using screen-printed carbon electrodes (SPCEs) modified with carbon-based nanodots. The performance of carbon nanodots (CNDs), carbon quantum dots (CQDs), and graphene quantum dots (GQDs) was compared as electrode modifiers for codeine detection. GQDs exhibited superior electrocatalytic properties, while CQDs functionalized with a Ru(bipy) complex demonstrated enhanced performance. The sensors were successfully applied to detect codeine in biological fluids (saliva and urine) and soft drinks. Various extraction techniques were employed for these complex matrices, including liquid-liquid and thin-film microextraction approaches, with the latter integrating sample preparation directly into the sensing step.

The developed sensors exhibit high sensitivity, selectivity, and cost-effectiveness, making them promising tools for point-of-care applications in forensic and clinical settings. This work addresses the challenges posed by matrix effects and interferences in complex samples, highlighting the potential of combining sample preparation with nanomaterial-modified SPCEs for rapid and accurate drug detection.

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MOF-MODIFIED GLASSY CARBON ELECTRODE WITH MOLECULARLY IMPRINTED POLYMER COATING - ELECTROCHEMICAL SENSOR DESIGN FOR GLYPHOSATE DETECTION

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Metal-Organic Frameworks (MOFs) are porous crystalline materials formed by the linkage of organic ligands with metal ions, creating a rigid periodic network. These materials exhibit exceptional properties, including large specific surface areas, uniform pore structures, controllability, and modifiability of surface characteristics. Incorporating rare earth elements (REE) as metal cations in the building blocks of MOFs presents an effective strategy for developing specific REE-MOFs that possess unique electronic, optical, luminescent, and magnetic properties [1]. In this work, we propose the development of an electrochemical sensor by integrating MOFs with Molecularly Imprinted Polymers (MIPs), while simultaneously employing a rational, strategic approach to MIP design. While MIP-based sensors demonstrate excellent target recognition capabilities, they often face challenges such as heterogeneous binding sites, random polymerization, and a rigid, dense network, which can result in reduced sensitivity and specificity. These limitations hinder the performance of MIPs in sensing applications. MOFs offer a promising solution to these challenges [2]. The synergistic combination of the distinct properties of both materials significantly enhances the sensitivity and selectivity of the resulting electrochemical sensor.

In this research, a novel electrochemical sensor MIP/Y-MOF@GO/GCE was developed by coating Y-MOF@GO on the surface of glassy carbon electrode (GCE) and covering molecularly imprinted membrane by electropolymerization for highly sensitive detection of pesticide glyphosate (GLY). The Y-MOF@GO composite was synthesized through a hydrothermal method and characterized using advanced techniques, including X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). By incorporating this synthesized material onto the surface of the GCE, we achieved high electrode surface porosity, which enhanced the binding capacity of the (MIP) film. We conducted quantum chemical calculations to analyze the interactions between various functional monomers and GLY molecules. This analysis allowed us to select the optimal monomer for polymer synthesis maximizing selectivity towards the target analyte. The calculations identified o-phenylenediamine (oPD) as the optimal monomer for subsequent electropolymerization experiments. Several key factors such as template to monomer ratio, number of polymerization cycles, polymerization scanning rate and elution time, during preparation of the imprinted sensor were optimized in detail. To characterize and study the electrochemical behavior of the MIP/Y-MOF@GO/GCE, we utilized electrochemical techniques – cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. Since GLY molecules are non-reactive and occupy the cavities within the MIP film, all measurements were conducted using a solution containing the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$, using differential pulse voltammetry as the driving method. The peak current density decreased with the rebinding of glyphosate molecules, thus serving as an indicative signal. The designed MIP sensor presented a wide linear range from 1.5 nM to 326 μM , and a low detection limit (1.3 nM). This newly developed method based on the synergistic effects of Y-MOF and GO combined with MIP technique offered outstanding selectivity, sensitivity, stability, and reproducibility.

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IONOGEL BARRIERS AND HYDROGEL REPOSITORIES FOR MICROFLUIDIC FLOW CONTROL

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Over the past decades, microfluidic systems have evolved to approach the ideal ASSURED guidelines with great success. However, passive liquid flow is not enough to address new analytical requirements and further research is needed in flow control systems [1]. Flow control strategies based on physical actions have been studied more than chemical ones [2]. Several stimuli-responsive materials have been developed and have attracted an outstanding attention because of their ability to mimic nature's responses to stimuli by changing volume, conformation or solubility [3]. These materials offer a great opportunity for use in flow control and reagent release in microfluidic systems.

In particular, thermoresponsive smart materials have enormous potential, as their actuation can be easily automated with a miniaturised heater. They are swollen in aqueous solution when the temperature is lower than a critical one, but when is exceeded, they collapse, releasing the aqueous solution. Poly(N-isopropylacrylamide) (NIPAM) is an example of thermoresponsive polymer, which can act as reagent repositories, barriers or flow retardants in μ PADs. Here, we present a μ PAD including these type of gel acting both as barriers and repositories for the determination of nitrites.

At first, the swelling/deswelling behaviour was studied, showing that for the same area the thicker NIPAM can store higher volumes. It was also found that NIPAM always returned to the same dry weight and did not degrade, and the absorption level oscillated depending on the time left absorbing and the ambient temperature. When the kinetics of swelling was analysed, it was proven that after 90 minutes it stabilised, and higher absorptions were not achieved by leaving the hydrogel immersed. In addition, the amount of water absorbed was significantly higher at 5°C than at 20°C. When studying the influence of pH, it was found that NIPAM absorbs less buffer solution at higher pH values. The NIPAM hydrogel was also shown to function as a reservoir for organic solvents or aqueous mixtures of organic solvents in NIPAM, such as methanol and ethanol. When evaluating the evaporation of the retained solvent at room temperature, water drying was not yet complete after two hours, but ethanol complete drying could be achieved within one hour. Results also showed that ionogels were capable of significantly slowing down the flow and even stopping it being very useful to prevent mixture of the reagents before desired or to control where the mixing takes place.

Once the hydrogel and ionogel were optimised, they were tested in a real application. For this purpose, a colorimetric sensor for the determination of nitrite in water was developed, based on Griess reaction chemistry. First, the μ PAD design was optimised by adjusting the size of the channels and the angle between them to facilitate the flow in the desired direction. Then, the location of the ionogel was optimised. The inclusion of both Griess reagents in the μ PAD was studied, with the best results obtained when sulfanilic acid was swollen in the NIPAM hydrogel and N-(1-naphthyl)ethylenediamine was deposited in the detection area. 5 μ l of the sample was added in the sampling area, flowing into the detection one. Then, NIPAM hydrogel was heated and deswelled allowing sulfanilic acid solution to flow into the detection area, where it mixed with the other reagent and the sample, taking place the recognition reaction. The detection was made using a camera and obtaining the Hue coordinate of the HSV colour space. The colour change could be correlated with the amount of nitrite in the sample. The LOD and LOQ obtained for the optimised sensor were 1.7×10^{-7} M and 9.0×10^{-7} M respectively. Figure shows the scheme of the μ PAD.

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DEVELOPMENT OF A MICROFLUIDIC-BASED COLORIMETRIC SENSOR FOR SMARTPHONE DETECTION OF NITRITES IN FRESH WATER

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Nitrites in drinking water pose significant health risks, particularly for infants under six months, as they can cause methaemoglobinaemia (blue baby syndrome), a condition that impairs oxygen transport in the blood and may lead to severe hypoxia. Long-term exposure has also been linked to an increased risk of colorectal and gastric cancer due to the formation of carcinogenic nitrosamines in the body [1]. The main sources of nitrite contamination include excessive nitrogen fertiliser use, improper livestock waste management, and untreated wastewater discharge. These activities contribute to nitrate leaching into water sources, where it can be reduced to nitrite [2].

To mitigate these risks, rigorous monitoring and treatment of drinking water are essential. The WHO and EPA have established maximum nitrite limits of 0.1 mg/L and 1 mg/L, respectively. Effective prevention strategies include regular water testing, advanced treatment methods such as reverse osmosis and biological denitrification and promoting sustainable agricultural practices to reduce nitrogen pollution. Ensuring safe drinking water is crucial for protecting public health and preventing serious diseases [3].

This work presents a novel centrifugal microfluidic platform for the determination of nitrites in fresh waters in combination with a low-cost optical detection system, i.e. a smartphone camera. The use of centrifugal forces to move fluids in centrifugal microfluidic discs allows the use of simple CD players to drive their operation in in-situ applications. The centrifugal microfluidic disc was designed using FUSION360 software and manufactured using 3D printing technology (Stratasys Objet260 Connex1) [4]. A colorimetric method based on the Griess reaction was used for the detection chemistry. In this reaction, nitrite reacts with a primary aromatic amine to form a diazonium salt. This then combines with another aromatic compound to form an azo dye with high absorbance [5].

To develop the methodology, solutions of sodium nitrite were prepared with different concentrations and the Griess reaction was performed into the centrifugal microfluidic discs to obtain a calibration curve in the 0-2 ppm concentration range with smartphone detection. Calibration curves were also obtained off-disc using a UV-VIS spectrophotometer as the reference method. ImageJ software and a smartphone camera app were used for image analysis. In this way, the colour change was measured according to the concentration of nitrite in these standard solutions using the RGB and HSV colour spaces, finally choosing the saturation (S) of the HSV space as the best analytical signal. Current work is in progress to improve image capture. Ultimately, this smartphone microfluidic-based method will provide a low-cost, portable system for on-site monitoring of nitrites in environmental waters.

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FABRICATION OF $\text{Eu}_2\text{O}_3/\text{rGO}$ -BASED ELECTROCHEMICAL SENSOR FOR DETECTION OF ESTRADIOL TOGETHER WITH THEORETICAL ILLUMINATION OF REDOX EVENTS

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Accurate detection and monitoring of endocrine disruptor estradiol (E2) in clinical and environmental settings are of critical importance [1] due to its significant health and ecological implications. This study presents an advanced electrochemical sensor based on europium oxide (Eu_2O_3) and reduced graphene oxide (rGO) for E2 quantification. The sensor was fabricated by incorporating Eu_2O_3 nanoparticles, synthesized via a hydrothermal method, with rGO, and immobilizing the composite on a screen-printed carbon electrode (SPCE). The electrochemical performance of the modified electrode was evaluated using cyclic voltammetry (CV) and square wave voltammetry (SWV), demonstrating a low detection limit of $0.06 \mu\text{M}$ and a sensitivity of $2.44 \mu\text{A} \mu\text{M}^{-1} \text{cm}^{-2}$. The developed sensor exhibited excellent repeatability, reproducibility, and stability, showing promising potential for real-world applications in water and biological samples.

To elucidate the fundamental redox mechanisms underlying the electrochemical response of E2, we conducted a comprehensive theoretical study using Density Functional Theory (DFT) [2]. The redox process was explored through calculations of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), along with the electron spin density of the radical cation formed upon oxidation. The HOMO localization analysis confirmed that oxidation primarily occurs at the phenolic hydroxyl group of E2, with electron density delocalization over the aromatic ring. This theoretical prediction aligns well with experimental voltammetric findings, which indicated a single anodic peak, suggesting a rapid two-electron oxidation process. Further insight into the oxidation mechanism was obtained through the Fukui function analysis [3], which revealed the most electrophilic sites susceptible to nucleophilic attack. The results highlighted the ortho and meta positions relative to the hydroxyl moiety as the most reactive sites, indicating a strong likelihood of forming a quinone-like structure upon oxidation. Two plausible oxidation pathways were proposed based on these theoretical findings: (i) a direct oxidation of the hydroxyl group to a ketone, leading to an extended conjugated system, and (ii) an alternative route involving nucleophilic water attack, forming a catechol intermediate that subsequently oxidizes to a quinone derivative. The absence of a reduction peak in the experimental voltammograms further supports the first pathway as the predominant oxidation process. Electrochemical impedance spectroscopy (EIS) and CV studies provided additional mechanistic insights by confirming a significant reduction in charge transfer resistance (R_{ct}) upon modification with $\text{Eu}_2\text{O}_3/\text{rGO}$, which enhanced electron transfer kinetics. The heterogeneous electron transfer rate constant (k_0) increased with the incorporation of the composite material, confirming improved redox activity. Additionally, the calculated electroactive surface area (EASA) of SPCE/ $\text{Eu}_2\text{O}_3/\text{rGO}$ was substantially larger than the unmodified SPCE, highlighting the synergistic role of Eu_2O_3 and rGO in enhancing the electrode's electrocatalytic efficiency.

The developed SPCE/ $\text{Eu}_2\text{O}_3/\text{rGO}$ sensor was successfully applied to detect E2 in tap water, river water, and human saliva samples, demonstrating strong agreement with UV-Vis spectrophotometry results. The integration of experimental electrochemical data with theoretical quantum chemical analysis provides a robust understanding of E2's redox behavior, establishing a foundation for further sensor optimizations. This work not only contributes to the advancement of electrochemical sensing technologies but also offers valuable theoretical insights into the redox mechanisms of E2, with broader implications for environmental and biomedical monitoring applications.

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A ZERO-GAP ELECTROCHEMICAL SENSOR WITH 3D-CONDUCTIVE GDES AND HYDROGEL ELECTROLYTE FOR HIGH-PERFORMANCE BREATH GAS ANALYSIS

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Portable gas analyzers play a critical role in medical diagnostics by enabling real-time monitoring of health status through the analysis of gaseous molecules in exhaled breath^{1,2}. Electrochemical sensing, with its two- or three-electrode configurations allowing both steady-state and transient measurements, offers superior advantages for gas detection: exceptional sensitivity, low detection limits, high precision, minimal power consumption, miniaturization capabilities, and ambient temperature operation³. Gas diffusion electrodes (GDEs), which facilitate electrochemical reactions at solid-liquid-gas interfaces, have consequently become instrumental in environmental monitoring, wearable electronics, and energy conversion applications^{4,5}.

In typical GDEs, a gas-permeable support is coated with a catalytic layer to facilitate electrochemical reactions at the gas-liquid interface. Conventional GDEs feature predominantly two-dimensional catalyst distributions, necessitating larger surface areas to create sufficient three-phase interfaces. These systems often rely on aqueous liquid electrolytes to ensure sufficient conductivity between electrodes. However, the use of liquid electrolytes introduces challenges such as signal instability due to evaporation or leakage, and poor tolerance to temperature and humidity fluctuations. These limitations substantially hinder their practical implementation in real-world applications such as breath analysis, where reliability and environmental adaptability are essential.

The work presents an innovative portable zero-gap electrochemical sensor with a "sandwich" architecture that addresses these fundamental limitations (Fig1 A). The innovative design features 3D-conductive noble metal catalysts conformally coated on nanofiber-based GDEs as sensing interfaces, creating sensing interfaces with optimized gas permeability that enhance electrocatalytic efficiency while amplifying Faradaic signals and suppressing interference. A novel hydrogel-based solid electrolyte replaces conventional liquid electrolytes, providing excellent ionic conductivity and long-term stability through a specialized polymer network that maintains efficient charge transport

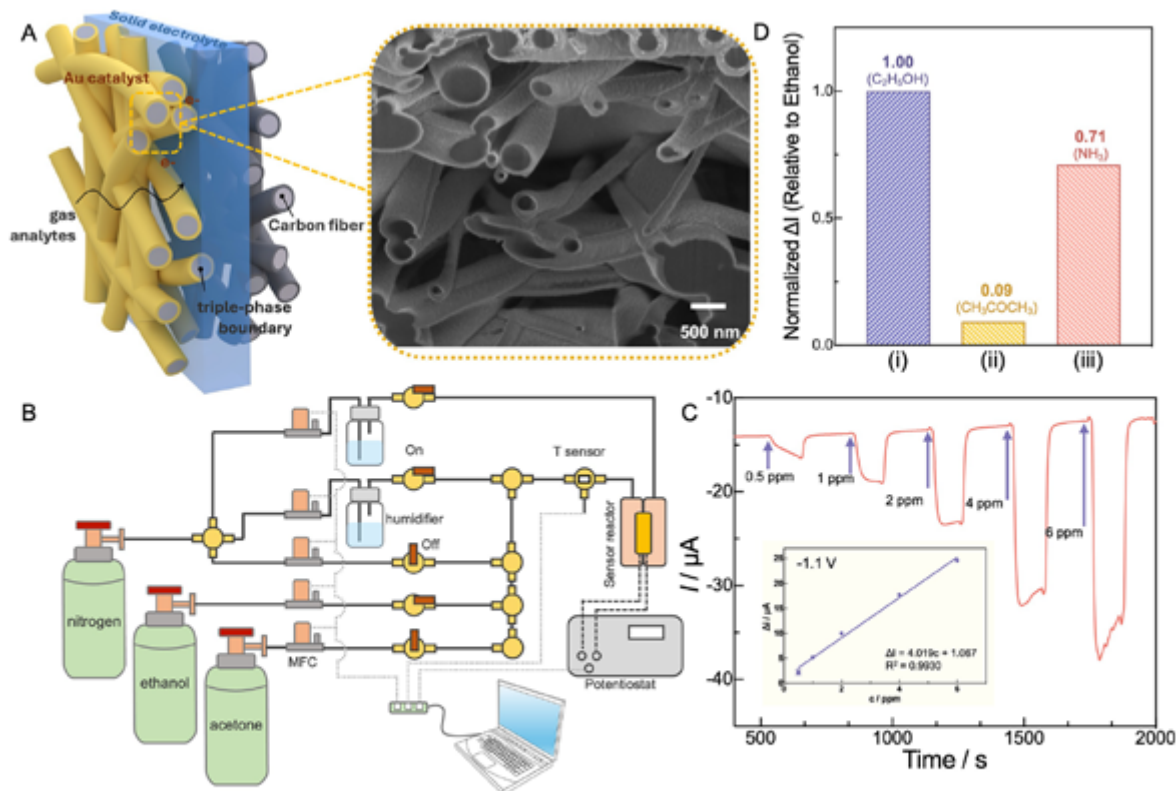


Fig.1: A) Schematic of the zero-gap gas sensor, with the cross-sectional SEM image of GDEs prepared by FIB milling. B) The workflow of the gas workstation. C) The response of the GDEs with different ethanol concentrations, and corresponding standard curve. D) The response values of the GDE sensor toward different gas species.

while eliminating evaporation and leakage concerns. The integrated assembly—combining this gel electrolyte with 3D-conductive GDEs and high-surface-area carbon paper counter electrodes in a zero-gap configuration—ensures rapid mass transport, enhancing both sensitivity and durability while simplifying fabrication.

The experiments were conducted using a custom gas generator system (Fig. 1B). Experimental results with the prototype demonstrate exceptional response to ethanol gas (Fig1 C), with significantly higher sensitivity compared to acetone and ammonia (Fig1 D), validating both the sensitivity and selectivity of the approach. This electrochemical sensing platform demonstrates potential for future integration into breath analysis devices, enabling non-invasive detection of volatile biomarkers in exhaled breath. Moreover, the sensor's exceptional selectivity and sensitivity make it particularly promising for real-time health monitoring in clinical settings and point-of-care diagnostics, potentially transforming how respiratory and metabolic conditions are diagnosed and monitored.

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P2-118**DEVELOPMENT OF AN ELECTROCHEMICAL BIOSENSOR FOR GLUTATHIONE REDUCTASE ACTIVITY USING SCREEN-PRINTED ELECTRODES MODIFIED WITH 3,5-DINITROBENZOIC ACID-FUNCTIONALIZED CARBON NANOTUBES**

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Oxidative stress is implicated in the pathogenesis of various human diseases due to an imbalance between reactive oxygen species (ROS) production and antioxidant defenses [1]. Among endogenous antioxidants, reduced glutathione (GSH) plays a central role in protecting cells against oxidative damage. The GSH/GSSG ratio is tightly regulated by glutathione reductase (GR), an NADPH-dependent flavoenzyme, whose abnormal activity has been associated with numerous disorders, including cancer, neurodegeneration, and immune dysfunction [2]. Therefore, sensitive and reliable methods for GR detection are of significant clinical interest.

This work describes the development of a disposable electrochemical sensor for the indirect determination of GR activity. A hybrid nanomaterial was synthesized by covalently linking 3,5-dinitrobenzoic acid (DNBA) to oxidized multi-walled carbon nanotubes using 1,6-diaminohexane as a cross-linker. This material was drop-cast onto screen-printed carbon electrodes (SPEs) and electrochemically activated to produce a redox mediator that enables NADPH oxidation at low potentials [3].

The sensor operates by measuring the residual NADPH concentration after enzymatic reduction of GSSG by GR, via amperometry at -0.05 V. It showed a linear response in the range of 1.6–174 $\mu\text{U}/\mu\text{L}$ GR, with high reproducibility and selectivity in the presence of potential interferents like glucose, uric acid, and dopamine. Characterization of the DNBA-CNT nanomaterial by Raman spectroscopy, electron microscopy, and electrochemical techniques confirmed successful functionalization and enhanced electrocatalytic properties.

The device was validated in diluted human serum samples, showing high recovery values (96–101%) and agreement with standard spectrophotometric assays. Its simplicity, portability, and low detection potential make it a promising platform for the clinical analysis of GR and potentially other NAD(P)H-dependent enzymes.

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P2-119**ENZYME-FREE AMPEROMETRIC APTASENSOR FOR CARCINOEMBRYONIC ANTIGEN BASED ON JANUS COLLOIDS**

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The development of reliable and highly sensitive diagnostic devices for early cancer detection is a key priority in biomedical research. Carcinoembryonic antigen (CEA), a glycosylated oncofetal protein involved in cell adhesion, proliferation, and migration, is considered a major biomarker for colorectal and other carcinomas due to its high overexpression in tumor tissues [1]. Among the emerging technologies, electrochemical biosensors have attracted growing attention thanks to their high sensitivity, rapid response, miniaturization potential, and cost-effectiveness. However, the performance of conventional biosensors can be limited by the use of enzyme-based labels, which are prone to denaturation and reduce long-term stability [2].

Herein, we report a novel enzyme-free amperometric aptasensor for the sensitive detection of CEA, based on functional Janus nanocolloids that act as dual-purpose labeling elements. These Janus colloids were prepared by a toposelective functionalization strategy using gold nanoparticles (AuNPs) asymmetrically modified with poly(amidoamine) (PAMAM) dendrons. Catalytically active platinum nanoparticles (PtNPs) were grown in situ within the dendritic matrix on one side of the particles, while the opposite hemisphere was functionalized with a ssDNA aptamer specific to CEA. This configuration enabled simultaneous signal amplification and selective recognition.

The sensing interface consisted of screen-printed carbon electrodes (SPCEs) coated with AuNPs and immobilized anti-CEA aptamers. Upon target binding, the Janus colloids formed a sandwich-type configuration, producing a catalytic response via the PtNPs in the presence of hydrogen peroxide. The resulting amperometric signal was directly proportional to the concentration of CEA.

The aptasensor exhibited a wide linear range from 25 pg/mL to 1.0 ng/mL and a limit of detection of 8.0 pg/mL. It also demonstrated high specificity against potentially interfering proteins and maintained full sensing functionality after 50 days of storage at 4 °C. The device was successfully applied to quantify CEA in reconstituted human serum samples, yielding recoveries between 92% and 106%, confirming its potential for clinical use.

Compared to conventional systems that rely on enzyme labels, our approach avoids the instability associated with biomacromolecular catalysts and provides a robust and cost-effective alternative for signal generation [2]. In addition, the use of aptamers as recognition elements offers advantages such as chemical stability, reusability, and synthetic accessibility, which further contribute to the simplicity and reproducibility of the biosensor fabrication process.

This work builds on our previous findings regarding the synthesis of anisotropically modified AuNPs with PAMAM dendrons through masking and toposelective methods [3], now applied to develop a high-performance biosensor with significant implications for cancer diagnostics. We envision that these functional Janus nanocolloids could serve as versatile components for the development of next-generation affinity biosensors targeting a wide range of clinically relevant biomarkers.

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P2-120**FORMULATION OF CONDUCTIVE FILAMENT FOR 3D PRINTED ELECTROCHEMICAL SENSORS****Serentill, Roger; Cetó, Xavier; Del Valle, Manel.***Universitat Autònoma de Barcelona, Bellaterra, Spain.*

Over the last years, additive manufacturing has emerged as a transformative technology, offering rapid, cost-effective and customizable solutions for real-time chemical analysis. Unlike screen printing, which requires expensive fabrication setups and generates significant material waste, 3D printing enables precise, on-demand production with minimal resource consumption. This technology reduces costs by eliminating the need for masks, stencils and multiple processing steps while allowing for customizable intricate electrode designs that enhance sensitivity and functionality. Additionally, the ability to use biodegradable or recyclable materials enhances sustainability of the developed products.

The fabrication of 3D-printed electrochemical sensors opens the door to rapid prototyping and groundbreaking applications across agri-food analysis, environmental monitoring and healthcare diagnostics, as both technologies align seamlessly on the emphasis of precision, customization and low-cost. In this context, electrodes can be fabricated by fused deposition modelling (FDM) from thermoplastics such as poly-lactic acid (PLA) or acrylonitrile-butadiene-styrene (ABS), commonly doped with different carbon-based materials such as carbon black, carbon nanotubes (CNTs) or graphene to overcome the insulating nature of PLA and ABS. Furthermore, the ease of fabrication of composite materials (through the incorporation of redox mediators/electrocatalysts during the extrusion of customized filaments) combined with the automatized fabrication and miniaturization of FDM makes 3D printing even more appealing.

In this regard, the development of a simple protocol for the preparation of bulk-modified conductive filaments for the printing of voltammetric sensors is explored herein. Firstly, optimum proportions of the composite material were found by percolation theory. Next, the process for the printing and activation of the filament were also optimized to ensure the highest reproducibility, sensitivity, stability and fast response. To this aim, devices were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), and their performance was benchmarked against commercial electrodes. Additionally, morphological characterization of the electrodes was conducted by scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX). Finally, the potential of the approach is demonstrated through the quantitative determination of ascorbic acid and melatonin, two different active pharmaceutical ingredients (APIs) found in over the counter (OTC) pharmaceuticals.

P2-121**FLOW ANALYSIS MONITOR OF MICROBIAL POPULATION FOR THE NEEDS OF MICROBIOLOGY AND BIOTECHNOLOGY**

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In this contribution a novel flow analysis monitor designed to quantify both the proliferation and spatial distribution of microbial populations during their growth is presented. The Multicommuted Flow Analysis (MCFA) system developed integrates an optoelectronic detector with microsolenoid devices, all controlled by an Arduino microcontroller board. This automated system enables precise and continuous monitoring of microbial growth, providing valuable insight into key population parameters, including potential multiplication rates and the duration of different growth phases such as lag, log and stationary phases [1]. A key advantage of this MCFA-based monitor is its flexibility. The system allows real-time monitoring of microbial cultures in multiple parallel growth chambers, making it highly applicable to laboratory-scale studies of microbial dynamics. In addition, it can track a single process at multiple sampling points of biotechnological reactor. Such opportunity is demonstrated by the monitoring of beer fermentation process [2].

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P2-122**DETECTION OF INDOOR VOCs: A CYCLODEXTRIN-DYE SENSING APPROACH**

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Volatile organic compounds (VOCs) are among the most prevalent indoor air pollutants, associated with adverse health effects and material degradation. Considering that individuals spend over 80% of their time in indoor environments [1], the development of reliable tools for indoor air quality monitoring is of significant analytical interest. Cyclodextrin-dye conjugates constitute a versatile class of supramolecular chemosensors, widely investigated for their ability to detect a range of analytes, including VOCs, by coupling molecular recognition with optical transduction mechanisms [2].

In this study, we report a set of chemosensors based on β - and γ -cyclodextrin-chromophore conjugates for the selective detection of VOCs in enclosed spaces. The systems exploit the host-guest inclusion capabilities of cyclodextrins and the optical sensitivity of covalently bound chromophores, such as methyl red and 1,8-naphthalimide derivatives. VOC perturbs the microenvironment of the dye moiety, inducing solvatochromic and fluorogenic responses that are characteristic of the analyte chemical nature.

These chemosensors offer a promising platform for low-cost, portable, and real-time VOC detection, with potential applications in environmental analysis, occupational exposure assessment, and smart building technologies. Moreover, their integration into 3D-printable polymer matrices enables the fabrication of solid-state optical sensors, expanding their applicability to customizable sensing surfaces, wearable formats, and embedded analytical components in indoor environments.

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INTEGRATED MICROFLUIDIC CELL LYSIS AND PLASMONIC ASSAY FOR DETECTING CIRCULATING TUMOR GENETIC BIOMARKERS IN LIQUID BIOPSY

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Liquid biopsy is a groundbreaking advancement in cancer diagnostics, offering a non-invasive alternative to traditional tissue biopsies. Liquid biopsies can be done with minimal discomfort, allowing for repeated tests without significant risk to patients, and have shown promise in detecting cancers at earlier stages, greatly improving treatment outcomes. [1,2]

This innovative method uses body fluids to identify cancer-related biomarkers, enabling early diagnosis and ongoing monitoring of disease progression. Technology mainly focuses on circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and other genetic materials, which provide insights into tumor heterogeneity and treatment responses.[3]

Current methods for analyzing liquid biopsy samples often involve complex procedures, such as pre-analytical processing and target isolation. These steps usually require a polymerase chain reaction to amplify sequences for genomic DNA analysis.

Liquid biopsy employs microfluidics to simplify these processes. Microfluidics improves the analysis of circulating tumor cells (CTCs) and tumor-derived nucleic acids, enabling efficient separation and enrichment of these biomarkers from blood. This provides comprehensive insights into tumor biology and patient-specific treatment responses. Combining microfluidics with advanced imaging and sequencing technologies has greatly enhanced the accuracy and usefulness of liquid biopsies.[4] To achieve this, we developed a microfluidic device for handling liquid biopsy samples, enhancing the processing of circulating tumor cells. The device features cellular lysis through two inlet channels for the sample and lysis reagent. It includes a serpentine zone for initial homogenization and a long channel for completion. The channel's width and depth—500 μm and 80 μm —allow processing of a few tens of microliters of sample. Lysis optimization used human wild-type (All-RAS WT) HT-29 and mutated (KRAS p. G13D) LoVo cell lines, isolated from colorectal adenocarcinoma tissues, expressing a mutation in exon 2 of the K-RAS oncogene. Genomic DNA from these cells was analyzed using optical fluorescence microscopy with dsDNA-specific intercalating dyes. gDNA detection was performed via a plasmonic assay based on surface plasmon resonance imaging, through target capture by functionalized superparamagnetic beads.[5,6]

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P2-124**IN-SYRINGE-AUTOMATED MATRIX PRECIPITATION AND SOLVENT EVAPORATION FOR HPLC ANALYSIS OF VITAMINS IN VEGAN MILK****Htet, Tar Tar Moe.***Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic.*

Sample preparation for vegan milk analysis is complex since deproteinization is required, which can imply further procedural steps such as centrifugation, supernatant withdraw, or solvent evaporation and reconstitution. In this work, the limiting factor for automation such as centrifugation step was omitted and automation of solvent evaporation inside the syringe was studied for the first time.

An overview is given on the development of an automated methodology for determination of selected 7 water-soluble vitamins in vegan milks, namely B1 (Thiamine), B2 (Riboflavin), B3 (Niacinamide), B3 (Nicotinic acid), B6 (Pyridoxine), B9 (Folic acid), and B12 (Cyanocobalamin) and theophylline as an internal standard. The chosen samples were oat, soy, almond, hazelnut, and coconut milk substitutes with fat contents ranging from 0.8 % to 3.5 %.

The method is based on a centrifugation-less milk deproteinization procedure taking advantage of salting out homogenous liquid-liquid extraction, which was automated via the flow-batch technique Lab-In-Syringe [1]. In a second step, the solvent was transferred into the void of a second automatic syringe for solvent evaporation to increase sensitivity and dilution with buffer.

The system was coupled online to high performance liquid chromatography with photodiode array detection. The separation process was carried out within 10 min in parallel to the subsequent preparative procedure and flow system cleaning. Parameters influencing the method performance including salt solution type and volume, solvent-to-sample ratio, extraction pH, stirring rate, stirring time, and evaporation conditions were optimized as well as the HPLC method for the separation of the analytes. A preliminary evaluation of the applicability of the developed procedure will be presented.

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ANALYTICAL EVALUATION OF MICELLAR AND MICROEMULSION ELECTROKINETIC CHROMATOGRAPHIC SYSTEMS USING A FAST ABRAHAM SOLVATION PARAMETER APPROACH

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Electrokinetic chromatography with micelles and microemulsion (MEKC and MEEKC) aggregates have proved to be versatile techniques, facilitating selectivity and surrogation of biological conditions through the application of customised separation media. Due to their flexibility and tunability, they are commonly well-standardized for physicochemical profiling and early-stage drug screening. The systematic characterization of solute-solvent interactions, which ultimately control retention and selectivity, is a crucial first step towards optimizing these separation systems.

In line with this goal, we adopt the Abraham solvation parameter model (a linear free energy relationship model, LFER) to elucidate retention on the basis of individual molecular interactions[1]. The model for neutral compounds is given as:

$$\log SP = c + eE + sS + aA + bB + vV$$

In this, SP stands for a property related to solvation (e.g., log retention factor), E denotes excess molar refraction, S dipolarity/polarizability, A and B the solute's hydrogen bond acidity and basicity, respectively, and V the McGowan volume. Chromatographic systems that are capable of the relevant interactions yield coefficients (e; s; a; b; v) and a term c (constant for the system being examined). This parameterization allows direct comparison between chromatographic and biological systems, as well as mechanistic insight into solute behaviour. To simplify this characterization, we developed a rapid Abraham LFER workup [2] that greatly minimizes the number of compounds needed to model the partition behavior of the system. In the current method, we used homologous series (such as n-alkyl phenones) to measure v coefficient and used nonanophenone as more practical micelle/microemulsion marker in place of traditionally used dodecanophenone. Apart from this, specific compound pairs allow for efficient calculation of the remaining coefficients (e, s, a, b). These pairs of compounds are substances that have similar descriptors except for one of them. It is possible to calculate solute solvent interactions for the selected pair of compounds with four identical molecular descriptors and different one X_i as:

$$x_i \approx \log \alpha_{1/2} X_{i,1} - X_{i,2} = \log k_1 - \log k_2 / X_{i,1} - X_{i,2}$$

In the present work, seven surfactant-based micellar systems have been characterized experimentally in MEKC [3]. These surfactants include four anionic surfactants—sodium dodecyl sulfate (SDS), lithium dodecyl sulfate (LDS), sodium cholate (SC), and sodium deoxycholate (SDC)—and two cationic surfactants—tetradecyltrimethylammonium bromide (TTAB) and hexadecyltrimethylammonium bromide (HTAB). On the other hand, MEEKC[4] was characterized three classes of microemulsions at different surfactant concentration at acidic (pH 2.0), neutral (pH 7.4) and basic (pH 12.0) conditions as, previously utilized for microemulsion system construction and application.

Interpretations of the resulting Abraham coefficients for these surfactant systems show important trends with respect to polar interaction, hydrogen bonding, and cavity effects across

a range of surfactant types micellar and microemulsion compositions. This work shows the power of the fast LFER approach, as an efficient and quantitative framework for the analytical assessment of electrokinetic systems for pharmaceutical and environmental applications in the early stages of chemical compounds behavior evaluation.

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COLLABORATIVE PEER EVALUATION APPLIED TO THE PREPARATION OF LABORATORY REPORTS

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This work presents the results of implementing collaborative peer evaluation, also known as P2P (Peer-to-Peer), in two experimental courses within the field of Analytical Chemistry, one at the undergraduate level and the other at the Master's level. The aim of this activity was to improve students' skills in writing scientific reports. In this case we used the format of a scientific article to expose them to world-class examples. We promote the critical thinking skills through the selection of a few appropriate previous works to be included in their reports and also through the revision of their peers' reports. The process utilized a "double-anonymized peer review" model, ensuring that students felt comfortable conducting their critical analyses. This was facilitated through the "Workshop" tool on the Virtual Campus (Moodle) of the University of Oviedo. Results indicated that students significantly improved their abilities to prepare scientific reports and were able to objectively evaluate the quality of their peers' work by using a rubric provided by the instructors. Overall, both students and instructors evaluated the activity positively, leading to the decision to implement it in subsequent courses with similar results.

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SPECTRAPP: A USER-FRIENDLY OPEN-SOURCE TOOL FOR CHEMOMETRIC ANALYSIS IN LEARNING AND RESEARCH CONTEXTS

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Chemometric techniques have become essential in modern analytical chemistry, offering powerful approaches to interpret complex, high-dimensional datasets. However, applying these methods effectively often requires programming skills—an area not commonly covered in traditional chemistry curricula. To address this challenge, we developed **SpectrApp**, an open-source R Shiny application designed to make chemometric analysis more accessible to students, educators, and researchers—no coding required.

SpectrApp provides an intuitive, interactive interface that supports the entire analytical workflow. Users can move seamlessly from data visualization—covering univariate, bivariate, and multivariate plots—to preprocessing, exploratory analysis, model building, and validation.

The app includes a comprehensive suite of tools for:

- **Unsupervised learning:** PCA, clustering, t-SNE, UMAP, DBSCAN.
- **Classification:** k-NN, PLS-DA, LDA, SVM, Random Forest, Logistic Regression, Naive Bayes.
- **Class modeling:** SIMCA.
- **Regression:** MLR, PCR, PLS-R.

Behind the scenes, SpectrApp integrates robust R packages such as *mdatools* [1] for multivariate analysis, *prospectr* for preprocessing spectral data, and *caret* [2] for supervised machine learning.

By combining a broad array of methods with an easy-to-use design, SpectrApp lowers the barrier to advanced data analysis. It encourages exploration and learning in educational settings and supports reproducibility and transparency in research workflows.

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P2-128

COLORIMETRIC ANALYSIS: INTEGRATION OF DIGITAL AND SPECTRAL COLOR MEASUREMENTS IN ANALYTICAL CHEMISTRY EDUCATION

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The growing trend toward decentralized analytical devices has established colourimetric analysis as a preferred technique. This progress allows the integration of smartphones and dedicated applications for quantitative color analysis across various measurement scales, opening new opportunities for teaching analytical chemistry. This study describes the implementation of smartphone-based colourimetric methods in courses within the Biochemistry programme at Universidad de la República (Montevideo, Uruguay), specifically in Analytical Chemistry, Nanochemistry, and Physical Chemistry.

Four different analytical applications were developed and assessed: (i) colour saturation analysis as an additive property for phycocyanobilin quantification in aqueous natural extracts and metallic nanoparticle systems (silver and gold); (ii) RGB channel monitoring for protein denaturation kinetics in *Spirulina* spp. extracts; (iii) CIE Lab parameter analysis for differentiating colloidal systems; and (iv) CIE Lab parameter discrimination between nanoparticle aggregates and large individual nanoparticles (>50 nm).

Students evaluated zero-intercept linear models for additive property determination through rigorous statistical analysis, comparing results with traditional Beer-Lambert Law applications. In colour space studies, students examined the significance of signs for *a* and *b* parameters to distinguish size-dependent effects on nanoparticle colouration. The correlation between colour space parameters and the dominant spectral wavelength was systematically studied. Thermal protein denaturation experiments involved parallel measurements of absorbance and colourimetry, with analysis of individual RGB channels and their linear combinations.

Results demonstrate the successful integration of smartphone-based analytical systems while preserving analytical rigour. This approach effectively introduces modern analytical technologies whilst reinforcing fundamental concepts, giving students practical experience in emerging methodologies suitable for resource-limited environments.

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P2-129

THE RELATIONSHIP BETWEEN MATERNAL HAIR OLEAMIDE AND POSTNATAL MENTAL HEALTH: ANALYSIS USING Nano-PALDI IMAGING MASS SPECTROMETRY

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Introduction

Depressive symptoms and bonding failure are significant maternal mental health concerns in the postpartum period. In recent years, studies have investigated the relationship between hormone levels and postpartum depressive symptoms or bonding failure (1). Most of these studies have relied on saliva or blood samples, capturing hormone levels at a single point in time. In contrast, hair samples allow for long-term data collection. Given that human hair grows at an average rate of approximately 0.4 mm per day, a 3 cm strand represents roughly the past three months of hormone exposure.

Oleamide has been reported to have a wide range of receptors and neurotransmitter systems, and it is suggested to have antidepressant properties. However, no studies to date have examined the relationship between oleamide and postpartum depressive symptoms. In this study, we investigated the association between maternal hair oleamide levels and postnatal depressive symptoms and bonding failure from single hair by nanoparticle-assisted laser desorption/ionization imaging mass spectrometry (Nano-PALDI IMS) (2).

Methods

Participants were recruited during the one-month postpartum check-up at a hospital. Eighteen participants completed the questionnaire. Depressive symptoms and bonding failure were assessed using the Edinburgh Postnatal Depression Scale (EPDS) and the Mother-to-Infant Bonding Scale (MIBS), respectively. Additionally, approximately five to ten strands of hair were collected from each participant, and the 3 cm lateral hair section from the root was analyzed. Hair oleamide levels were measured using Nano-PALDI IMS. This study was reviewed and approved by the Fukui Prefectural University Research Ethics Committee.

Results

The mean age of participants was 30.83 years (± 4.18). Six participants (33.3%) were primiparas, and twelve (66.6%) were multiparas. Fifteen women (83.3%) had vaginal deliveries, while three (16.7%) underwent Caesarean sections. Based on the EPDS and MIBS cutoff scores (≥ 9 and ≥ 5 , respectively), four participants (22.2%) were classified as having postpartum depressive symptoms, and seven (38.9%) were identified with bonding failure.

Oleamide was successfully detected and spatially imaged (Fig.1). Each participant's hair oleamide intensity was corrected for control hair intensity. The imaging results showed that women with bonding failure (Fig.1a) tended

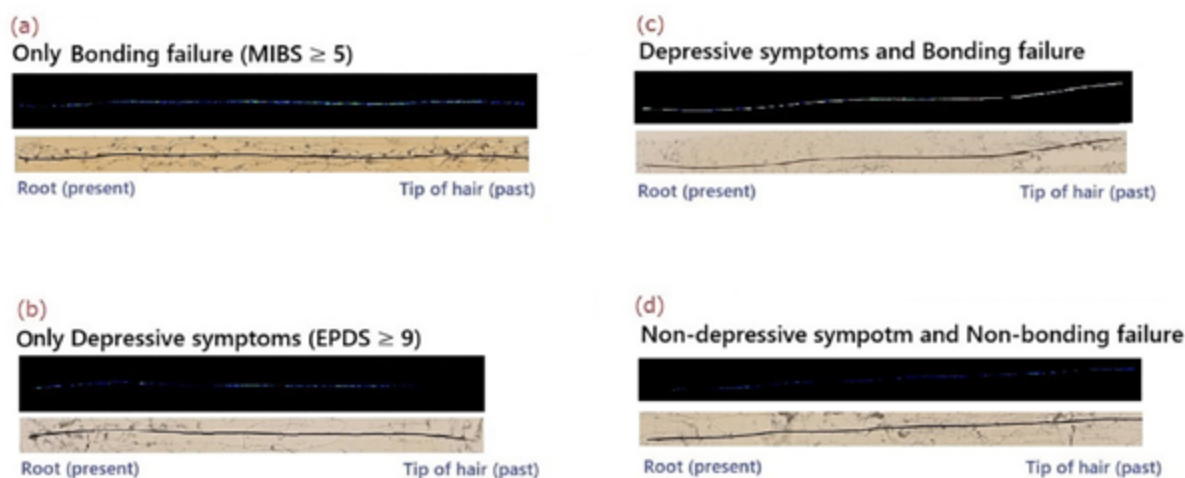


Fig.1 Visualization of hair oleamide levels by Nano-PALDI imaging mass spectrometry

to have higher oleamide intensity than women without. Furthermore, although differences between depressed women (Fig.1b) and non-depressed women were not clear, women with both depressive symptoms and bonding failure (Fig.1c) showed stronger intensity than women without both (Fig.1d). Given that oleamide has antidepressant properties, depressed women would be expected to have lower hair oleamide levels than non-depressed women, however imaging results showed the opposite. One reason for this might be that any oleamide that isn't used in the body travels through the bloodstream to the hair due to depression and bonding failure. These mechanisms need to be investigated in the future.

Furthermore, results over time show that the hair of non-depressed, non-bonding failure women had no oleamide intensity near the roots, whereas the hair of women with depressive symptoms, bonding failure, and combined both had continuous intensity from the roots. These results suggest that women who consistently have detectable oleamide in their hair during the late pregnancy may be at risk for postpartum depressive symptoms and bonding failure, regardless of the intensity of oleamide. Spatial visualization of hair oleamide in pregnancy may be useful as non-invasive for early detection of possible postpartum depressive symptoms and bonding failure.

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P2-130**PROSTATE CANCER DIAGNOSIS BASED ON CHEMICAL ANALYSIS OF HUMAN SCENT**

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Human scent comprises a variety of chemical compounds that can provide valuable information about an individual. By using sensitive analytical methods, such as gas chromatography, which accounts for the volatile nature of these substances, and appropriate detectors, the characteristic scent profiles of individuals can be identified. This facilitates the identification of an individual's gender, blood type, and Rh factor. It is believed that these characteristics originate primarily from what is known as the primary scent, which remains largely unchanged throughout a person's life and is probably genetically determined.

Genetically determined diseases include conditions such as Parkinson's disease, diabetes types I and II, as well as various forms of cancers. The most diagnosed cancer in men is prostate carcinoma. With early diagnosis, which follows a tissue biopsy and blood tests, the survival rate for this type of cancer can reach up to 99%. Given that cancer is a degenerative disease, it is plausible to assume changes in genetic material that could also alter the primary scent of a patient. This variation could potentially be used to differentiate between patients based on their health status.

Scent samples were collected from both ill and healthy individuals by absorbing scent components onto a suitable sorbent, in this case, glass beads. These samples were then extracted from the sorbent using a special methodology. The prepared substrates were analysed using a chromatographic technique (GC×GC-TOF, Pegasus BT-4D, LECO, USA). Eventually, the resulting chromatographic data were evaluated using a combination of statistical methods, including PCA (Principal Component Analysis), CA (Cluster Analysis), KNN (k-nearest neighbors algorithm), and SVM (Support Vector Machine).

This method of obtaining scent samples is non-invasive and stressless compared to surgical procedures, which have associated health risks such as infections and anaesthesia. The process of obtaining the sample itself is undemanding; therefore, it would be possible to do the sampling directly at the doctor's office.

Acknowledgement: This work was supported by grant no. VJ01010123.

P2-131

ANALYTICAL PROFILING OF HUMAN VOLATILOME FOR INDIVIDUAL DISCRIMINATION USING HS-SPME-GC-MS AND HS-GC-IMS COUPLED TO MACHINE LEARNING

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The human volatilome, or human scent, represents a complex and dynamic chemical signature shaped by physiological, genetic, and environmental factors. It holds significant potential in diverse fields such as forensic science, clinical diagnostics, personalized medicine, and security¹. Human scent consists of a complex mixture of volatile, semi-volatile (VOCs and SVOCs), and non-volatile compounds released by the body, which can be used to discriminate and individualize between subjects. These compounds can be extracted from textile materials commonly found in clothing, such as cotton².

Gas chromatography combined with mass spectrometry (GC-MS) is commonly used to identify marker molecules in odor analysis. In this study, two non-target analytical methods were evaluated to distinguish individuals based on characteristics such as assigned sex and age, through the analysis of cotton patches worn on the lower back for a defined period. Headspace solid-phase microextraction with GC-MS (HS-SPME-GC-MS) and headspace gas chromatography–ion mobility spectrometry (HS-GC-IMS) were optimized using a Box-Behnken design and Response Surface Methodology. The distances between two independent samples by using the total ion sum spectrum (TIS) and the ion mobility sum spectrum (IMSS) were used as response variables, respectively³. HS extraction parameters—including incubation temperature, incubation time, and agitation—were optimized. Repeatability and intermediate precision were also assessed for both methods.

The extracted data were subjected to various machine learning techniques, including principal component analysis (PCA) and hierarchical cluster analysis (HCA) as unsupervised learning techniques, and support vector machine (SVM), random forest (RF), linear discriminant analysis (LDA), and partial least squares–discriminant analysis (PLS-DA) as supervised learning techniques. These approaches aimed to evaluate and compare classification models for the discrimination of individuals according to sex, diet, ethnicity, and age.

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P2-132**ANALYSIS OF CUTTING AGENTS IN SEIZED COCAINE SAMPLES AND THEIR INFLUENCE ON THEIR NIR PROFILES**

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Cocaine is one of the most relevant substances of abuse globally and regionally, with an increase in its detection of 376% between 2012 and 2022, and ranking as the second most seized substance with 84000 registrations in 2022 [1], only behind cannabis and its derivatives. In the context of the European Union, record quantities of this substance are being seized [2], with Spain being one of the most affected countries with a total of 58.3 tons of cocaine seized in 2022 [3]. According to the latest annual report of the European Union Agency for Drugs, cocaine is one of the substances most frequently found in transit of intermodal containers (those that are transported by sea and by road transport), with the largest seizure of this drug to date having been carried out in 2023, estimated at 9.5 tons [2].

Analytical methods commonly employed to characterize these forensic samples involve separation methods, like chromatography, making use of different detection methods. These techniques allow not only to quantify the active compound -cocaine- but also to identify and, if necessary, quantify the different cutting agents that come along. More recently, the development of spectroscopy-based methods has emerged as a potential in-situ analytical tool. However, this approach is often hindered by the complex composition of the samples, with different particle sizes and a variety of concurrent cutting agents.

In this work, a chromatographic determination based on liquid chromatography has been carried out in a sample set consisting of 172 seized cocaine samples. Additionally, NIR spectra of the samples were acquired using a portable NIR spectrometer directly within the vial.

Spectral processing was performed using PLSToolbox in MATLAB. For preprocessing, a first derivative (Savitzky-Golay, window width = 3; degree = 2) was applied to account for baseline variations, and spectra were mean-centered in the column direction. Concentration data were autoscaled column-wise. Subsequently, an exploratory analysis was carried out using Principal Component Analysis (PCA) on both concentration and spectral data, allowing the evaluation of how the concentration of each of the seven cutting agents influences the obtained spectral signals.

The PCA applied on concentration data revealed the distribution of the different samples in the scores plot according to their concentration variability, showing groups of samples with similar composition. Based on this analysis, cocaine and caffeine were identified as the ones contributing the most to the variability in PC1, while phenacetin dominated in PC2. Thus, these species were identified as the ones contributing the most to compositional variability.

To assess the impact of these compounds' concentrations on the NIR spectra, an additional PCA was performed on the preprocessed spectral data. Phenacetin showed a clear correlation with the sample distribution along PC1, suggesting a strong influence of its concentration on the spectral signal. In contrast, cocaine and caffeine exhibited a partial influence on the spectra: higher cocaine concentrations (above 50 % mass concentration) led to sample clustering in a specific direction, whereas samples with lower concentrations were uniformly distributed. In contrast, for caffeine, an opposite trend was observed, with lower concentration samples showing a clearer distribution along PC2. When it comes to the other investigated adulterants —levamisole, lidocaine, tetracaine, and procaine—, no clear trends were identified in the PCA, suggesting that their contribution to spectral variability is minor or overlapped with other factors.

Given the need to develop fast and portable methods based on spectroscopy, the investigation of relevant cutting agents becomes fundamental. Thus, the results presented here are not only important given the compositional information, but because they show how cutting agents can exert influence the development of fast and in situ analytical approaches in forensics.

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P2-133**FORENSIC ANALYSIS OF PACKAGING MATERIALS: LIBS EXAMINATION OF PAPER SUBSTRATE AND COVERING MATERIALS**

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The manufacturing and distribution of counterfeit goods represent a significant issue in today's world, creating potential risks to consumer health and safety. Counterfeiting undermines the economic growth of legitimate businesses and affects consumers through financial losses, operational delays, and costs associated with replacements. The proliferation of counterfeit cosmetics and perfumes presents serious health and safety risks. When applied directly to the skin, these illicit products, including perfumes, cosmetics, and personal care items, can cause a significant harm. As consumers, we are becoming more inclined to pay premium prices for quality products. Unfortunately, this trend is being exploited by individuals who flood the market with substandard goods of an unknown, hidden identity. In 2019, perfumes and cosmetics were ranked as the third most commonly confiscated goods at the EU border for suspected violations of intellectual property rights. The top spot was occupied by clothing, accessories, and luxury items, followed by electronic devices such as phones and food products. In 2020, EU authorities seized approximately 66 million counterfeit goods, with nearly 69% of these items intercepted within the EU and the remaining 31% at the border. That same year, counterfeit perfume packaging materials emerged as one of the most frequently confiscated items at the EU's external border, apart from cosmetic products, aroma compounds, fixatives or solvents [1]. Given the widespread prevalence of counterfeit cosmetics and perfumes, there is an ongoing search for effective ways to combat this issue. Research efforts often focus on analyzing the contents of the packaging. However, examining packaging materials offers a promising alternative approach to identify potential counterfeits.

In this study, the laser induced breakdown spectrometer (J200, Applied Spectra) was used to analyze cosmetic packages. The assessment of the potential authenticity of the products was made based on differences in the elemental composition of the covering material and the paper substrate. The main objective of the study was to develop a practical methodology that would generate fast, sensitive, repeatable semi-quantitative results. Due to the very large data sets, it was necessary to use multidimensional methods to verify the degree of similarity between objects in terms of the parameters considered. In total, 59 packages by 6 different brands were analyzed in this study. For each selected element within a given package, three independent measurements were performed. The intra-subject variability proved to be negligible and did not notably influence the determination of the cosmetic product's origin. The covering material and the paper substrate were important discriminating elements for the analyzed packages. The studies have shown that the obtained standardized semi-quantitative data supported by multivariate methods (PCA) can be successfully used in verification of the authenticity of cosmetic products and perfumes. In the future, this approach could help expand the focus of analyses to encompass the packaging itself, rather than being limited solely to the products inside.

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P2-134**INDIVIDUAL AND CLASS IDENTIFICATION OF PERSONS BY COMPUTER USING DIGITAL HUMAN SCENT EVIDENCE****Urban, Štěpán; Bouška, Luděk; Malá, Ulrika; Čechová, Jana; Kamynskyi, Oleksii; Škeříková, Veronika.***University of Chemistry and Technology in Prague, Prague, Czech Republic.*

Human scent is very important evidence that is an accessible alternative to traditional crime scene facts, such as fingerprints etc. So far, police have only exploited human scent signatures exclusively using specially trained police canines for an identification of persons. The reliability of this identification is often questioned due to possible misinterpretation and subjectivity. Therefore, recently, an objective method based on analytical chemistry (olfactronic) has been under development. However, it was found that canine olfaction is several orders more sensitive than the best laboratory devices and therefore, two-dimensional gas chromatography coupled with time-of-flight mass spectrometer as the best solution. With this GCxGC/TOF more than 60,000 various chemical compounds can be observed in a scent sample.

The digital scent evidence was created from several thousand chromatographic peaks of the selected primary scent compounds that were digitalized as the intensity ratios of the selected pairs of compounds. The digital scent evidence of different persons were used to create a database of these digital signatures.

Our comparative software enables the comparison of a digital scent signature of an unknown person with all the scent signatures in database. This computer comparison of the digital human scent signatures has worked very well so far. It is obvious that the digital scent signatures and the corresponding software have a huge application potential in police service on the national as well as international levels.

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P2-135**CANINE TRAINING AIDS SUBSTRATE OPTIMIZATION: ADSORPTION AND NON-TARGETED CONTAMINATION**

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This study investigates the contamination resistance of canine training aid substrates as well as their adsorption, desorption and selectivity capabilities, critical factors that affect the efficacy of detection training[1]. Canine detection proficiency is directly influenced by the quality of training aids, and contamination or using the wrong adsorbant can rapidly degrade their performance[2]. This study addresses the problem of contamination in canine training aids by comparing the extent of contamination across various common substrate materials in two real-world scenarios: training and storage.

The first part of the project focused on assessing non-target contamination during training. Substrate materials, including GetXent polymer tubes, cellulose-based materials, PDMS, and novel FPSE materials, were exposed to highly odorous environments to simulate training conditions. The headspace above these substrates was sampled and analyzed using SPME-GC/MS. Results indicated significant differences in non-contamination levels among the substrates suggesting variability in the suitability of different substrates for training aids. Additionally showed how different methods of handling training kits affect contamination levels.

The second part of the project investigated cross-contamination during storage. Substrates impregnated with specific odors were placed in containment vessels, and their headspace was sampled over time. Analysis using SPME-GC/MS revealed variability among substrates' resistance to cross-contamination and replicability of odor integrity over extended storage periods, especially PDMS and FPSE, indicating the need for improved storage protocols for certain substrate types.

The findings from this study provide valuable insights into the strengths and weaknesses of these substrate materials, informing future manufacturing processes and guiding the development of cautionary measures and mitigation strategies. It also showed no correlation between cost and effectiveness. Finally, while GetXent tubes and PDMS training aids are gaining popularity in the operational community, there has been limited research on their strengths, limitations, and contamination potential.

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P2-136**MICROFLUIDIC BLISTERS FILLED WITH NANOZYME-ANTIBODY CONJUGATES****Furmanek, Wiktoria¹; Stańczak, Paweł²; Żukowski, Kamil³; Chudy, Michał¹; Pietrzak, Mariusz²; Dybko, Artur¹.**

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The paper presents the design of an antibody-filled blister. The aim of the work was to check if the nanozyme-antibody conjugate could be encapsulated inside a blister, stored in a laboratory or a refrigerator, and then used in biochemical experiments. We have used a hybrid polymeric foil, which is made of nylon, aluminium, and polyethylene with a total thickness of 152 μm . The polyethylene layer allows thermal bonding of the foil. The combination of aluminium, and nylon assures very low gas permeability, a low evaporation coefficient, and a solution inside a blister is isolated from ambient light. The blisters were formed by the cold forming of domes in the foil applied. Special aluminium moulds were prepared by the micromilling technique. The hybrid foil was inserted between the forms with polyethylene layer upwards, and the whole set was put into a hydraulic press for 5 minutes under 2 atm pressure. After this time, the domes with volumes of 100 μl were formed in the foil. Then the domes were filled with a solution of antibodies and thermally sealed at a temperature of 165 $^{\circ}\text{C}$ for 5 s. In order to seal the blister, we designed a special heating tool in the form of a metal pipe with a heating wire wound outside. A special strain sensor was used to control the pressing force of the heating tool onto the hybrid foil. The temperature of thermal bonding was set experimentally, and it was governed by the thermal characteristic of polyethylene. It was obvious that during the thermal bonding the solution containing antibodies will warm up and high temperature could cause antibody denaturation. We have placed a miniature temperature sensor inside the antibody-filled dome in order to monitor the exact temperature of the solution during the thermal bonding process. The temperature was found to rise to 92 $^{\circ}\text{C}$ within 3 s and then drop. The prepared blisters were stored in a laboratory (temperature 22 $^{\circ}\text{C}$) and a fridge (temperature 4 $^{\circ}\text{C}$) and a typical NLISA tests were performed. It was found that antibodies did retain their ability to form sandwich type assays even after the thermal bonding of the blister. The NLISA test confirmed that they could be released from the blister and used for the experiments. The paper will compare the two blister storage temperatures and the efficacy of the encapsulated antibodies.

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ASSESSMENT OF BETWEEN-BOTTLE HOMOGENEITY, PARTICLE SIZE AND ELEMENT CONTENT OF CANDIDATE REFERENCE MATERIALS OF BLACK CRICKET (*GRYLLUS ASSIMILIS*) IN NATURA EMPLOYING WAVELENGTH DISPERSIVE X-ray FLUORESCENCE SPECTROMETRY

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Cricket contains approximately 65% protein and minerals such as K, Mg, Ca, Zn and P, among other chemical elements, considered a significant source of nutrients and one of the principle type of edible insect most consumed in the world. In this respect, the cricket powder has emerged as a viable alternative to conventional animal proteins, more sustainable, healthy, accessible and palatable food source, contributing to the human health and nutrition¹. It is estimated that crickets are consumed by approximately 2 billion people in approximately 80% of countries, due to their nutritional value, and it is therefore essential to ensure food safety through analytical methods that provide precise and accurate data on the elemental composition of these insects². Worth to emphasize that crickets have a complex chemical composition and may present a matrix effect, being necessary to use Reference Material (RM), which have characteristics similar to the sample matrix and are important tools for quality control in laboratory analysis. To prepare a RM, a validated procedure is followed, according to internationally accepted criteria, ensuring the reliability, quality and traceability of measurements of one or more properties, in addition to enabling the identification of possible sources of contamination. However, there is no provider of cricket RM in Brazil, and there are few in the world, which makes it difficult the study of the insect matrix as raw material. Therefore, The objective of this work was to prepare a RM candidate of metals for Ca, Cu, Cl, Fe, K, Mg, P, Zn and S with proposed concentrations varying from 22.9 to 10745.5 mg/kg, aiming its application in the monitoring of these parameters in cricket samples. The study was based on the characterized and evaluated the influence of particle size on the concentrations of chemical elements and homogeneity of a national RM candidate, using the black cricket (*Gryllus assimilis*) in natura. The production process was adapted to the characteristics of the matrix, consisting of the steps of grinding, lyophilization and packaging, resulting in a lot of 25 bottles. The homogeneity between lot was based on ANOVA and was evaluated for the chemical elements Ca, Cu, Cl, Mg, P and S, through the random selection of 5 bottles of the lot. From each lot, 3 portions of 0.2 g of material were sampled. The material characterization was performed for the elements, with quantification of the mass fractions (mg kg⁻¹) using the Energy Dispersive X-ray Fluorescence (EDXRF) technique. The expanded uncertainty of the RM candidate was calculated for a coverage factor (k) equal to 2.00, at a coverage probability of approximately 95%. The results indicated that the material presented homogeneity between the bottles (p-value > 0.05) and that the particle size smaller than 500 µm did not influence the concentration values. The samples of the RM candidate produced presented the following average concentrations for the chemical elements: Ca (1531.0 ± 116.4 mg kg⁻¹), Cl (5559.4 ± 1373.1 mg kg⁻¹), Cu (22.9 ± 1.4 mg kg⁻¹), Fe (67.9 ± 18.5 mg kg⁻¹), K (10745.5 ± 3696.4 mg kg⁻¹), Mg (1083.1 ± 289.2 mg kg⁻¹), P (10397.0 ± 1008.5 mg kg⁻¹), Zn (159.3 ± 5.1 mg kg⁻¹) and S (7608.1 ± 365.2 mg kg⁻¹), with coefficients of variation obtained for the analytes with values between 2.1% and 10.9% and expanded uncertainty below 35%. Furthermore, the mass fractions observed for the analytes are in accordance with data from previous studies. The achievement of these results indicates the feasibility of the continuity of the work regarding evaluations of intra-bottle homogeneity, stability and characterized, intending to make this material available to the laboratories that perform analysis of cricket samples.

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TOWARDS GREENER WORKFLOWS WITH CLEVER METHOD DEVELOPMENT

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Analytical labs face growing demands to reduce energy use, solvent waste, and costs without sacrificing performance. Traditional workflows often rely on time-consuming sample prep and multiple methods, increasing environmental impact and complexity.

Our solution combines virtual method development tools and advanced chromatography phases to create greener, more efficient workflows. Tools like the Pro EZLC Chromatogram Modeler and Method Translator enable remote optimization and modernization of methods, cutting instrument time, solvent use, and energy consumption.

Key to multi-method development is selecting the right stationary phase. While conventional C18 phases struggle with diverse analytes, Biphenyl phases offer enhanced selectivity through π - π and hydrophobic interactions, allowing consolidation of multiple analyses on a single column. This simplifies workflows, reduces material costs, and minimizes errors.

By integrating innovative tools and versatile columns, laboratories can streamline operations, save resources, and support sustainability—all without compromising analytical quality.

P2-153**ESTIMATION OF THE THEORETICAL MAXIMUM DAILY INTAKE OF PESTICIDE RESIDUES AND CHRONIC DIETARY RISK ASSESSMENT IN FRUIT****Alotaibi, Sarah.***Saudi, Riyadh, Saudi Arabia.*

The National Theoretical Maximum Daily Intake (NTMDI) for 53 pesticides in fruits: apples, bananas, berries, grapes, mango, and strawberries, was calculated for the first time using the Maximum Residue Limits (MRLs). The TMDI was evaluated for three age groups of the Saudi population by comparing it with the Acceptable Daily Intakes (ADI). These calculations were conducted following the procedure recommended by the World Health Organization (WHO). In this study, the TMDI for all examined pesticides in fruits was less than 65% of the ADI for children and pregnant women. However, for the adult cluster, the overall result of TMDI was less than 65% of the ADI except for chlorpyrifos and fenpropimorph in bananas, dimethoate in mango, and difenoconazole in grapes. Furthermore, the TMDI was recalculated using a probabilistic approach for these exceeded compounds, and the value was less than 65% of the ADI. On the other hand, the hazard quotients (HQ) value for the risk assessment of exposure to pesticide residue based on monitoring program data was less than 1 for all fruits in the three age groups, except for fenpropimorph in the banana for the adult sub-population. When it is recalculated using a probabilistic way, it is reduced to fall within the acceptable level.

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P2-154

DEVELOPMENT OF A SENSOR FOR LEAD DETECTION IN GUNSHOT RESIDUAL FOR FORENSIC SCIENCE APPLICATIONS

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In today's society, misuse of information, such as tampering with crime scene evidence, poses challenges to justice. Firearm-related crimes require rapid identification of suspects through gunshot residue (GSR) detection, but traditional methods are slow. This study explores electrochemical sensors as a faster alternative for detecting lead (Pb) in GSR. Samples from individuals who fired 9mm ammunition were analyzed and compared across brands. The method's accuracy was assessed against the standard ICP technique.

P2-155

INVESTIGATION OF MICROSTRUCTURAL CHANGE AND CORROSION OF STEEL DRUMS FOR FORENSIC EVIDENCE INCINERATION

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In recent years, crimes have become increasingly complex, often involving sophisticated methods for evidence destruction. Among these, incineration is a commonly employed and highly effective technique, which may include the burning of physical objects, or, in severe cases, human remains in homicide investigations. A notable case in Thailand in 2019 involved the disappearance and murder of Billy Porlajee Rakchongcharoen, an ethnic Karen activist. A 200-liter oil drum containing bone fragments was discovered by forensic officers in a reservoir area. DNA tests confirmed the bones were Billy's, indicating that he had been inside the drum. The findings suggested an attempt to eliminate forensic evidence by burning materials at temperatures between 200 and 300 °C. This incident highlights the importance of understanding how heat affects the physical and microstructural properties of steel containers used in such crimes. The analysis of thermal damage and material degradation is essential for improving the accuracy of forensic investigations. Knowledge of heat-induced changes in materials such as steel drums contributes to estimating the exposure temperature, duration, and destruction methods used by criminals. This study investigated the changes in a 200-liter steel drum exposed to varying temperatures and heating durations. At 600 °C, the outer protective coating degraded, and a gray-black oxide layer formed on the inner surface. At 800°C, grain recrystallization and growth occurred, resulting in a reduction in grain size number over time, although the hardness of the steel remained relatively stable. Corrosion rates increased following thermal exposure but did not show a consistent relationship with temperature or duration. Microstructural analysis thus provides valuable evidence regarding the thermal history of such containers. The findings of this study reveal the importance of forensic understanding of heat-induced material transformations. However, further research is necessary to fully interpret the complex interactions between thermal exposure, material degradation, and microstructural changes.

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P2-156

PoliBrush – A DIDACTIC SOFTWARE FOR ILLUSTRATING THE MULTIVARIATE PROCESSING OF SPECTRAL IMAGES

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Since the first introduction of hyperspectral imaging in chemistry, the need for dedicated software was recognized by the chemometric community. Nonetheless, considering that such an introduction has been relatively recent, availability of software for multivariate analysis of hyperspectral images is still limited, if compared with general chemometric applications. PoliBrush is a freely distributed, stand-alone software designed for teaching exploratory multivariate analysis in the frame of color RGB and spectral imaging. PoliBrush implements principal component analysis (PCA) as its core method. It features a single main window that provides users with essential tools for spectral image preprocessing and exploration. The software emphasizes an interactive brushing approach, enabling users to gain a comprehensive understanding of the relationships between PCA score space and image pixel space. PoliBrush usage and its operational procedure will be demonstrated through two case studies, whose data will be made available for download. The software has already been successfully used in various educational settings and workshops on multivariate approaches for spectral imaging, proving its effectiveness in teaching key concepts.

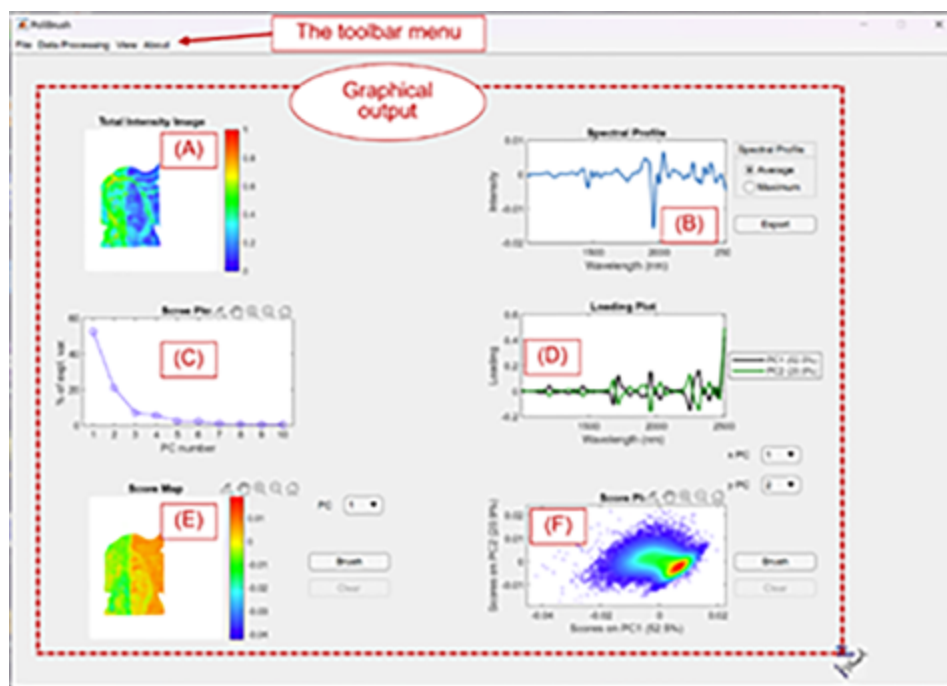


Figure 1: Main window of PoliBrush with graphical output panels: total intensity image (A), average/maximum spectral profile (B), PCA scree plot (C), loading line plot (D), score map (E), and score scatter plot (F).

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P3-001**ASSESSING *DAPHNIA MAGNA* CAPACITY FOR REMOVING SILVER NANOPARTICLES FROM WATERS IN PRESENCE OF MICROPLASTICS. LABORATORY STUDIES****Torrent, Laura; Villacis, Mary Isabel; Hidalgo, Manuela; Iglesias, Mònica; Marguí, Eva.***Department of Chemistry, Faculty of Sciences, University of Girona, C/ M. Aurèlia Capmany, 69, 17003 Girona, Spain.*

Nowadays, nanoparticles (NPs) are widely used in various commercial applications, leading to an increase of their presence in wastewaters. Moreover, they can interact with other contaminants present in the aqueous media such as microplastics (MPs). Both emerging contaminants can be persistent in the aquatic environment, posing a potential threat for living organisms and humans [1,2]. Existing conventional wastewater treatments are ineffective for completely removing these pollutants due to their persistence and characteristics [3,4]. Therefore, efforts are made to develop sustainable wastewater treatments for improving water depuration. In fact, previous studies have demonstrated the capacity of *Daphnia magna* (*D. magna*), employed as a tertiary water filtration system, to ameliorate water quality of reclaimed water, particularly the reduction of pharmaceuticals and, per- and polyfluoroalkyl substances [5]. Nevertheless, there is lack of information on the efficiency of *D. magna* in removing NPs from water in the presence of MPs. The aim of this work was to perform preliminary investigations at laboratory scale for evaluating the potential of *D. magna* to eliminate silver NPs (AgNPs) from water in the presence of MPs.

As a first step, NP and MP stability tests were performed considering the wastewater complex matrix. Wastewater contains humic acids, hence aqueous AgNP suspensions were prepared containing different concentrations of humic acids (0, 2, 4, 10 mg·kg⁻¹). After 24 hours, the suspensions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Similar silver concentrations were determined within the humic acid concentrations tested, thus 4 mg·kg⁻¹ was chosen. To assure MPs homogeneity in suspension for 24 hours, surfactant concentrations of 0.01, 0.05 and 0.1% were assessed considering the survival of *D. magna*. It was observed that at a surfactant concentration of 0.1% *D. magna* mortality increased considerably, hence 0.05% was the surfactant concentration selected.

As a second step, various filtration strategies were evaluated (cellulose acetate, nylon and glass wool) to remove the MPs from AgNPs suspensions before their analysis by ICP-MS. The filtration with glass wool showed higher AgNPs recoveries in comparison to the other two filtration approaches.

As a third step, *D. magna* were exposed to AgNPs and MPs for assessing their potential for eliminating these contaminants from waters. The survival of *D. magna* after 24 hours was lower when water contained MPs, indicating that their presence reduced *D. magna* capacity to survive. Moreover, the individuals were submitted to an acid digestion and the resulting digests were analyzed by ICP-MS. Results showed that *D. magna* accumulated more silver when they were exposed to AgNPs without the presence of MPs.

In conclusion, this contribution gives a first overview of the potential of *D. magna* for removing AgNPs from water in the presence of other contaminants. Furthermore, it has been demonstrated that the presence of MPs can play a role in the filtration efficiency of *D. magna* for AgNPs.

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P3-002**APPLICATION OF A ZVI-FUNCTIONALIZED BIOCHAR FROM ORANGE PEEL FOR THE ADSORPTION AND DEHALOGENATION OF PICLORAM****Vidal, Jorge¹; Godoy, Mariel¹; Sepúlveda, Pamela²; Báez, María³.***¹Universidad de Santiago de Chile, Santiago, Chile; ²Universidad Mayor, Santiago, Chile; ³Universidad de Chile, Santiago, Chile.*

The agricultural sector frequently relies on the use of pesticides to control pests and diseases in crops. However, excessive application of these chemicals poses significant environmental risks, such as contamination of aquifers through surface runoff or percolation¹. To mitigate this issue, the use of a biochar derived from orange peels and functionalized with zero-valent iron (ZVI) nanoparticles is proposed. This material is designed to simultaneously promote the adsorption and dehalogenation of the chlorinated herbicide picloram (PCM).

The study included the synthesis and characterization of the ZVI-functionalized biochar, along with kinetic and isotherm evaluations, desorption analyses, and tests conducted in the presence of different soil matrices. Results indicated that the adsorption kinetics followed a pseudo-second-order model and did not fit the intraparticle diffusion model, suggesting the involvement of multiple processes such as external and internal diffusion. Moreover, the adsorption process was best described by the Freundlich isotherm, indicating multilayer adsorption on a heterogeneous surface. Soil amendment with biochar significantly increased the distribution coefficient (K_d) of the soils, although the adsorption percentage decreased due to the matrix effect.

In conclusion, the functionalized biochar exhibited lower adsorption capacity compared to the unmodified biochar but successfully promoted contaminant dehalogenation, making it a promising material for the remediation of chlorinated pesticides in soils.

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P3-003**EVALUATION OF THE IMPACT OF CROP IRRIGATION WITH NATURE-BASED RECLAIMED WATERS: LABORATORY STUDIES**

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Climate change and population growth are increasing global water demand, requiring the optimization of water regeneration processes as an alternative for various uses, including agricultural irrigation. However, to meet the regulatory limits on water reuse, more intensive wastewater treatments—particularly tertiary treatment systems—are required. In this field, new alternatives to conventional wastewater treatment methods are being investigated through nature-based solutions, which represents a more sustainable strategy [1].

This study presents an experimental design using *Lactuca sativa* cultivated in a volcanic soil under irrigation with wastewater subjected to different tertiary treatments, both conventional and nature-based, to analyze the effects of different water sources on plant development [2]. Specifically, the study evaluated the use of two types of regenerated waters from nature-based tertiary treatment systems: the Daphnia Reactor, which employs *Daphnia magna* for microcontaminants filtration, and the Biosolar Reactor, which integrates sunlight and biofilm beneficial effects. Additionally, water from a conventional wastewater treatment plant using a sand filter and UV radiation as tertiary system was included for comparison.

Initially, the physicochemical characterization of the waters was performed according to the parameters established in RD.1620/2007. Although the regenerated waters complied with regulatory limits except for the amount of *Escherichia coli*, the one from the Daphnia Reactor exhibited a slightly lower nitrate concentration.

There were two phases of cultivation, each lasting for a period of over one month each. Morpho-physiological parameters were measured and then the lettuce leaves were harvested and dried for microwave-assisted digestion followed by Inductively Coupled Plasma Mass Spectrometry and Inductively Coupled Plasma Optical Emission spectroscopy analysis to determine the total metal content in these plant tissues. ANOVA analysis revealed no significant differences in leaf number of leaves, plant biomass (leaves and roots), root system architecture, plant water status, or photosynthesis-related parameters across the different irrigation treatments. This suggests that regenerated water does not adversely affect crop development. However, variations in metal absorption by lettuces were observed, with notable increases in Fe and Na in plants irrigated with regenerated water.

Soil parameters were also determined before and after the cultivation periods using standard methods [3], Energy Dispersive X-ray Fluorescence Spectroscopy and granulometry, to identify potential alterations in its natural properties.

The application of nature-based solutions in water treatment presents a promising approach to ensure safe and efficient water reuse while minimizing public health risks. This study underscores the importance of understanding the environmental implications of using regenerated water, evaluating its suitability for irrigation, and assessing the advantages and limitations of these treatment systems as a sustainable alternative for improving water quality.

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P3-004**DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE DETERMINATION OF FREQUENTLY (AB)USED PHARMACEUTICALS AND THEIR METABOLITES IN WASTEWATER**

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The consumption of pharmaceuticals is usually determined on the basis of sales statistics and prescription rates. However, the figures obtained using these methods may not be accurate, especially for drugs that are frequently abused, such as anxiolytics, hypnotics, sedatives and erectile dysfunction drugs. The alternative approach, wastewater-based epidemiology (WBE), is now recognized as an objective, cost-effective and timely method that involves the chemical analysis of specific human biomarkers in municipal wastewater. WBE has been widely accepted by health authorities worldwide to assess illicit drug use, but is increasingly being used to assess the consumption of licit drugs and other substances.

The aim of this research is to develop a method for the determination of selected pharmaceuticals and their metabolites in municipal wastewater. The method will include more than 30 compounds – several benzodiazepines, zolpidem, zopiclone, methylphenidate and some phosphodiesterase type V inhibitors, with a focus on metabolites and conjugates. The target compounds will be analyzed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), with prior enrichment by solid-phase extraction (SPE).

The first step in method development was to optimize the parameters of chromatographic separation and mass spectrometric detection using Multiple Reaction Monitoring (MRM). This detection mode involves the selection of at least two specific transitions for each compound and the optimization of a number of parameters to achieve the highest possible sensitivity and selectivity required for the reliable determination of traces of the target compounds in municipal wastewater. All analytes were ionized in positive polarity on a triple quadrupole mass spectrometer. The chromatographic conditions were optimized by varying the column type, mobile phase composition and gradient. HSS T3 and BEH C18 columns were tested as stationary phases and the different mixtures of polar solvents as mobile phases. The gradient was optimized by varying the initial conditions and the gradient slope. Optimal separation was achieved on an HSS T3 column with 0.1% formic acid and acetonitrile, and the total run time was 11 minutes.

The solid-phase extraction conditions were optimized by varying the sorbent type, extraction pH and sample volume. Different columns were tested, including Oasis HLB, Oasis HLB Prime, Strata-X, Oasis MCX and Strata-X-C, with either one-step or sequential elution (methanol + 0.5 % ammonia solution in methanol). The final method will be used to assess the consumption of selected pharmaceuticals in cities from six Croatian regions using the WBE approach. These data will also be compared with the official data on pharmaceutical consumption in Croatia.

P3-005**CHEMICAL CHARACTERIZATION AND BIOLOGICAL IMPACT OF PM₁₀ AIR POLLUTANTS: LINKING ANALYTICAL METHODS TO NEUROTOXIC OUTCOMES**

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Air pollution is a significant environmental risk to human health, causing around 4.2 million premature deaths annually due to cardiovascular, respiratory, and cancer-related conditions. Despite this, few studies have explored the neurotoxic effects of atmospheric pollutants, even though fine particles (PM_{2.5} and PM₁₀) reach the brain through the bloodstream or olfactory bulb, leading to neuronal damage, oxidative stress, and neurodegenerative diseases like Alzheimer's and Parkinson's [1].

This study investigates the neurotoxic effects induced by atmospheric pollutants extracted from PM₁₀ filters collected at various air quality stations in Catalonia, including rural background location of Bellver and suburban locations like Manlleu and Mollet del Vallès. Neurotoxicity was studied using SH-SY5Y neuroblastoma cells to assess cell viability, reactive oxygen species (ROS) production, xenobiotic stress response (XRE) activation, and acetylcholinesterase (AChE) inhibition. Neurocortical brain organoids were used for lipidomics to investigate lipid involvement in the neurotoxic mechanisms. The chemical composition of the filters was analyzed via GC-MS and ICP-MS, identifying thirty organic compounds, including biomarkers for biomass burning (e.g., levoglucosan, mannosan) and incomplete combustion products (e.g., polycyclic aromatic hydrocarbons). Winter samples had distinct chemical profiles, separating them from other seasons due to increased biomass burning and reduced atmospheric dispersion.

Biological analysis showed a concentration-dependent reduction in cell viability, with extracts from Bellver and Manlleu being the most toxic. Oxidative stress was notably observed, characterized by increased ROS production and XRE activation in cold-season samples. Additionally, pollutants inhibited AChE activity in warm-season samples, which could disrupt neuronal signaling. Seasonal patterns revealed that cold-season samples caused more neurotoxicity, correlating with higher pollutant concentrations.

Brain organoids were used as three-dimensional model to better replicate the human brain's complexity. Lipid profiling revealed seasonal variations in membrane composition. These lipid alterations suggest adaptations in membrane stability, signaling, energy regulation, and neuroprotection. Interestingly, changes were observed in lipids such as gangliosides, which play a critical role in neuroprotection and development, emphasizing the importance of such adaptations in maintaining neural integrity and functionality [2]. These findings provide valuable insights into the biochemical mechanisms linking air pollution to neurotoxicity and neuronal dysfunction.

The joint analysis of chemical composition and the biological effects observed, provided information about the relationship of some pollutants or sources of pollution with the neurotoxic outcomes. This study underscores the importance of interdisciplinary research by integrating chemical analysis and biological models to better understand and address the neurotoxic risks of air pollution.

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P3-006**DEVELOPMENT AND VALIDATION OF A NEW SEMI-EMPIRICAL DEPOSITIONAL MODEL TO ESTIMATE THE CONCENTRATION OF BLACK CARBON DEPOSITED ON SNOW**

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A new semi-empirical depositional model (SEDM) is proposed to estimate the concentration of black carbon (BC) deposited on snow, based on in-situ measurements of atmospheric BC and critical meteorological variables in the Chilean central Andes (ChicA).

BC is an atmospheric aerosol emitted during the incomplete combustion of fossil fuels, biofuels, and biomass. It is currently the second most important climate change (CC) agent after CO₂, due to its high absorption capacity of solar radiation. BC is deposited on the cryosphere, modifying the albedo of snow and ice, accelerating melting and glacier retreat, and decreasing the seasonal snow accumulation capacity. This has important hydrological consequences for regions where snowmelt is key to the water balance, such as Chile, which has the largest portion of the Andean cryosphere, strongly affected by CC, decreasing water availability in the country. Currently, the effects of BC on the Andean cryosphere are not considered in climate and hydrological models, mainly due to the scarcity of observations and detailed knowledge of the variables and processes involved. Considering the high difficulty and cost of collecting snow samples to measure BC concentrations in large areas of the cryosphere, and given that BC measurements in air can currently be performed with easy-to-operate and more affordable automated equipment, the implementation and validation of a BC SEDM was proposed.

Currently, there are no experimentally validated depositional models for BC that incorporate and couple dry (DD) and wet (WD) deposition of these aerosols on snow and ice. This research integrated air and snow BC sampling campaigns using the NUNATAK Laboratory-Shelter, located in Portillo (3,000 masl), Andes Mountains, belonging to CETAM-UTFSM, the processing of these samples in the laboratory and statistical analysis of the atmospheric variables measured in situ, to develop, calibrate and validate the new BC SEDM. The model was designed using MATLAB software and programming language and allows calculating BC concentration in snow from the BC concentration in air and critical meteorological variables (pressure, humidity, temperature, etc.), incorporating semi-empirical mathematical equations, i.e., equations developed from theory and empirical observations, associated with WD and DD processes. The model is capable of calculating the rate of BC removed from the atmosphere due to WD or DD, with high temporal resolution (minutes). Finally, the model was validated under field conditions with field data from 2022-2024, verifying its performance.

An important finding was that, during precipitation events, only 3.2% of the BC present in the atmosphere actually manages to deposit on the snow and be detected in the samples, while the remaining 96.8% is retained in the clouds. When testing the model with data from 2024, it was observed that the simulated BC concentration in snow presented an average difference (underestimation) of 64% with respect to the values measured in snow samples from Portillo, representing a notable improvement compared to current international models, which underestimate actual data by between 200% and 300%.

This model allows easy-to-install and operate airborne BC monitoring instruments to determine BC concentrations in snow, compared to sampling snow in the field and subsequently processing them in the laboratory. The SEDM can be coupled with radiative transfer models such as SNICAR, which are used to calculate albedo and radiative forcing in snow and ice with high precision and temporal resolution. This allows for estimating snow and ice melt rates in a catchment with greater temporal resolution and accuracy, allowing for improved hydrological forecasts.

Acknowledgements

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P3-007**CALIBRATION OF CERAMIC PASSIVE SAMPLERS FOR THE MEASUREMENT OF CONTAMINANTS OF CONCERN IN WELLS AND RIVER WATERS****Moro, Giacomo; Santana Viera, Sergio; Pérez, Sandra; Lacorte, Silvia.***Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18, 08034 Barcelona, Spain, Barcelona, Spain.*

Contaminants of emerging concern (CECs) are pollutants that are not yet fully regulated, but are susceptible of causing environmental and health effects, often due to their environmental persistence and ubiquity [1]. This calls for the development of new techniques to measure their concentration in environmental samples such as groundwater and rivers. Measuring them through grab sampling poses limitations, as it is a punctual measurement and therefore vulnerable to fluctuations in concentration. [2]

Passive samplers, on the other hand, are continuously exposed to water contaminants for a period of days, weeks or months. They are an attractive alternative as they offer a time-averaged concentrations of multiple contaminants. Well-established passive sampling devices such as POCIS exist in the market. [3] Alternatively, ceramic passive samplers (CPSs) can be used. [4] These are ceramic cylinders with high porosity: contaminants diffuse across the ceramic membrane and are retained on a sorbent material placed inside.

However, in order to obtain a meaningful measurement, their rate of uptake needs to be linear, and it needs to be determined for each analyte. With such calculated rate, the total amount of contaminant accumulated can be converted into an estimated concentration of the contaminant in the sampled water. [5]

In this work, the absorption and diffusion of 56 CECs was studied. 20 MCPSs were submerged in water spiked with the studied CECs at a concentration representative of the higher range of typical river- and groundwater concentrations (0.5 µg/L). 2 CPSs were sampled and extracted every 2-3 days, for a duration of 20 days. As a result, the majority of the compounds showed linear absorption and linear diffusion through the ceramic wall, and are therefore calibrated. In parallel, CPSs were deployed in 12 wells and 2 river locations in the catchment area of the river Besòs in Barcelona, Spain, for a period of 2-3 weeks. The concentration of linearly-absorbing CECs in these sampling locations was estimated by using the table with diffusion coefficients (D_e) and rate of uptake (R_s) resulting from the calibration experiment.

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P3-008**ANALYTICAL APPLICATIONS BASED ON INORGANIC As(V) AND As(III) ADSORPTION BY Fe-based MOFs AND EDXRF**

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Arsenic contamination of water endangers the health of millions of people worldwide, affecting certain countries and regions with particular severity. Arsenic toxicity is related to its oxidation state: in natural waters the inorganic species arsenate As(V) and arsenite As(III), which is 60 times more toxic than As(V), are particularly common. The stability and adsorptive properties of Fe-based metal organic frameworks (MOFs), such as iron-1,3,5- benzenetricarboxylic (Fe-BTC) MOFs, make them suitable for the effective sorption of arsenic species. The sorption performance to As(V) and As(III) of Basolite® F300 and Nano-{Fe-BTC}, synthesized following green chemistry principles, was compared in water. Sorption efficiencies of over 90% for As(V) were found for both Fe-BTC MOFs, Nano-{Fe-BTC} showed its maximum adsorption capacity at pH 2 whereas for Basolite®F300 this was at pHs > 6.5. As(III) species, on the other hand, could not be adsorbed at pHs < 8.

The different effects of the pH on the sorption performances of As(III) and As(V) are attributed to the their speciation. This fact was used to determine the different inorganic As species in water samples. The water matrix effect on the extraction efficiency was studied, resulting in adsorption efficiencies >90% for As(III) at pH 8 whereas at pH 6.5 it was not adsorbed. However, in the case of As(V) using Basolite®F300, efficiencies of >90% were obtained allowing separation factors of » 100% between arsenate and arsenite to be achieved at acidic pHs.

Taking into account the good adsorption performance of both Fe-BTC MOFs in terms of efficiency and equilibrium time (1 hour), they were tested as preconcentration sorbents for the determination of As by ICP-OES. However, the efficiency of the desorption using different acidic solutions was low. To overcome this problem, X-Ray Fluorescence (XRF) was evaluated as an alternative given that the As adsorbed on the Fe-BTC MOFs could directly be determined.

The procedure consisted of the adsorption of As species on few mg of the solid sorbent (Fe-BTC MOFs) dispersed in the aqueous sample. After the dispersive micro solid-phase extraction process, the loaded sorbent was collected onto a filter and directly measured by Energy-Dispersive XRF (EDXRF). The effect of various experimental parameters such as the amount of sorbent (2-5 mg), aqueous sample volume (25-50 mL) and type of sample holder (paper filter, glass filter and cellulose nitrate filter) were investigated. The homogeneity of As and Fe of the loaded material on the filters was also tested by μ -XRF analysis. As and Fe mappings were created using a grid of 10x10 points, a focal spot of 1mm and a measurement time of 100 seconds/point).

EDXRF measurement conditions were also selected to obtain the best sensitivity for As determination (50kV, 500 μ m Al filter, 200 seconds). It is interesting to mention that the presence of Fe in the MOF did not interfere the signal of As and iron was used as internal standard in the As determination.

Seven aqueous standard solutions containing As (V) in the range of 5 to 200 μ g/L were analyzed at the best conditions and used for calibration purposes. Results obtained showed a good linearity for both Fe-BTC MOFs in the studied concentration range. The precision of the methodology was evaluated in terms of relative standard deviation (RSD) of duplicate analysis of aqueous solutions at different initial As(V) concentrations and, in all cases, RSDs values calculated were less than 10%. Limit of quantification obtained for both adsorbents met also the requirements for the regulation of drinking water.

Finally, the developed method was applied in the analysis of spiked well water samples obtaining quantitative recoveries that confirmed the good analytical performance of the developed method.

P3-009**ASSESSMENT OF THE IMPACT OF ROAD TRAFFIC AND INDUSTRY ON THE CONTENT AND SPATIAL DISTRIBUTION OF MERCURY IN SOIL SAMPLES FROM THE LODZ REGION**

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Urban soils accumulate pollutants, such as toxic elements, from various anthropogenic sources [1]. Among these, mercury (Hg) is of a particular concern because of its toxicity, persistence in the environment and bioaccumulation capacity. With the increase in production and industrial use of mercury, mercury pollution has become an increasingly serious problem. Mercury can enter the food chain, leading to serious health consequences. Natural sources of mercury in the atmosphere are the burning of forests or the process of weathering of rocks. In contrast, industrial activities including ore processing, cement production, coal combustion or vehicle emissions of mercury associated with fuel-type petroleum products are among the main anthropogenic sources of Hg release to the atmosphere. The increase in mercury emissions over recent decades has contributed to its global spread, and the problem was tragically demonstrated in the Minamata disaster of 1956, where the consumption of contaminated fish led to numerous deaths and serious neurological disorders. In response to the scale of the threat, The Minamata Convention was established to limit globally mercury emissions and use its globally. Poland joined the signatories of this agreement in 2014 and ratified it in 2021 [2,3]. In the European Union, mercury emissions are estimated to be around 80 tons per year, representing less than 5% of global mercury emissions. In Poland, according to the Global Mercury Assessment report for 2015, annual mercury emissions were at 11.387 kg, of which 9409 kg came from coal combustion and 881 kg from cement production [4], while according to a report from the National Balancing and Emissions Management Centre in 2022, total mercury emissions decreased to about 6 tons [5].

The aim of the study was to assess the changes of Hg levels within 5 year period of time. The results of Hg content in surface soil collected from the same sampling points obtained in 2020 and in 2025 were compared and graphically visualized. The study material consisted of 480 samples collected over five years from 80 measurement points distributed around the city. The samples were prepared according to a standardized procedure: drying, sieving (2 mm) and then homogenization in an agate mortar. Total mercury content was determined by the cold vapor of atomic absorption spectroscopy technique using an automatic mercury analyzer, the MA-3000, from NIC. The influence of different parameters, such as road type or soil on mercury levels, was verified by statistical tests using Statistica software. Data were also spatially interpreted and visualized using a Geographical Information System (GIS), and maps were generated using Esri ArcGIS Pro 3.3.2 software (Esri Polska sp. z o.o., Warsaw, Poland).

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P3-010

THE EFFECT OF AGEING ON THE QUALI-QUANTITATIVE DETERMINATION OF MICROPLASTICS BY ANALYTICAL PYROLYSIS

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Microplastics (MPs) are increasingly recognized as widespread and persistent contaminants, raising concerns for both ecosystems and human health. Once released in the environment, they undergo ageing driven by UV exposure and other environmental stresses that lead to physical and chemical changes affecting both their environmental behavior and analytical determination. This study investigates the effects of ageing by UV irradiation on MP (< 120µm) of polymers representative of non-polar polyolefins (PS and PVC) and polar condensation polymers (PA66, PET). Virgin and aged particles were characterized by FTIR, Py-GC-MS, and EGA-MS. The effect of ageing on the performance of Py-GC-MS was investigated considering linear regression, precision, and recoveries following Fenton pre-treatment for the oxidation of organic matter.

Thermal profiles by EGA-MS remained unchanged for PET and PVC after ageing. In contrast, aged PS showed a slight decrease in thermal stability, while PA66 exhibited notable changes in curve shape and peak temperatures, highlighting important structural alterations.

Qualitative Py-GC-MS analyses showed no significant differences in the pyrogram profiles of virgin and aged PET, PS, and PVC, instead, loss of the pyrolytic marker hexamethylenediamine was observed for PA66, indicating some structural modification in accordance with EGA-MS. Identification of all the polymers was not affected by aging when pyrolysis conducted under thermal hydrolysis/methylation with tetramethylammonium hydroxide (THM-TMAH).

Quantitative analysis by THM-TMAH showed changes in calibration parameters and statistical significance for PS and PVC. Ageing influenced recoveries of MP of all the polymers, both decreasing (PA66 and PVC) and increasing (PET and PS) trends. The process also had an impact on precision.

In conclusion, THM-TMAH markers for polymer identification were maintained, while analytical sensitivity, recoveries, and precision, were affected to different degrees for various polymers.

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P3-011**QUANTIFICATION AND CHARACTERIZATION OF UV FILTERS IN COASTAL SEAWATERS OF ADRIATIC BEACH ENVIRONMENTS**

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UV filters are a class of emerging contaminants (ECs) that are widely used in personal care products and have been detected at low concentrations ($\mu\text{g L}^{-1}$ - ng L^{-1}) in aquatic environments. Detection of contaminants in the natural waters is a critical step to develop precise environmental risk assessment strategies for ECs. However, the diverse origins and varying physicochemical properties of these compounds make their detection complicated. Moreover, their occurrence in the aquatic environments at trace concentrations requires sensitive and selective analytical procedures.

In this study, a solid-phase extraction (SPE) method coupled with GC-MS/MS was optimized for the favorable determination of 12 UV filters of different chemical natures in seawater samples. Recovery studies were conducted demonstrating the suitability of the method. The method was validated in terms of linearity, accuracy, and precision with limit of detection (LOD) in the low ng L^{-1} range. The method was applied to the analysis of 14 seawater samples collected from Rimini and Riccione beaches during peak recreational periods. In total, 12 targeted UV filters were detected, among which the most frequent were ethylhexyl salicylate (EHS), homosalate (HMSa), octocrylene (OCR), trans-ethylhexyl methoxycinnamate (EHMC), and its transformation product cis-EHMC. Higher concentrations of the transformation product cis-EHMC in natural environments are likely due to photochemical degradation. This study provides for the first time the occurrence of sunscreen UV filters in the coastal waters of Adriatic Sea confirming the widespread occurrence of these ECs and contributing to better understanding of their exposure for environmental risk assessment.

P3-012**EXPRESS DETERMINATION OF AVIATION FUEL IN SOIL BY GC-MS COUPLED WITH SPME: GREENNESS ASSESSMENT VIA GAPI****Alimzhanova, Mereke; Abdykarimov, Bauyrzhan.***Al-Farabi Kazakh National University, Almaty, Kazakhstan.*

Aviation fuel contamination in soil resulting from aircraft and rocket incidents poses significant environmental and forensic challenges. In the last 5 years 1090 aviation incidents have occurred all over the world [1].

Conventional analytical methods for quantifying aviation fuel contamination typically require extensive sample preparation, long analysis times, and involve the use of toxic solvents, which conflict with green analytical chemistry principles. In response, this study developed an express analytical method utilizing gas chromatography-mass spectrometry (GC-MS) coupled with solid-phase microextraction (SPME) for the rapid, sensitive, and environmentally sustainable detection [2] of aviation fuel in soil samples.

Optimization of critical parameters, including extraction temperature (90°C), extraction time (10 min), fiber type (PDMS 100 µm), and sample preparation conditions, significantly reduced analysis time and eliminated the need for chemical solvents. The developed method demonstrated robust analytical performance, exhibiting a detection limit (LOD) of 1.27 µg kg⁻¹ and a quantification limit (LOQ) of 4.24 µg kg⁻¹, with relative standard deviation (RSD) values of 2.21%. [3].

Environmental sustainability of the analytical procedure was assessed using the Green Analytical Procedure Index (GAPI) [4]. Results indicated excellent environmental compatibility, highlighting significant advantages in terms of reduced solvent use, minimized waste production, and decreased energy consumption compared to traditional extraction methods. Thus, the GC-MS coupled with SPME approach emerges as an efficient, green, and highly reliable analytical technique suitable for routine environmental monitoring and forensic analysis of aviation fuel contamination in soil matrices [5].

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P3-014

METHOD DEVELOPMENT FOR THE DETERMINATION OF GBCA'S (GADOLINIUM-BASED CONTRAST AGENT, DOTAREM) IN VEGETABLE SAMPLES

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The current global drought crisis is increasing the need to reuse water in agriculture.

However, wastewater treatment plants are unable to fully eliminate certain contaminants, including gadolinium-based contrast agents (GBCA's) such as Dotarem used in (MRI) Magnetic Resonance Imaging [1]. As a result, these compounds persist in irrigation water and may accumulate in crops. This highlights the necessity of developing analytical methodologies to detect and quantify their presence in plants.

The present study focuses on the determination of Dotarem in escarole and tomato samples that have been grown in a greenhouse. The irrigation water was fortified at two concentration levels (0.5 and 5 ppb), and samples were analysed after three months of irrigation. A water-based extraction method was optimized and validated using escarole and tomato fortified samples at an equivalent concentration of 5 and 25 ng g⁻¹. Quantification was performed with an LC-ICP-MS system. Separation was achieved using a Hamilton PRP X100 anion exchange column, with a mobile phase consisting of NH₄NO₃ and EDTA at pH 9.4.

The method showed satisfactory recoveries and RSD values below 20% in most cases.

No significant matrix effects on signal response or changes in retention times were observed. The results obtained for escarole and tomato provide insight into the potential accumulation of dotarem in crops. Preliminary observations indicate that the amount detected in plant samples increases with the concentration of Dotarem in the irrigation water.

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P3-015**DETERMINATION OF PHARMACEUTICALS IN SEDIMENT SAMPLES BY MOLECULARLY IMPRINTED MATRIX-SOLID PHASE DISPERSION EXTRACTION TECHNIQUE COUPLED WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**

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The technology of molecularly imprinted polymerization (MIP) has been developed for decades to increase the sensitivity and selectivity of analytical methods in various fields of application. One of the recognized global problems is the limited water resources that are disrupted by various small, invisible molecules that have short- and long-term effects on living organisms in the environment. Pharmaceuticals are among the micropollutants that have been present in various parts of the environment (water, soil, sediment) for many years. Advances in the continuous development of new analytical techniques and equipment allow the detection of this type of micropollutants even at trace levels. In this work, the focus was on a solid sample, specifically sediment, contaminated with twelve different pharmaceuticals for which a MIP-MSPD-HPLC-DAD method was developed and optimized for analysis. A matrix solid-phase dispersion was combined with an imprinted polymer to increase the selectivity of the target molecules and minimize the matrix effect. The sediment sample taken from the area of the Republic of Croatia, the C18 sorbent, and the MIP were mixed and packed into a cartridge. MIP was prepared from torasemide as the template molecule and methacrylic acid as the monomer unit, and the polymerization reaction was supported by the addition of ethylene glycol dimethacrylate as the crosslinker, and azobisisobutyronitrile as the initiator (60 °C, 24 h). The MIP-MSPD-HPLC-DAD method was used to determine the optimal ratio between the sorbents, where 50 mg of each (sediment, C18, and MIP) gave the best efficiency for the mixture of twelve pharmaceuticals, eluted with 5 mL of solvent consisting of 0.1M NaOH and methanol in a ratio of 2:8 (v/v). In addition, validation experiments were performed to determine the suitability of the optimized method – by examining linearity, repeatability, and reproducibility as well as the matrix effect. Finally, the MSPD-HPLC-DAD method was successfully applied on another real sediment sample.

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P3-016**MULTI-TEMPLATE MOLECULARLY IMPRINTED POLYMER FOR SORPTION OF SULFAMETHOXAZOLE, TORASEMIDE AND PROCAINE****Tolić Čop, Kristina¹; Čolo, Ivana²; Petek, Karla²; Mutavdžić Pavlović, Dragana².**

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Pharmaceuticals are active substances synthesized to cure, prevent or treat various diseases in human and veterinary medicine. In addition to their benefits to society and global industry, their enormous production and consumption have led to negative environmental impacts. Pharmaceuticals enter the environment both in unaltered form and in the form of metabolites through various routes (including households, improper disposal, industry, hospitals), with the highest yield in wastewater treatment plants, which are generally unable to remove these compounds. Pharmaceuticals are among the emerging pollutants that are mostly unregulated but whose effects on living organisms are of increasing concern, even at low concentrations. Many of these substances are already being detected in various areas of the environment, including soil, groundwater, surface water and even drinking water supplies. Advances in analytical techniques over the years have enabled the detection and identification of these small molecules, whose trace levels in complex matrices make them difficult to analyze. Therefore, analytical methods are often combined with various extraction techniques. In order to increase the selectivity and sensitivity of the methods, it is necessary to develop new types of sorbents in addition to commercially available ones, which facilitate the analysis taking into account the above-mentioned aspects. One such technology that has been frequently mentioned is the preparation of molecularly imprinted polymers (MIPs), which are designed to form specific bonds with formed cavities whose dimensions correspond to the molecule of interest. This type of sorbent can effectively remove not only the template molecule but potentially other impurities present in the sample. In this work, MIP was prepared by bulk polymerization using three different template molecules to create a sorbent with multiple recognition sites. The molecules of torasemide, procaine and sulfamethoxazole were mixed in a batch with methacrylic acid as monomer unit, ethylene glycol dimethacrylate as crosslinking agent and azobisisobutyronitrile as initiator, dissolved in DMSO and polymerized at 60 °C for 24 hours. After washing the templates and grinding them into small particles, MIP was ready for sorption experiments. The sorption capacity of MIP towards a pharmaceutical mixture was evaluated through material characterization and batch sorption studies by investigating the effects of initial pollutant concentration, pH and sorbent dosage. All experiments were performed simultaneously for the non-imprinted polymer (NIP). In addition, the optimal eluent for both polymers was determined, followed by regeneration experiments to test the reusability of the materials. Besides calculating the imprinting factor for each pharmaceutical, the β -selectivity was also estimated based on a sorption experiment for the structural analogs of sulfamethoxazole, torasemide and procaine (sulfamethazine, furosemide, and lidocaine), showing the expanded possibilities of applying MIP not only to target molecules. Finally, the applicability of the synthesized MIP and NIP is tested using solid-phase extraction and real wastewater samples. The experimental sorption data best fitted the linear, Freundlich and Langmuir isotherm with correlation coefficients, R^2 higher than 0.92. The results showed better sorption affinity of pharmaceuticals on MIP than on NIP, with the efficiency occurring in the following order: sulfamethoxazole>torasemide>procaine.

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P3-017**DETERMINATION OF CONTAMINANTS OF EMERGING CONCERN IN RECLAIMED WASTEWATER FROM THE CANARY ISLANDS**

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The Canary Islands are characterized by arid conditions and severely limited water resources, posing significant challenges for agricultural practices. Among the seven islands, Lanzarote, and Fuerteventura are the most affected by water scarcity. In this context, the use of reclaimed wastewaters (RWWs), originating from treated domestic and industrial effluents, has emerged as a viable alternative for crop irrigation. However, this practice may introduce potential risks that should be assessed, including the presence of microorganisms and diverse chemical contaminants.

Contaminants of emerging concern, also known as emerging contaminants (ECs), are mainly organic compounds, such as pesticides, pharmaceutical and personal care products (PPCPs), hormones, phenols, and flame retardants, among others. The widespread occurrence of ECs in water sources may cause adverse effects on human health, especially considering that water is one of the main exposure routes.

The aim of this work was the development and validation of an analytical method to determine 29 ECs, including antibiotics, pesticides, pharmaceuticals, and related compounds in RWWs. The method was based on automated solid-phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS).

Twenty RWWs were collected per sampling campaign from five agricultural sites located on four islands (Tenerife, Gran Canaria, Lanzarote and Fuerteventura), comprising a total of 80 RWWs samples. Additionally, water samples from different wastewater treatment plants (WWTPs) in each island were also collected during the last two sampling periods, comprising a total of 15 WWTPs water samples, to compare contamination profiles between both types of water.

The obtained results revealed consistent EC contamination profiles across the islands, highlighting the need for ongoing monitoring efforts to ensure the safe reuse of RWWs in agriculture.

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P3-018**PRESENCE OF PHARMACEUTICALS, PFASs, AND METALS IN SURFACE AND GROUNDWATER OF THE PALANCIA RIVER BASIN: A HYDROGEOCHEMICAL AND ENVIRONMENTAL RISK APPROACH**

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The increasing presence of contaminants of emerging concern (CECs) and trace inorganic elements (metals) in aquatic systems poses a threat to water quality and ecosystem health. In this context, a hydrogeochemical study was conducted in the Mediterranean basin of the Palancia River, a semi-arid region subjected to agricultural and urban pressures. The aim was to characterize pharmaceuticals, PFASs, and metals of both geogenic and anthropogenic origin. Additionally, physicochemical and hydrogeochemical parameters were evaluated to understand the factors influencing the distribution and mobility of these compounds. To achieve this, a network of 40 sampling points was established in surface and groundwater, including environmentally sensitive areas such as coastal marshes.

Samples were processed using solid-phase extraction (SPE) and analyzed by High-Performance Liquid Chromatography with High-Resolution Mass Spectrometry (HPLC-HRMS/MS) for CECs. Metals were extracted using microwave-assisted acid digestion, following the method U.S. EPA 3052 and total of concentration was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Preliminary results reveal the presence of compounds as tramadol with concentrations up to 71.5 ng/L in surface waters, and PFBS (a short-chain PFAS) detected in both surface (29.5 ng/L) and groundwater (36 ng/L), indicating active anthropogenic pressure and high mobility. Inorganic elements showed levels consistent with the geological background, highlighting strontium as a geogenic tracer, with values up to 6334 µg/L, related to the evaporitic geology of the area.

Ecological risk indices (RQs) and hydrogeochemical indicators will be applied to identify areas of greater environmental vulnerability. These results will allow for an initial diagnosis of the chemical quality status of the basin and will serve as a foundation for the development of strategies aimed at the sustainable management of water resources in areas under high anthropogenic pressures.

Keywords: Contaminants of emerging concern, PFASs, pharmaceuticals, metals, groundwater, ecological risk, HPLC-HRMS/MS.

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P3-019**INNOVATIVE WATER SAMPLING STRATEGY FOR THE SIMULTANEOUS SAMPLING AND SAMPLE PREPARATION FOR CADMIUM QUANTIFICATION****Ribas, Tânia; Rodrigues, Francisco; Mesquita, Raquel; Rangel, António.***Universidade Católica Portuguesa, Porto, Portugal.*

The content of certain metal ions in aquatic ecosystems has emerged as an environmental concern, posing significant risks to both aquatic life and human health. Some metal ions, including cadmium, are usually present at a trace level in environmental waters. Nevertheless, even at low exposure levels, cadmium can lead to serious health problems. Its increasing presence in natural waters is largely attributed to its widespread use in products such as batteries and electronic components. Consequently, regular monitoring of water quality is crucial for effective pollution control.

Traditional methods for metal ions assessment in environmental waters usually involve bottle sampling, the addition of some preservatives, and sample treatment, and analyte quantification. However, the sampling and sample preparation procedures can compromise sample integrity due to factors such as temperature variation, UV radiation, oxygen exposure, changes in pH, use of organic solvents, microbial activity, among others. Consequently, sample degradation can introduce uncertainty in the analyte. Besides the potential sample degradation, these processes may increase the time required for analysis and the complexity of the process.

Solid phase extraction (SPE) is one of the widely used techniques for sample preparation, aimed at sample matrix clean-up and/or analyte enrichment. This work presents a novel SPE-based method that integrates both sampling and sample preparation into a single step for cadmium quantification in freshwater. This was achieved by devising a portable column packed with Chelex-100 resin, a weak cation exchange resin that offers high selectivity towards metal ions. For sample collection, the water passes through the column, using a syringe, and, during this step, the metal ions are retained and the water matrix discarded (matrix clean-up). Subsequently, a lower volume of eluent is used to recover the metal ions, promoting in this way the enrichment of the analyte for further analysis (ICP or AAS). This approach takes advantage of the resin's properties to enrich cadmium, which is typically present in concentrations between 3 and 5 $\mu\text{g L}^{-1}$, often below the limit of detection of several traditional methods.

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P3-020**COMBINATION OF SPE AND pH ADJUSTMENT FOR THE SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF COPPER, ZINC AND MANGANESE IN WATER****Ferreira, Francisca; Ribas, Tânia; Mesquita, Raquel; Rangel, António.***Universidade Católica Portuguesa, Porto, Portugal.*

Copper, zinc, and manganese are commonly found in both natural and treated water sources. Although these metals are essential micronutrients, their accumulation, even at low concentrations, can lead to serious health issues. Therefore, continuous and accurate monitoring of these elements in environmental and drinking water is crucial to ensure public safety.

Solid Phase Extraction (SPE) is a widely used sample preparation technique that relies on sorbent materials to separate and concentrate specific analytes from complex matrices. In heavy metal analysis, SPE offers several advantages, such as enhanced sensitivity, selectivity, and compatibility with automated systems. Ion-exchange resins selectively bind to ions based on their charge and therefore are one of the most effective sorbents for metal ion separation. While cation-exchange resins retain positively charged metal ions like Cu^{2+} , Zn^{2+} , and Mn^{2+} , anion-exchange resins bind to negatively charged species. This selectivity allows for cleaner separation and more accurate quantification in multi-parametric analysis.

The objective of this work was to develop an automatic sequential injection analysis (SIA) system integrated with an in-line cation-exchange resin column (Toyopearl) for the simultaneous determination of copper (II), zinc, and manganese in water samples. This system employs a cationic resin coupled with a colorimetric reagent (Zincon) whose selectivity varies with pH, enabling the separation of target metal ions and consequent individual quantification. This method presents a reliable and reagent-efficient alternative to conventional techniques, with strong potential for real-time or on-site monitoring applications.

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P3-021

METHODOLOGIES FOR ULTRASHORT-CHAIN AND COMPREHENSIVE PFAS ANALYSIS IN WATER SAMPLES

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Abstract: Per- and Polyfluoroalkyl substances (PFAS) have been a trending global issue for a number of years now. As more research is being carried out on these compounds, their breakdown paths, and their bioavailability, additional methods are required to quantify at lower concentrations, and to look for novel chemistries. As this search has expanded, shorter chain, and particularly ultrashort chain (USC) PFAS with carbon backbone lengths shorter than C4 have become areas of focus. In the most recent EU Commission Notice for Drinking Water (C/2024/4910) the fact TFA was not included in their definition of "PFAS Total" is highlighted as potential cause for concern, and both TFA and USC PFAS more generally are highlighted as areas for more specific monitoring.

In this poster we examine two separate approaches to examining USC PFAS compounds. The first is a comprehensive look at a panel of PFAS compounds (C1-C14) with a dilute and shoot method using an inert coated, polar embedded alkyl LC column. The second is a more targeted screen via direct injection, focussed upon USC PFAS utilising a novel HILIC/ion exchange LC column. These methods were then confirmed utilising a variety of fortified potable and non-potable water samples (industrial effluents, along with bottled, tap and spring waters). Both workflows demonstrate accuracy, sensitivity and linear calibration ranges of ppt levels, within industrially acceptable recovery values (70-130%).

P3-022

MONITORING AND PREDICTING HEAVY METAL CONTENTS OF URBAN ROAD DUSTS: METHODOLOGY AND ANALYTICAL INSIGHTS IN SUPPORT OF GEOSPATIAL MODELS

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With the continued expansion of the global urban population, the monitoring of urban environments has become increasingly critical to public health. Road dust, composed of fine solid particles deposited on road surfaces, represents a key vector for airborne pollutants. The resuspension of these particles—particularly those in the finer fractions—can lead to elevated concentrations of heavy metals in the ambient air.

In the municipality of Anderlecht (Brussels-Capital Region, Belgium), extensive street residue collection campaigns were conducted annually between April and September from 2019 to 2021. A standardized sampling protocol was employed, whereby a one-meter square stencil was used to delineate a rectangular surface area of 6 m² (2 m × 3 m), with the longer edge aligned along the interface between the roadway and the gutter. This area was exhaustively cleaned using an industrial-grade filtered vacuum system to recover both fine and coarse particulate matter. The collected material was subsequently subjected to granulometric processing (sieving) to isolate the <250 µm fraction for further analysis.

An initial compositional characterization was conducted using X-ray fluorescence (XRF) and X-ray diffraction (XRD) spectroscopy. XRF enabled the quantification of major and trace elements, including Pb, Zn, Cu, Cr, and Ni, in the <250 µm fraction. These preliminary results informed the design of analytical batches for subsequent elemental analysis using inductively coupled plasma optical emission spectrometry (ICP-OES). The known elemental composition of the analytical matrix was used to assess and mitigate spectral interferences.

XRD analysis was employed to elucidate the mineralogical composition of the dust, facilitating the identification of crystalline phases and the association of metallic elements within specific compounds. This provided indirect insights into the presence of amorphous phases and the physicochemical states of the analytes.

The particle size distribution of the fine dust was determined using laser diffraction, and the organic and volatile matter content was assessed via loss on ignition at 1000°C. A macroscopic examination of the samples was also performed to support qualitative observations.

In terms of heavy metal analytical characterization per se, the study was structured in two main phases. The first phase focused on the development and comparative evaluation of three chemical extraction methods. The second phase encompassed the quantitative analysis of Pb, Zn, Cu, Cr, Ni, and Cd using ICP-OES. Cadmium was further analyzed by graphite furnace atomic absorption spectroscopy (Zeeman-GFAAS) to enhance sensitivity and accuracy. This relied on an alternative (fourth) extraction method.

All analytical results were georeferenced using the Belgian Lambert 1972 coordinate system, enabling spatial representation within a Cartesian framework (datum: WGS84). The ultimate aim is to develop a valid geospatial model predicting heavy metal levels of dusts on each and every streets and roads of the Brussels-Capital Region.

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P3-023

USING A POLYMER INCLUSION MEMBRANE FOR THE ANALYSIS OF MERCURY AND METHYLMERCURY IN NATURAL WATERS

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Abstract: We developed a polymer inclusion membrane (PIM) composed of cellulose triacetate as the base polymer and the task-specific ionic liquid trioctylmethylammonium thiosalicylate (TOMATS). This PIM was specifically designed for the analysis of mercury (Hg) and methylmercury (MeHg) in natural waters [1,2]. Preliminary laboratory experiments were promising, demonstrating the PIM's efficiency in both the uptake and determination of these mercury species. Furthermore, we integrated the PIM as a resin gel into a passive sampling system known as the Diffusive Gradient in Thin-Film (DGT) [3]. This DGT configuration, which also included an agarose diffusive gel, a polyethersulfone membrane filter as the protective top layer, and a plastic support for optimal thickness and functionality, allows for the assessment of the bioavailable fraction of Hg in environmental matrices. The final device was assembled in a plastic mold with a simple, tight-fitting piston mechanism and a 2-cm diameter exposure window (Figure 1a). Time-series experiments using Hg^{2+} and MeHg^+ solutions were carried out under controlled laboratory conditions with PIM membranes and the PIM-DGT system. We demonstrated, for the first time, the effectiveness of the PIM in the uptake of MeHg (Figure 1b). Furthermore, initial experiments showed a clear linear relationship for Hg uptake, confirmed by a laboratory calibration curve obtained within 24 hours (Figure c). This finding suggests that Hg concentrations in natural waters can be accurately determined with only a short deployment time. A significant advantage of this membrane is its high stability, allowing for storage at room temperature for extended periods without degradation, making it very practical for field applications.

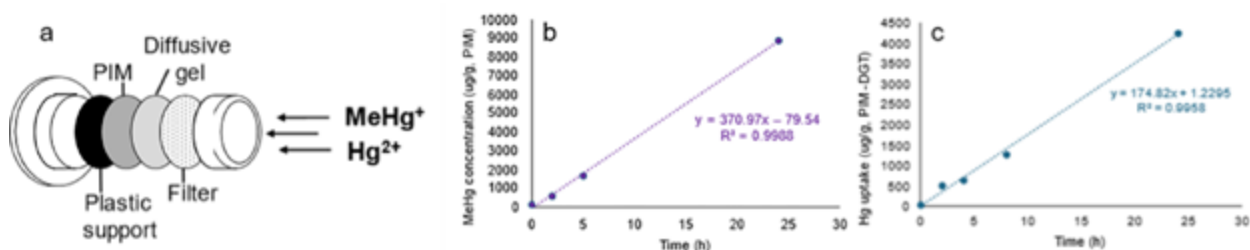


Figure 1. a) Structure of a piston-type sampling device used for the application of the DGT technique in aquatic and terrestrial systems; b) MeHg uptake solely by PIM for 24h; c) Hg uptake by PIM in a DGT system.

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P3-024

EVALUATION OF A SARCOSINE-BASED CORE-SHELL SORBENT FOR SOLID-PHASE EXTRACTION OF ACIDIC AND BASIC PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

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Pharmaceutical residues have been identified as emerging environmental contaminants due to their extensive consumption and subsequent excretion by humans. These compounds have been detected in both wastewater and surface water at very low concentrations ($\mu\text{g/L}$ and ng/L), which requires appropriate sample treatment such as solid-phase extraction (SPE), followed by a sensitive analytical technique like liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)[1]. Solid-phase extraction (SPE) is a widely used and well-established sample preparation technique to extract the pharmaceutical compounds from water samples.

The development of advanced SPE sorbents is essential to improve sensitivity and selectivity in sorptive extraction. Mixed-mode ion-exchange sorbents, which combine reversed-phase and ion-exchange properties, enhance capacity and selectivity of ionic analytes. To enable the simultaneous extraction of both acidic and basic compounds within a single SPE cartridge, zwitterionic sorbents containing cation- and anion-exchange groups have been developed [2,3]. Recently, core-shell polymer microspheres with ionic-exchange properties have also been developed for SPE sorbents [4]. These materials, characterized by hypercrosslinked and relatively thin functional shells, offer high specific surface areas and sorption capacity, while shortened diffusion path improve mass transfer. Additionally, they contribute to maintain stable pressure during the extraction process, resulting in greater reproducibility.

This study presents for the first time the development and application of a zwitterionic material based on core-shell polymer microspheres functionalized with sarcosine (HXLPP-WAX/WCX), which combines weak anion-exchange (WAX) and weak cation-exchange (WCX) moieties. This material was evaluated in SPE followed by liquid chromatography-diode array detection for the simultaneous extraction of acidic and basic pharmaceuticals from ultrapure water, river water and effluent wastewater samples. Different parameters in the SPE protocol were tested in order to promote the selective interactions among the sorbent and the target analytes. Thus, the sample was adjusted at different pHs and at pH 6, the analytes (acidic and basic) and the sorbent's functional groups (a tertiary amine and a carboxylic acid) were ionized, enabling ionic interactions that improved retention of both acidic and basic compounds. The washing step improved selectivity by removing matrix interferences, while the elution step, using only 5 mL of 5% NH_4OH in methanol was sufficient to efficiently recover all target compounds. After optimization of the SPE conditions, the recoveries after percolating 100 mL of sample for most analytes were from 31% to 81% for river water and from 41% to 89% for effluent wastewater. The method also showed good precision, with %RSD ($n=3$) values below 15% in river water and below 14% in effluent wastewater for analytes with satisfactory recoveries.

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P3-025**BENEATH THE SURFACE: EMERGING CONTAMINANTS IN GROUNDWATERS OF ANAGA AND EL TEIDE PARKS (TENERIFE, CANARY ISLANDS)**

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Emerging contaminants have increasingly been detected in diverse environmental matrices over the past three decades [1]. Although typically present at trace concentrations, many of these substances exhibit persistence, bioactivity, and potential for bioaccumulation, raising significant concerns regarding both ecological integrity and human health [2]. Among environmental compartments, groundwater –historically considered as a clean and protected resource– can be recognized as vulnerable to contamination due to increasing anthropogenic pressures and diffuse pollution sources.

The Canary Islands, a volcanic archipelago located off the northwest coast of Africa, rely extensively on groundwater resources for domestic supply, agricultural, and tourism-related uses, especially in the more mountainous islands such as Tenerife. The island hosts two major protected natural areas: Anaga Rural Park and El Teide National Park. These regions are not only biodiversity hotspots and UNESCO Biosphere Reserves but are also integral to the hydrological balance of the island. While these areas are safeguarded from direct human development, they are still susceptible to the infiltration of emerging contaminants through long-range atmospheric transport, recreational activities, and other diffuse inputs.

To the best of our knowledge, this study presents the first comprehensive analysis of emerging contaminants, including pesticides, hormones, antibiotics, pharmaceuticals, phenols, antibacterial agent, stimulants and UV filters, in groundwater samples collected from wells, springs, and galleries within Anaga Rural Park and El Teide National Park. A total of 31 samples were collected during 2024 and analyzed using automated solid-phase extraction followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. The results revealed the presence of various emerging contaminants in several groundwater samples, with concentrations varying across locations.

In this context, this work provides crucial baseline data on groundwater quality in two of the most ecologically valuable areas of Tenerife. It also emphasizes the need for routine monitoring of emerging contaminants in insular environments, where hydrological systems can be sensitive to external disturbances. In addition, the methodology applied demonstrates a robust and sensitive approach for trace-level detection of a wide array of contaminants, supporting its application in future monitoring programs.

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P3-026

DEVELOPMENT OF MK-015: A SELECTIVE AND SENSITIVE GUAIAZULENE-BASED CHEMOSENSOR FOR ON-SITE N_2H_4 DETECTION: SPECTROPHOTOMETRIC, COLORIMETRIC, AND SMARTPHONE-BASED ANALYSIS

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Hydrazine (N_2H_4) is a potent reducing agent and a strong base known for its high reactivity¹. Due to its highly flammable and explosive properties, N_2H_4 is a highly reactive and hazardous compound extensively used in aerospace propulsion, pharmaceuticals, and polymer industries^{2,3}. Despite its industrial relevance, hydrazine poses serious environmental and human health risks due to its hepatotoxic, neurotoxic, and potentially carcinogenic nature^{2,4,5}. Given these significant health hazards, effective detection and monitoring of hydrazine are critically important for safeguarding human health. A variety of analytical techniques have been developed for N_2H_4 detection, including surface-enhanced Raman spectroscopy (SERS), gas chromatography–mass spectrometry (GC–MS), and electrochemical methods^{6–8}. While these methods offer excellent selectivity and low detection limits, they are often constrained by high cost, complex and bulky instrumentation, the need for specialized personnel, and time-consuming sample preparation. Moreover, such methods frequently lack suitability for on-site applications and require pre-treatment steps that hinder practical deployment¹.

In this study, a cost-effective, portable, and user-friendly chemosensor, MK-015, was developed for the selective detection of N_2H_4 . The probe was synthesized via a straightforward and efficient procedure and fully characterized using NMR, IR, MS, and single-crystal X-ray diffraction to confirm its molecular structure. Exceptional selectivity toward N_2H_4 was observed, with no significant interference from common competing ions or their mixtures. In spiked drinking water, lake water, and tap water samples, recovery rates ranging from 78% to 99% were obtained. The sensor also exhibited long-term stability for over 100 days and enabled rapid spectrophotometric and colorimetric responses within seconds.

To facilitate practical implementation, an Android-based mobile application, “MK-015 N_2H_4 Detector”, was designed to quantify N_2H_4 levels by analysing Green/Red intensity ratios in captured images. This system allows for on-site measurements without the need for specialized equipment or technical training. The integration of the MK-015 sensor with the smartphone application provides a reusable, affordable, and portable platform for environmental monitoring and public health protection.

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P3-027**EMERGING CONTAMINANTS IN SURFACE WATER: TARGETED AND SUSPECT ANALYSIS BY UHPLC-HRMS/MS AND IMS-HRMS/MS**

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Contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, endocrine disruptors and industrial chemicals, are increasingly detected in aquatic environments. Their persistence, bioactivity, and potential toxicity pose significant environmental and public health risks. The structural diversity and typically low concentrations of CECs in complex matrices demand advanced analytical methodologies to enable their comprehensive detection, accurate quantitation, and confident structural elucidation.

In this study, a multi-technique analytical workflow was developed for the screening of CECs in surface water samples from Girona (Catalonia, Spain), integrating both targeted and suspect screening strategies. Sample treatment involved a multilayer solid-phase extraction (SPE) protocol using a combination of HLB, WAX, WCX, and PPL sorbents, enabling broad-spectrum retention of analytes with diverse physicochemical properties and minimizing matrix effects. Instrumental analysis was carried out using two complementary platforms: a UHPLC-Q-Orbitrap system, operating in high-resolution full-scan and all-ion fragmentation modes (HRMS/MS), and a UHPLC-IMS-QTOF system incorporating ion mobility spectrometry (IMS) for enhanced separation and structural characterization. The IMS provided experimental collision cross section (CCS) values and cleaner fragmentation spectra, significantly improving the confidence in compound identification.

The combined application of HRMS/MS and IMS enabled the confident quantitation of target compounds and identification of a wide range of CECs in surface waters. Most were confirmed at Schymanski levels 1 and 2, supported by accurate mass measurements, characteristic fragmentation pattern, and experimental CCS values. This integrated approach demonstrates high effectiveness for detecting known, suspect, and unknown contaminants in complex environmental matrices.

Overall, the integration of optimized SPE, high-resolution MS and IMS provides a powerful platform for environmental monitoring, supporting the development of more informed water quality assessments and remediation strategies.

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P3-028

FROM ULTRASONIC TO MOKA-LIKE DEVICES: A CRITICAL COMPARISON OF EXTRACTION STRATEGIES FOR EMERGING POLLUTANTS IN ANTARCTIC MARINE SEDIMENTS

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The analysis of marine sediments is a critical tool for evaluating the extent of anthropogenic impacts on aquatic environment. Marine sediments can accumulate various contaminants, with particular concern directed towards lipophilic compounds. These substances can be stored over time within sediment matrices and may subsequently be released into the aquatic environment, posing significant risks to the ecosystem integrity and biodiversity [1]. Among these contaminants, particular attention has recently been given to the emerging contaminants (ECs), a wide group of chemicals that are not subjected to regulatory control. This category includes some lipophilic substances that are the focus of the present investigation [2,3].

Traditional approaches for the extraction of contaminants from sediments and soil, such as Soxhlet extraction, vortex-assisted extraction, and ultrasonic baths, have been long employed in environmental monitoring. These methods usually require the use of large volumes of organic solvents, extended extraction times, and often necessitate additional clean-up steps, such as solid-phase extraction [1]. Furthermore, they offer limited flexibility in the adjustment of critical operational parameters, including solvent consumption. Consequently, there is growing interest in exploring alternative extraction strategies that are both effective and resource efficient.

In this context, devices originally designed for everyday use can be repurposed for analytical applications. A notable example is the domestic coffee maker, the Moka pot, which enables solid-liquid-vapor extraction under elevated temperatures and moderately high pressures [4]. However, conventional Moka pots afford limited control over extraction parameters. To overcome these limitations, the present study explores the use of a Moka-like coffee machine, the Kamira (Image 1), which enables more precise manipulation of the extraction variables and significantly shorter extraction times (approximately 30-40 seconds) [5]. This device also allows for adjustable solvent volumes, further enhancing its applicability in the analytical workflow.

This study presents a comparative evaluation of three extraction techniques: traditional ultrasonic bath extraction, standard Moka-pot extraction, and Kamira extraction. Antarctic sediment samples, sourced from the Banca Campioni Ambientali Antartici (BCAA) at the University of Genoa, were artificially spiked with known amounts of selected ECs. The samples were then subjected to the three pre-treatment protocols. For the ultrasonic bath, 40 mL of acetonitrile-water (90:10, v/v) mixture was employed during the 2 minutes sonication. For the Moka and Kamira methods, 40 mL of the same solvent mixture were used to perform extraction directly from the coffee-holder unit, following procedures adapted from Baglietto et al. (2024) [4]. Extracts were then diluted to 50 mL, 1 mL was evaporated to dryness, and reconstituted using 100 μ L of methanol-water (50:50, v/v) prior to analysis using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

The results obtained indicate that the Kamira-based approach exhibits superior recovery rates and similar matrix effects compared to the ultrasonic bath and traditional Moka-pot methods. These findings highlight the potential of repurposed domestic devices as cost-effective and efficient alternatives for contaminant extraction in environmental analysis. Ongoing work is focused on the optimisation of key operational parameters through the application of experimental design methodologies. To our knowledge, this is the first study to systematically compare these three extraction strategies for the determination of ECs in Antarctic marine sediments. The proposed approach offers the perspective of a simple, low-cost, and effective tool for the monitoring of ECs in the environment, thereby contributing to a more comprehensive understanding of their distribution and ecological impact in polar regions.

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P3-029

QUANTIFICATION OF ANTIBIOTICS IN AQUACULTURE WATERS USING SPE-LC-HRMS: INTEGRATING ONE HEALTH INSIGHTS

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The sustainability of aquaculture partly depends on intensive farming practices, including the use of antibiotics, whose residues in aquacultural products are strictly regulated. However, unmetabolized antibiotics excreted by fish can contaminate surface waters within and around farms. This is particularly concerning in open systems, where water flows through the farm and is returned downstream after limited treatment [1], potentially releasing antibiotics into the environment without control. Such discharges contribute to a major public health threat: antimicrobial resistance.

Therefore, the main goal of this study was the development of a fit-for-purpose method based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) for the quantification of antibiotics present in surface waters from fish farms. The analytes under study were selected based on two criteria: (i) antibiotics permitted in French fish farms (i.e., florfenicol, oxytetracycline, oxolinic acid, flumequine and trimethoprim) and (ii) antibiotics associated with antimicrobial resistance in France according to the Agence Nationale de Sécurité Sanitaire (ANSES) (i.e., sulfamethazine, sulfamethoxazole, metronidazole, amoxicillin, doxycycline, clindamycin, erythromycin, clarithromycin, roxithromycin, ciprofloxacin, and ofloxacin) [2].

Extraction of antibiotics from water samples was performed using solid-phase extraction (SPE) with OASIS HLB cartridges (500 mg, 6 cc), achieving a 33-fold concentration factor. Chromatographic separation was conducted on a ZORBAX Eclipse Plus C18 Rapid Resolution HD (50 x 2.1 mm, 1.8 µm) column using an Agilent 1290 Infinity II device. The gradient elution employed water and methanol, both acidified with 0.1% (v/v) formic acid, as mobile phases over a 15-minute run. Mass spectrometric detection was performed using an Agilent 6550 iFunnel Q-TOF mass spectrometer equipped with a Dual AJS ElectroSpray Ionization (ESI) source operating in both positive and negative ionization modes. Acquisition involved simultaneous scan events under a non-targeted mode, with each cycle lasting 0.2 s: a Full Scan followed by an All-Ion MS/MS scan, generating product ions with a fixed collision energy of 30 V. For compound identification, retention time, exact mass, and at least one product ion, were used. Diagnostic ions presented a signal-to-noise (S/N) ratio above 3, relative intensities over 10%, and ion ratios within ± 40% across the study.

The method was successfully validated in accordance with Commission Implementing Regulation 2021/808 [3]. Matrix effects were negligible, with all values under 15.2%. Consequently, external calibration curves were used for all the analytes investigated ($R^2 > 0.99$), with linear ranges extending from the LOQs to 30 ng mL⁻¹. Limits of quantification (LOQ) ranged from 0.09 to 1.5 ng mL⁻¹. Accuracy, assessed through trueness and precision, was evaluated at 1, 2 and 3 times the LOQ, with 18 replicates per level. All trueness values fell within the range 66-119%, with relative standard deviations (RSDs) for precision were consistently below 19%.

This method was applied to samples collected from fish farms in France, with several samples testing positive for oxolinic acid, flumequine, ofloxacin, florfenicol, doxycycline, and oxytetracycline at concentrations ranging from below LOQs to 5.9 ng mL⁻¹—even in cases where the use of some of these antibiotics had not been reported by the farms. Several hypotheses regarding their origin are currently under investigation; notably, fish farms may act as bioaccumulators of antibiotics introduced from external sources via interconnected surface waters.

Taken together, these findings demonstrate the applicability of the developed method for detecting antibiotics in environmental waters and underscore the need for broader monitoring efforts to identify release sources and implement effective remediation strategies.

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P3-030**FLOW-MODULATED COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR NON-TARGET ANALYSIS OF ENVIRONMENTAL POLLUTANTS IN WATER****Santos, Francisco Javier; Trigueros, Francisco; Moyano, Encarnación.***ChroMS-EnviFood Research Group. Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Barcelona, Spain.*

Water pollution remains a major environmental and public health concern, primarily driven by the continuous introduction of new hazardous substances from anthropogenic sources. Over the years, regulatory frameworks have been established to reduce or restrict the release of these contaminants into aquatic environments, accompanied by the implementation of monitoring programs aimed at detecting and identifying a wide spectrum of pollutants, including their transformation products [1]. Despite the effectiveness of these measures, numerous contaminants remain unidentified, underscoring the need for advanced monitoring strategies capable of determining a broad range of compounds within a single analysis. In this context, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC–TOFMS) has emerged as a powerful technique for the in-depth analysis of volatile and semi-volatile organic compounds in complex environmental samples. It has demonstrated superior performance over conventional one-dimensional GC, offering enhanced separation capacity, structured chromatograms, improved resolution, and reduced matrix-related interferences [2].

This study presents the development and application of a robust analytical method based on flow-modulation comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC–TOFMS) for suspect and non-target screenings of organic pollutants in aquatic environments, including river water, groundwater, sediments, and wastewater effluents. The method combines solid-phase extraction (SPE) using Oasis HLB cartridges with GCxGC separation employing a dual-column configuration (DB-5MS × Rxi-17SilMS) and a new-generation flow modulator (Flux™ – LECO Corp.). Operating parameters of the flow modulator, such as injection/divert steps and the modulation time, were optimized to achieve maximum peak capacity. The detection and identification of the suspect and non-target compounds were accomplished using a time-of-flight mass spectrometer (LECO Pegasus BT 4D TOFMS), operating in electron ionization and full-scan mode (m/z 45–650), enabling high acquisition rates (100–200 spectra/s) and sensitive detection. Suspect and non-target screening workflows were established for the identification and structural confirmation chromatographic information (^1D and ^2D retention times), mass spectral deconvolution, and mass spectral library data. The developed method was applied to the analysis of surface water and effluents of wastewater treatment plants, allowing the (tentative) identification of more than 300 semi-volatile contaminants per sample, most of them considered emerging contaminants. The main detected pollutant groups included pharmaceuticals, flame-retardants, personal care products, linear and aromatic hydrocarbons, byproducts, and metabolites, among others. The results and conclusions of this research are presented here.

Acknowledgments

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P3-031**HYDROCHEMICAL INVESTIGATION OF PERSISTENT POLLUTANTS IN THE BIFERNO RIVER KARST SYSTEM WITHIN MATESE NATIONAL PARK: CHEMOMETRIC ANALYSIS IDENTIFYING KEY ENVIRONMENTAL AND ANTHROPOGENIC DRIVERS OF WATER QUALITY**

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Water resources are critical for life, agriculture, and industry but increasingly threatened by human activities and climate change. Over five billion people depend on surface and groundwater for drinking. However, water quality is declining due to persistent pollutants from urban, agricultural, and industrial areas. Surface water is vulnerable, and groundwater pollution can last decades, complicating sustainable management and risking access to drinking water [1]. A growing concern is the presence of per- and polyfluoroalkyl substances (PFAS) alongside heavy metals in the environment and human body. These contaminants are persistent, bioaccumulative, toxic, and linked to health problems, especially cardiovascular diseases, through oxidative stress, inflammation, and lipid metabolism disruption. Simultaneous exposure to PFAS and heavy metals may cause stronger, synergistic health effects, increasing risks beyond their individual impacts [2].

The Matese Mountains karst system plays a key role in Southern Italy's water supply. Situated in the Apennines between Molise and Campania, it is one of Europe's most important karst areas. The Biferno River springs, emerging from karstified carbonate rock on the Matese's eastern slope, are vital for millions [3]. The recent designation of the Matese National Park (Italian Law No. 205/2017) has boosted protection. Hydrogeological studies examined hydrographic boundaries, infiltration rates, and dynamics to promote sustainable management and safeguard water quality. Through a complex capture and distribution network, Biferno springs supply populous provinces in central Italy, including Termoli, Naples, and Ischia [3]. A study assessed human impacts on surface water quality and persistent pollutants in the karst system supplying the Biferno springs in Matese National Park.

The Bojano springs, a drinking water site, and the river's upper course, along with the hydrological sub-basins, were included among the 17 sampling sites. The inorganic fraction in water samples was determined using ICP-OES after microwave-assisted digestion with nitric acid, following EPA 3015A. Twenty-three elements, including metals and metalloids, were analyzed. To assess the fluorinated organic fraction, a targeted analysis of 41 fluorinated organic compounds, including carboxylates, sulfonates, and sulfonamides, was conducted by direct injection of 100 µL of sample using LC-HRMS (Dionex 3000RS coupled with Q Exactive, Thermo), employing a Kinetex XB-C18 column maintained at 40 °C.

The results were statistically processed using unsupervised methods to reduce data dimensionality and identify main variables causing variability. A Principal Component Analysis (PCA) was performed. The scree plot showed that the first two principal components together explain 55 % of the total variance in the dataset, while the first three components account for 68.4 %. The variables that contribute most to the first component (PC1) are Co, PFPeA, and K; for the second component (PC2), they are Fe, Ni, and Cr; and for the third component (PC3), Pb, Ba, and B are predominant.

Lead is a toxic metal whose presence has increased mainly due to human activities such as mining, battery production, lead-based paints, and contaminated fertilizers. These sources release Pb into the air, soil, and water, causing environmental and health damage [4]. Regarding PFPeA, it is primarily formed from the degradation of 6:2 diPAP, a compound found in contaminated compost, household dust, and wastewater treatment sludge [5]. Iron in the environment mainly comes from natural sources like desert dust (about 95 %), with smaller contributions from volcanic eruptions and wildfires (less than 5 %). It can travel through the air and deposit on soil, water, and snow [6].

Even if all samples comply with Legislative Decree 23/2023, continuous monitoring is necessary to manage potential pollution risks. Water quality, in this case, is influenced by both natural and anthropogenic sources, mainly deriving from iron in the former and lead and PFPeA in the latter.

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P3-032

FLOWER-PAPER SHAPED RECYCLED POLYMER-CELLULOSE COMPOSITES FOR ECO-FRIENDLY THIN-FILM MICROEXTRACTION OF VOLATILE ORGANIC CONTAMINANTS IN WATER

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Abstract

The accumulation of polymeric waste from daily consumption demands urgent sustainable solutions to mitigate environmental risks. This study introduces an innovative thin-film microextraction (TFME) platform using "paper flower"-shaped cellulose paper coated with recycled polymers for isolating volatile organic compounds (VOCs) from water matrices. The design leverages discarded low-density polyethylene (LDPE) and expanded polystyrene (PS) residues as sorptive phases, dip-coated onto a cellulose substrate to create an eco-sustainable, reusable system. The "paper flower" configuration enhances mass transfer through efficient fluid dynamics while minimizing resource use.

PS-coated phases outperformed LDPE, achieving rapid analyte isolation (toluene, xylenes, ethylbenzene, styrene) within 20 minutes. Critical synthesis parameters—paper size, polymer type, precursor concentration, and dip cycles—were systematically optimized alongside extraction variables (time, temperature, elution solvents). Coupled with gas chromatography-mass spectrometry (GC-MS), the method achieved quantification limits of 9–10 $\mu\text{g L}^{-1}$, intra-/inter-day precision <7.6% RSD, and recoveries of 92–110% across spiked samples.

Applied to real-world drinking waters, the approach detected styrene ($18 \pm 3 \mu\text{g L}^{-1}$) in tap water and identified contaminant profiles in commercial packaging: mineral water in biobased cartons contained styrene, toluene ($15.2 \pm 0.3 \mu\text{g L}^{-1}$), and o-xylene ($5.5 \pm 0.2 \mu\text{g L}^{-1}$), while polyethylene terephthalate (PET) bottles showed residual toluene and styrene. This work bridges waste valorization with analytical innovation, offering a cost-effective, environmentally conscious strategy for water quality monitoring and microplastic pollution studies. The method's simplicity, scalability, and dual waste-to-resource applicability position it as a transformative tool for addressing both environmental and public health challenges.

Acknowledgments

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P3-033**UNIVERSAL MODULAR IMMUNOCHROMATOGRAPHIC PLATFORM FOR MONITORING ANTIBIOTIC RESIDUES IN FOODS WITH SIMPLE ADOPTION TO DIFFERENT ANALYTES**

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Monitoring antibiotic residues in food products has become increasingly in demand in recent years, accompanied by an extension of the list of compounds to be controlled. In this regard, the use of immunochromatographic analysis (ICA), which provides rapid and methodologically simple testing, requires new solutions to simplify the transition from one group of controlled antibiotics to another and reduce the cost of testing.

To address this challenge, we propose a modular principle for assembling sets of compounds used for ICA implementation. This modular approach is based on combining a universal test strip for lateral flow processes with changeable solutions of specific immunoreagents for detecting antibiotics from different groups. The universal test strip's analytical zone is formed by applied streptavidin. Before analyzing a sample, specific antibodies and a biotinylated conjugate of the antibiotic being analyzed with a carrier protein are mixed in a well of the plate, incubated, and then the universal test strip is immersed in the reaction mixture. To detect the specific immune complexes formed, a conjugate of gold nanoparticles (GNPs) and an antibody-binding reactant (such as staphylococcal protein A) is applied. This allows for the same control of assay results as in traditional ICA, including quantitative assessment of antibiotic content based on the intensity of coloration of the zone with bound GNP labels. Importantly, this approach simplifies adaptation of the testing procedure for different controlled antibiotics without the need to stock up on a complete set of test strips for all substances, ultimately reducing the cost of testing significantly.

Using this modular platform, we conducted multifactorial optimization of reagent concentrations and durations of the analysis stages to achieve minimum detection limits with reliable assessment of assay results for all controlled compounds, including visual assessment. The proposed test system, utilizing analyte-independent test strips, successfully detected antibiotics from different chemical classes such as tetracycline, streptomycin, neomycin, tylosin, and more. For example, the tetracycline assay demonstrated instrumental and visual detection limits of 0.02 and 5.5 ng/mL, meeting regulatory demands. These tests were effectively used for detecting antibiotics in meat products, providing reliable and reproducible results. The proposed ICA had a duration of 12 minutes.

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P3-034**HIGH-SENSITIVE LATERAL FLOW IMMUNOASSAY FOR CONTROLLING RESIDUES OF GROWTH PROMOTER CLENBUTEROL IN MEAT PRODUCTS****Zvereva, Elena A.; Hendrickson, Olga D.; Zherdev, Anatoly V.; Dzantiev, Boris B..***A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation.*

Growth promoters are popular tools among meat producers to increase productivity in manufacturing. However, their presence in final food products poses significant threats to consumer health. Therefore, strict requirements have been established for the maximum permissible content of these toxicants in food products of animal origin, and highly sensitive methods are required for their control. Simple and rapid analytical techniques for out-of-laboratory testing are in demand during the initial wide screening. Lateral flow immunoassays (LFIAs) meet these criteria, but their sensitivity is often insufficient for monitoring toxicants in food matrices. For these reasons, we conducted a multifactorial study of LFIA conditions for the sensitive detection of beta-agonist clenbuterol (CLE) in meat products.

Gold nanoparticles (GNPs) with an average diameter of 30 nm were found to be the most efficient labels in the competitive format of LFIA. The advantages of replacing commonly used conjugates of GNPs with specific antibodies with a combination of non-modified specific antibodies and conjugates of GNPs with anti-species antibodies were demonstrated. By varying parameters such as immunoreagent concentrations and the composition of the reaction mixture (buffer type and pH, detergents used, ionic strength, presence of additional organic components), we found an assay mode with high intensity of detected coloration and maximal response to the presence of CLE in tested samples. Different protocols for preparing meat samples for LFIA testing were compared, and conditions were optimized to reduce duration while maintaining a high degree of CLE extraction.

The LFIA implementation regime we found allowed for the detection of CLE in concentrations up to 1.0 ng/mL in qualitative mode (visual results assessment) and up to 0.02 ng/mL in quantitative mode (photometric registration of coloration intensity by a scanner or smartphone camera). This level of sensitivity meets regulatory demands for CLE control in food products. The assay duration was 15 minutes. The manufactured test strips were used to detect CLE in real meat samples, showing a high degree of analyte revealing (78-119%) and reproducibility of measurements (standard deviation no more than 22%). The results obtained demonstrate the promising potential and competitiveness of the developed analysis for the primary control of clenbuterol in meat products.

This study was financially supported by the Russian Science Foundation (grant number [25-26-00214](#)).

P3-035**IMPACT OF COLD STORAGE AND ON-TREE MATURATION ON THE CARBOHYDRATE COMPOSITION OF HASS AVOCADO PULP**

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Avocado (*Persea americana* Mill.) is highly popular due to its exceptional nutrition and associated health benefits. However, increasing demand frequently exceeds local production, leading to increased reliance on imported avocados. These imports often undergo prolonged cold storage during transport, which can compromise fruit quality. As a climacteric fruit, avocado ripens post-harvest, with non-structural carbohydrates playing a key role in fruit development. Despite their importance, the biochemical mechanisms governing these metabolic changes remain only partially understood.

To better understand how post-harvest conditions influence the fruit's carbohydrate profile, this study examines the effects of cold storage versus prolonged on-tree maturation on the composition of carbohydrate in Hass avocado pulp at edible ripeness. Approximately 125 fruits were harvested and stored under controlled temperature and humidity conditions in refrigerated chambers, simulating typical import logistics within the continent. Every 10 days, subsets of fruits from the cold chamber were allowed to ripen at room temperature until reaching edible ripeness. Simultaneously, freshly harvested avocados were collected and left to ripen under identical conditions (simulating domestic conditions). For each time point, five biological replicates consisting of five fruits each were analysed, yielding 45 representative samples in total.

All avocado freeze-dried mesocarp samples underwent ultrasound-assisted solid-liquid extraction using a 60:40 (v/v) ethanol:water solution. The extracts were then analysed using hydrophilic interaction liquid chromatography coupled with mass spectrometry (HILIC-MS) to rapidly and accurately determine several primary metabolites in the mesocarp.

The analysis revealed notable differences in sugar profiles depending on the management approach employed. Avocados subjected to cold storage exhibited a consistent increase in seven-carbon (C7) sugars, such as perseitol and D-mannoheptulose, throughout the storage period. Conversely, fruits that matured longer on the tree accumulated higher levels of six-carbon (C6) sugars, including fructose, glucose, and sucrose. To further interpret these differences, Principal Component Analysis (PCA) was employed as an unsupervised statistical tool. The PCA results demonstrated a clear separation between cold-stored samples and those freshly harvested from the trees, reinforcing the idea that post-harvest methods significantly influence the mesocarp's carbohydrate composition.

The results of this study offer new perspectives on the ways in which harvest timing and post-harvest handling distinctly modulate the metabolic profile of avocado pulp, particularly in relation to sugar composition. Understanding these changes is crucial for optimizing supply chain strategies, improving post-harvest quality management, and promoting more sustainable production and distribution models. These findings may contribute to the development of enhanced storage protocols and inform consumer-focused marketing approaches based on fruit quality and nutritional value.

P3-036

DEVELOPMENT AND VALIDATION OF THE EXTRACTION METHODS FOR LINGONBERRY AND LINGONBERRY-ENRICHED COOKIES

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In this study, two extraction methods of phenolic compounds from two different matrices: lingonberry and lingonberry-incorporated cookies were developed and optimized. The optimization was done with the employment of Box-Behnken design (BBD) using three factors: solvent-to-sample ratio, solvent composition, and extraction temperature.

The ultrasound-assisted extraction (UAE) conditions were optimized to maximize the extraction yield of phenolic content from lingonberry. The optimized parameters for the UAE extraction were: 1:50 solid-to-liquid ratio, 30 °C extraction temperature, 5 min extraction time, and 100% methanol as an extraction solvent.

The maceration extraction method was optimized for the polyphenol extraction from cookie matrix using BBD experimental design. The optimum extraction conditions were: 1:10 sample-to-solvent ratio, 10% aqueous methanol as an extraction solvent, 50 °C extraction temperature, and 20 min extraction time.

The UPLC-MS methods were developed and validated according to ICH guidelines for the determination of phenolic compounds from lingonberry and lingonberry-fortified cookies. The methods showed good precision: the coefficient of variation (CV) for repeatability for lingonberry was 0.69%, while for lingonberry enriched-cookies it was 3.53%. The intermediated precision was 1.91% for lingonberry to 4.31% for cookies, both expressed as CV.

The robustness study conducted using face-centered Central Composite Design (CCD) revealed the significant impact of two factors: temperature of the column and flow rate, emphasizing the importance of the control of these parameters to ensure reliable results.

The developed methods have shown satisfactory levels of accuracy: $98.53 \pm 0.44\%$ for lingonberry and $78.82 \pm 0.02\%$ for the cookies, good linearity (with coefficients of determination ranging from 0.9938 to 0.9999), limit of detection (LOD) ranging from 0.30 to 0.73 mg/L, and limit of quantification (LOQ) varying from 0.91 to 2.2 mg/L.

P3-037**DEVELOPMENT AND VALIDATION OF ULTRASOUND ASSISTED EXTRACTION OF ANTHOCYANIN AND PHENOLIC COMPOUNDS IN CRANBERRY AND COOKIES**

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Cranberries are known to be associated with many health benefits including protection against cardiovascular diseases and anticancer activity as a result of their high dietary flavonoids, including anthocyanins, and phenolic content. In order to have more data about their effects on human health, reliable and fast analytical methods are needed. The analytical methods for the determination of these compounds include extraction procedures, so fast and reliable extraction methods are convenient. The ultrasound-assisted extraction technique is a unique one as it can obtain components with a high recovery, faster rate and at the same time has lower solvent consumption when compared to other conventional liquid-solid extraction techniques. However, it must be carefully evaluated in order to avoid the compounds to be degraded under the working conditions. Additionally, recovery must be carefully determined to obtain reliable results. The study was aimed at optimizing and validate ultrasound assisted extraction and UPLC separation method of phenolic compounds in cranberry and cookies produced from cranberry. UAE extraction procedure was developed using a Box Behnken Design (BBD) and a Factorial Design (FD) and optimal extraction conditions for determining phenolic compounds and anthocyanin in cranberry and cookies were established. The optimum conditions for extraction of phenolic compounds and anthocyanin in cranberry were discovered at solvent (methanol) composition of 100%, solvent to sample ratio of 25:1 in mL/g and temperature of 30 °C. However, 5 minutes optimum time was discovered for anthocyanin and 10 minutes was established as the optimum extraction time for phenolic compounds in cranberry. On the other hand, optimum conditions were obtained at temperature 37.5 °C, solvent to sample ratio 25:1 and 15 minutes extraction time for extraction of anthocyanin in cookies. The method developed showed high repeatability and reproducibility with short extraction time.

P3-038

ASSESSMENT OF ROLE OF GLUCOSE OXIDASE, FLAVONOIDS, COPPER AND IRON ON THE GENERATION OF HYDROGEN PEROXIDE IN HONEY

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Glucose oxidase (GOx) is responsible for the generation of hydrogen peroxide in honey. However, several studies have shown a lack of correlation between the amounts of GOx and H_2O_2 in honey, indicating contributions from other hidden factors; such as the autoxidation of flavonoids catalyzed by metals or the inhibition of GOx activity by themselves (Bucekova et al., 2018; Brudzynski 2020; Brudzynski 2023). Thus, the aim of this study was to investigate the generation of H_2O_2 in honey attributable to GOx activity and flavonoid autoxidation, and to evaluate the effects of Cu^{2+} and Fe^{3+} on this process. To this end, the research plan considered: (I) Evaluate the effects of Cu^{2+} , Fe^{3+} and flavonoids on the activity of GOx in an artificial honey as simplified model. (II) Survey the generation of H_2O_2 in a semi-natural model system consisting of isolated GOx and flavonoid fractions from honeys. (III) Relate the generation of H_2O_2 to the activity of GOx, flavonoids, copper and iron in natural honey. The results show that flavonoids, Cu^{2+} and Fe^{3+} inhibit the activity of GOx; with the decrease being more important in the case of flavonoids than in the case of metals. However, the formation of complexes between them attenuates this effect, without a complete restoration of GOx activity. Moreover, metals promote prooxidant action of flavonoids, particularly flavonols, contributing to the generation of H_2O_2 in a lesser extent than GOx activity in honey. The evidence indicates that the generation of hydrogen peroxide in honey is due to a complex network of reactions.



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P3-039

PORTUGUESE EMERGING BIOINDUSTRY: SUSTAINABLE EXTRACTION AND CHARACTERIZATION OF OILS FROM INSECT FLOURS

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The continuous increase in the global human population poses a problem of nutrient scarcity. According to the Food and Agriculture Organization of the United Nations (FAO), approximately one in three people in the world, 2.37 billion, did not have access to adequate food in 2020, and this represents an increase of almost 320 million people in just one year [1]. This problem can be solved by finding sustainable and natural sources of nutrients in insects [2]. This area of research is currently expanding rapidly, and there are companies in Portugal dedicated to insect farming [3]. Through a partnership project [4] with these companies' insect flours were obtained to improve the extraction of oil and protein from such biomass through sustainable green technology (Figure 1a).

In this work, the extraction of oil from the (i) *Tenebrio molitor* (mealworm) larvae, (ii) *Acheta domesticus* (domestic cricket), and (iii) *Hermetia illucens* (black soldier fly) flours was optimized. Solid-liquid extraction procedures were developed using green solvents at room temperature, and the extracts were characterized by advanced analytical techniques such as, Nuclear Magnetic Resonance Spectroscopy (NMR), Gas Chromatography coupled to Flame Ionization Detection (GC-FID) and Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS). The extraction yields were remarkably close to the total oil content expected for the insect flours. The oils from *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) were essentially composed of triacylglycerols (TAGs, Figure 1b), while the oil from *Acheta domesticus* (AD) was primarily made of free fatty acids.

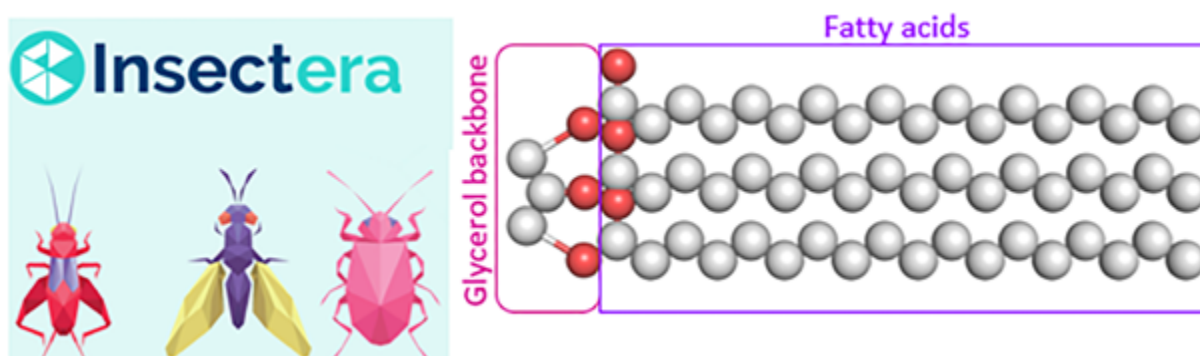


Figure 1: (a) InsectERA partnership; (b) TAG generic structure.

Acknowledgements

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P3-040

INVESTIGATING ACETOGENINS IN AVOCADO PULP, PEEL AND SEED FROM DIFFERENT VARIETIES BY LC-IMS-MS

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Avocados (*Persea americana* Mill.) are known for their rich composition of bioactive compounds, with acetogenins standing out due to their diverse biological activities. Derived from C-18 fatty acids, these compounds are found in various avocado tissues, including the peel, pulp, seeds, and leaves. They act as natural defenses against herbivores and fungal infections, and their antimicrobial properties, such as inhibiting bacterial endospore germination, have attracted food industry interest. As natural preservatives, acetogenins offer a promising alternative to synthetic additives, meeting the growing demand for sustainable food solutions.

The aims of this study were (i) to optimise the extraction of acetogenins, (ii) to develop an analytical method for their separation and detection, (iii) to establish their distribution in different avocado tissues and (iv) to evaluate their potential role in fruit ripening. To investigate these aspects, avocado tissues (peel, pulp and seed) of three different varieties (*Bacon*, *Fuerte* and *Hass*) were studied at different stages of ripening using an advanced LC-IMS-MS platform. A total of 180 samples were processed, each sample consisting of six avocados. There were five biological replicates per tissue, variety and ripening stage.

Several parameters were tested to ensure an efficient extraction of the targeted analytes, including the type of solvent (isopropanol, methanol and a 50:50 methanol:ether mixture), the number of extraction cycles and the need for an additional clean-up step. The final optimised method consisted of a single-cycle solid-liquid extraction with methanol, which excluded the need for further cleaning. Chromatographic conditions such as column selection, flow rate and temperature were also adjusted to improve the separation and detection of acetogenins. After validation, the method was applied to the avocado samples.

A comprehensive assessment of the full acetogenins profile across different avocado tissues led to the identification of 21 acetogenins, many of which were structural isomers, along with three additional fatty acids consistently present in the profiles. Afterwards, the most abundant acetogenins were quantitatively evaluated in each tissue and variety. *Bacon* seeds were rich in AcO-avocadene, whereas *Fuerte* and *Hass* seeds contained higher levels of persenone B. In the peel, the dominant acetogenin varied among varieties: *Bacon* had the highest levels of persenone A, *Fuerte* of AcO-avocadene, and *Hass* of persin. Similarly, in the pulp, AcO-avocadene was predominant in *Bacon* and *Fuerte*, whereas persin was the major constituent of this chemical class in *Hass*.

To explore potential varietal differences, Principal Component Analysis (PCA) was applied to the data. The results showed a clear distinction between avocado varieties when each tissue type was analysed separately, suggesting that acetogenin composition could serve as a chemical marker for cultivar differentiation.

This work advances the study of acetogenins in avocados by optimising extraction and detection methods, and provides a comprehensive analysis across tissues and cultivars. The findings also have practical implications for the avocado industry, highlighting the potential of typically discarded tissues for value-added applications.

P3-041**CHARACTERIZATION OF BIOACTIVE PEPTIDES OBTAINED BY ENZYMATIC VALORIZATION OF CORN WASTE**

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Corn waste, including corn gluten meal (CGM), is a significant environmental challenge resulting from corn processing due to its large-scale production and limited disposal options. To promote sustainability and reduce waste, a green enzymatic approach was applied for the valorization of proteins CGM. In this study, proteases capable of degrading zein, the main corn protein, were produced from the *Bacillus subtilis* 24B strain through solid-state fermentation. Zein-derived biopeptides are utilized in the food, pharmaceutical, cosmetic, and agricultural industries for their antimicrobial, antioxidant, and biological properties

The obtained enzymes were purified using Nuvia™ hydrophobic interaction chromatography and subsequently identified by mass spectrometry. Identified proteases were used for zein hydrolysis, generating bioactive peptides. Hydrolysis products were fractionated using ultrafiltration membranes (3-10 kDa) to obtain peptide fragments of different molecular sizes, which were further characterized by LC-MS/MS analysis. The bioactivity of the obtained peptides was evaluated through antimicrobial assays, including the Kirby-Bauer test and minimal inhibitory concentration (MIC), as well as antioxidant assays, such as FRAP, DPPH, and ABTS tests.

The obtained results demonstrate an environmentally friendly approach to converting corn waste into valuable bioactive compounds, aligning with sustainable biotechnology and waste valorization strategies. By integrating enzymatic processes and green methodologies, this research contributes to the development of eco-conscious solutions for industrial applications.

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P3-042

ANTIDIABETIC POTENTIAL OF GALICIAN EXTRA VIRGIN OLIVE OILS: FROM SIMULATED DIGESTION TO HUMAN INTERVENTION IN TYPE 2 DIABETES MELLITUS (OILDIABET PROJECT)

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Diet is one of the most critical lifestyle factors influencing the development, prevention, and treatment of diabetes. Among various dietary patterns, the Mediterranean diet (MedDiet) has garnered particular scientific interest for its preventive and therapeutic effects.

Olive oil—especially extra virgin olive oil (EVOO)—is considered a hallmark of the MedDiet, serving as its primary source of fat. The health benefits attributed to EVOO stem from its unique composition, particularly its fatty acid profile and high content of phenolic compounds.

EVOOs obtained from native Galician olive varieties stand out for their remarkable sensory, nutritional, and health-promoting properties, largely due to their high phenolic content (>700 mg/kg) (Figueiredo-González et al., 2022; Reboredo-Rodríguez et al., 2015a, 2015b, 2016, 2018, 2020, 2021, 2023). Preliminary studies by our research group, Food and Health Omics, demonstrated the strong antidiabetic potential of these oils compared to other Spanish varieties. In particular, their extracts exhibited α -glucosidase inhibitory activity stronger than acarbose (the reference drug used to delay intestinal glucose absorption) (Figueiredo-González et al., 2019, 2022; Reboredo-Rodríguez et al., 2020).

With support from the research project “Antidiabetic potential of phenolic-rich olive oil: from an in vitro dynamic gastrointestinal simulator to patients with type 2 diabetes mellitus (OILDIABET)” (Ref.: RTI2018-098633-B-I00), we evaluated the health benefits of phenolic-rich EVOOs from native Galician varieties in both in vitro and in vivo models.

In vitro models included the simulation of:

- Gastrointestinal digestion of EVOO to identify phenolic compounds in the bioaccessible fraction (i.e., released and hydrolyzed compounds available for intestinal absorption) and in the residual oil fraction reaching the colon. The initial antidiabetic potential of the EVOO increased by 267-fold during the digestion process due to the hydrolysis of complex phenolics (secoiridoids) into simpler structures (mainly tyrosol, hydroxytyrosol, and hydroxytyrosol acetate).
- Absorption and biotransformation of the bioaccessible fraction using Caco-2 cell monolayers in Transwell plates to simulate the intestinal epithelium. Phase I and II metabolites were identified, formed through enzymatic transformations by epithelial cells (viz. hydroxytyrosol-3'-sulphate).
- Colonic fermentation, performed with the oil fraction reaching the colon and fecal microbiota from two diabetic donors (one individual with obesity). DNA was extracted from fermentation pellets and 16S rRNA gene sequencing was conducted. The data revealed a shift in the relative abundance of dominant bacterial genera. Several genera inversely correlated with type 2 diabetes and obesity—increased in the presence of EVOO—were associated with greater short-chain fatty acid production, particularly butyric acid. Significant increases were observed for Akkermansia, Alistipes, Bacteroides, Bifidobacterium, Butyrivibrio, Butyrivibrio, Clostridium sensu stricto 1, Parabacteroides, Roseburia, Slackia, and Senegalimassilia.

In vivo models involved a dietary intervention in which 108 diabetic subjects consumed 30 mL/day of Galician EVOO. This randomized, prospective, parallel-group, controlled trial lasted six months. Two key responder groups were identified:

- Obese diabetic individuals (BMI ≥ 30 kg/m²) showed significant reductions in fasting plasma glucose, estimated average glucose, and glycated hemoglobin after sustained EVOO intake.
- Individuals with high insulin resistance (HOMA-IR ≥ 3.8) showed significant reductions in fasting insulin levels and HOMA-IR index.

These findings demonstrate a strong concordance between in vitro and in vivo models, supporting the validity of in vitro assays as effective preliminary tools to predict physiological responses and to guide the design of more complex dietary intervention studies.

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P3-043

NOVEL TRIFLUOROACETIC ACID-FREE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR COMPREHENSIVE PROTEIN PROFILING OF BOVINE MILK

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Milk proteins, primarily caseins and whey proteins, play a crucial role in both the nutritional value and functional properties of milk [1,2]. Gaining a detailed understanding of their composition and genetic variability demands robust and reliable analytical techniques. In this study, we describe for the first time a trifluoroacetic acid (TFA)-free method for comprehensive protein profiling of bovine milk using liquid chromatography with ultraviolet absorption and mass spectrometry detections (LC-UV and LC-MS, respectively).

A reversed-phase LC-UV method was initially developed using a wide-pore C4 column and acetonitrile:water mobile phases with 0.1% (v/v) formic acid (HFor), replacing the commonly used ion-pairing agent TFA, which is widely accepted to be unsuitable for LC-MS due to ion-suppression and potential contamination of the mass spectrometer [2-4]. The substitution of TFA with HFor allowed full LC-MS compatibility and led to a 10-minute reduction in total analysis time, with little impact on separation efficiency. The TFA-free method demonstrated high analytical performance, yielding low relative standard deviations (%RSD) in retention times and peak areas across extended analytical sequences, while preventing mass spectrometer contamination.

LC-MS analyses were conducted using a high-resolution, accurate-mass time-of-flight (TOF) mass spectrometer, enabling accurate identification and quantification of major bovine milk proteins. The method allowed the detection of proteoforms with subtle structural differences, such as β -casein A1 and A2 [2], and enabled clear differentiation among β -casein genotypes (A1A1, A1A2, and A2A2). It also proved effective in quantification of low-level contamination of A2A2 milk with A1A1 milk, which contains β -casein A1 that releases during digestion b-casomorphin 7 (BMC-7), a peptide with opioid activity linked with several health conditions. These findings highlight the method's strong potential for high-throughput, reliable, and efficient applications in dairy research, quality control, and product authentication, without compromising the performance of the mass spectrometer.

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P3-044

SCREENING ANALYSIS OF POTENTIALLY HAZARDOUS ORGANIC COMPOUNDS IN ENERGY DRINKS BY GC-MS COUPLED WITH SPME

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Due to the annual increase in energy drink consumption among young people, there is a growing need for comprehensive studies of their chemical composition and an assessment of potential health risks [1].

Recent studies have identified the presence of volatile and semi-volatile organic compounds in these drinks, including caffeine, taurine, water-soluble B vitamins, phthalates, aldehydes, and esters, some of which are known to exhibit toxic, sensitizing, or endocrine-disrupting properties [2–4].

Within the scope of this study, a screening analysis of eight available energy drinks was carried out using solid phase microextraction (SPME) [5] coupled with gas chromatography–mass spectrometry (GC–MS). As a result, the complete chemical composition of the energy drinks available on the market of Republic of Kazakhstan was identified.

The selected extraction parameters (temperature: 50 °C; extraction time: 30 min; DVB/CAR/PDMS fiber: 50/30 µm) provided high analytical sensitivity while completely eliminating the need for toxic solvents. As a result, more than 220 compounds were identified in the 8 energy drinks, including furfural, methyl salicylate, benzaldehyde, limonene, methyl benzoate, and phthalates, some of which are classified as potentially hazardous according to ECHA and PubChem [6–8].

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P3-045**GEOGRAPHIC ORIGIN CLASSIFICATION OF EXTRA VIRGIN ARGAN OILS USING RAMAN, NIR SPECTROSCOPY AND CHEMOMETRIC FUSION APPROACHES**

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Accurate determination of the geographic origin of food products is a critical step toward ensuring quality, authenticity, and traceability, especially for high-value commodities such as Extra Virgin Argan Oil (EVAO)[1-2]. In this study, a comprehensive analytical approach combining Raman and Near-Infrared (NIR) spectroscopy, along with chemical profiling and advanced chemometric modeling, was used to classify EVAO samples from five distinct Moroccan Argan forest regions.

A total of 95 authentic EVAO samples (19 per region) were analyzed using both Raman and NIR spectroscopy. These spectral datasets were processed using standard preprocessing techniques, including baseline correction, Savitzky-Golay smoothing, and normalization. Beyond spectroscopy, the oils were chemically characterized through measurements of acidity, peroxide value, spectrophotometric indices (K232, K270), and detailed composition analyses including fatty acids, tocopherols, and sterols key indicators of oil quality and origin.

To assess the capability of each technique independently and in combination, classification models were developed using Support Vector Machines (SVM), Random Forests (RF), and k-Nearest Neighbors (k-NN). Model training and evaluation were performed using stratified cross-validation and external validation to ensure generalizability. The study also implemented low-level data fusion (concatenation of NIR and Raman features) to evaluate whether a multi-sensor approach could improve classification performance.

Results showed that both Raman and NIR spectroscopy independently provided moderate-to-high classification accuracies for geographic origin prediction, with RF consistently outperforming SVM and k-NN in both modalities. NIR spectra captured broader compositional trends, particularly in the fatty acid and sterol regions, whereas Raman spectroscopy highlighted specific molecular vibrations associated with unsaturation and phenolic compounds. Data fusion significantly improved classification outcomes, with the RF model achieving over 92% accuracy across all five regions, highlighting the synergistic benefit of combining spectral data sources.

Furthermore, regression models were applied to predict individual chemical quality parameters from Raman, NIR, and fused spectra. RF and SVR yielded high R^2 values (>0.90) for key predictors such as oleic acid and total tocopherol content, demonstrating the models' ability not only to classify origin but also to serve as predictive tools for compositional analysis.

This work underscores the utility of combined vibrational spectroscopy and machine learning in high-resolution origin authentication of plant-based products. The integration of Raman and NIR in a unified chemometric workflow presents a powerful, non-destructive alternative to classical analytical methods. In the context of global markets and increasing cases of food fraud, such systems can support regulatory frameworks, consumer protection, and sustainable labeling practices for Geographical Indication (GI)-certified products like Moroccan Argan oil[3].

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P3-046**DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF PRIMARY AROMATIC AMINES BY LC-MS/MS AND LC-HRMS AND THEIR STABILITY UNDER DIFFERENT MIGRATION CONDITIONS**

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Primary aromatic amines (PAA) can occur in food contact materials (FCM) as a contamination from azo dyes synthesis or as a result of azo bond cleavage under reducing conditions. Besides colorants, other sources of PAA include e. g. residues of isocyanates in polyurethane-based adhesives, which can be hydrolyzed to PAA in aqueous environments, or co-monomer addition in polyamide manufacturing. Thus, plastic kitchenware, e. g. colored polyamide utensils, but also paper and board FCM – especially those made from recyclates containing e. g. printing inks – can be relevant sources of PAA exposure for consumers. When PAA migrate from FCM into food, this may raise toxicological concerns as some congeners of this diverse substance class are classified as carcinogenic to humans according to Regulation (EC) No 1272/2008 (CLP Regulation). Therefore, migration into food is restricted according to Regulation (EU) No 10/2011 for plasticware and the BfR Recommendation XXXVI for paper and board FCM to be non-detectable, with a specified limit of detection (LOD) of 0.002 mg/kg food per individual PAA. PAA migration from plasticware is typically tested using 3 % acetic acid, as this is considered to be the worst-case food simulant. However, previous studies reported considerable degradation of PAA under acidic conditions [1, 2] which might result in an underestimation of PAA migration.

In this work we present a multi-analyte method for the determination of 42 PAA using liquid chromatography-mass spectrometry (LC-MS) on a biphenyl analytical column (150 x 2.1 mm ID, 3 µm particle size) within a total run time of 18 min. The method is suitable for both, triple quadrupole (QQQ) MS instruments as well as high-resolution Orbitrap systems. For most analytes, both setups yielded LODs below 2 ng/mL as defined in Regulation (EU) No 10/2011. The tandem-MS system was typically more sensitive, achieving detection limits of ≤ 0.1 ng/mL. The method was successfully applied for the direct analysis of PAA in cold water extracts of paper FCM and was characterized by high inter-day precision (< 10 %) and recoveries (95–105 %) for most analytes. Additionally, we compare migration of PAA from polyamide kitchenware utensils into 3 % acetic acid and water, and present data on the stabilities of PAA standards during storage in solvents and cold water extracts. The results will help to further optimize the strategy for migration testing of PAA from FCM including the definition of realistic worst-case conditions.

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P3-047**COMPREHENSIVE METABOLOMIC EVALUATION OF NADES-BASED EXTRACTION OF *CROCUS SATIVUS* L. FLORAL BY-PRODUCTS****Campmajó, Guillem; Gjoni, Hilva; Torrijos, Raquel; Dall'asta, Chiara.***Department of Food and Drug, University of Parma, Parma, Italy, Parma, Italy.*

Crocus sativus L. is widely cultivated in various Mediterranean and South-Central Asian countries for the production of saffron, which is derived from its stigmas. However, its floral by-products are usually discarded, despite being a source of phenolic acids, flavonoids, and, to a less extent, crocins. In the context of the current transition toward a circular economy, natural deep eutectic solvents (NADES) have demonstrated great potential for recovering bioactive metabolites from agri-food system by-products [1]. Composed of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), NADES have emerged as a cost-effective, biodegradable, and less toxic alternatives to conventional solvents for phytochemical extraction [2].

The present study aimed to evaluate the use of NADES for extracting phytochemical compounds from a mixture of *Crocus sativus* floral by-products (primarily tepals and stamens), in comparison with conventional organic solvent mixes (water, ethanol, methanol, acetonitrile, and acetone). Twelve different NADES were prepared by thermal mixing at 70°C, combining one of two HBAs — proline or choline — with one of six HBDs: urea, glucose, citric acid, glycerol, ethylene glycol, or 1,2-butanediol. The molar ratio of HBA to HBD and the amount of added water were optimised for each mixture using a central composite design (CCD), based on spectrophotometric analyses (Folin-Ciocalteu, FRAP, and DPPH) performed on the floral by-product sample. The extraction procedure consisted of a solid-liquid extraction (SLE) assisted by magnetic stirring at 30°C.

Furthermore, extracts obtained using the optimised NADES and conventional solvents were analysed by liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC–TWIMS–HRMS) to comprehensively characterise their phytochemical composition. Chromatographic separation was carried out under conventional reversed-phase conditions, using an Acquity BEH C₁₈ column and a mobile phase consisting of water and acetonitrile, both acidified with 0.1% (v/v) formic acid. For HRMS detection, each sample was acquired in both positive and negative electrospray ionisation (ESI) modes using a quadrupole-time-of-flight (Q-TOF) mass analyser, operating in full-scan MS and data independent acquisition (DIA) modes (*m/z* range from 100 to 1,200). Suspect analysis was conducted using UNIFI software, based on an in-house database containing HRMS and TWIMS information of phytochemicals commonly associated with *Crocus sativus*. In addition, non-targeted analysis was conducted using Progenesis software.

As a result, the spectrophotometric analyses not only enabled the optimisation of each NADES combination but also provided a preliminary comparison of their extraction efficiency with that of conventional solvents. For instance, most proline-based NADES exhibited higher extraction capacities according to the DPPH results. Moreover, LC–TWIMS–HRMS allowed an in-depth metabolomic analysis, tentatively identifying more than 50 metabolites through the suspect approach and annotating several others at the compound class level via the non-targeted approach. Overall, the present study highlights the potential of applying LC–TWIMS–HRMS to better understand the phytochemical profiles obtained through NADES extraction and to evaluate their effectiveness as green solvents for recovering bioactive compounds from *Crocus sativus* L. floral by-products.

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P3-048**PHYTOCHEMICAL PROFILING OF RED BEETROOT PRODUCTS: A COMPARATIVE STUDY OF POWDERS AND JUICES****Campmajó, Guillem; Biagi, Marco; Bruni, Renato; Dall'asta, Chiara.***Department of Food and Drug, University of Parma, Parma, Italy, Parma, Italy.*

In recent years, interest in substances that can enhance well-being and improve athletic performance has expanded from professional to amateur athletes. In this context, the consumption of red beetroot (*Beta vulgaris* L.) products has been associated with ergogenic effects, such as improved fatigue management and faster recovery, mostly attributed to their high nitrate content [1]. Moreover, red beetroot is also rich in pigments like betalains (including both betacyanins and betaxanthins), as well as phenolic compounds and organic acids. In fact, betalains may also contribute to endurance capacity, suggesting a potential synergistic interaction with nitrates [2]. Currently, various red beetroot products — mainly in the form of powders, food supplements, or juices — are commercially available for these purposes. However, the lack of standardisation among these products is a critical issue, as the content of bioactive compounds can vary significantly between them.

In this study, the phytochemical profiles of 25 red beetroot products (13 powders and 12 juices) were determined to assess their quality, with emphasis on the standardisation levels of betacyanins, betaxanthins, and phenolic compounds. Samples were extracted using an ethanol:water (70:30, v/v) mixture prior to analysis by liquid chromatography–traveling wave ion mobility spectrometry–high-resolution mass spectrometry (LC–TWIMS–HRMS). LC separation was carried out on an Acquity BEH C₁₈ column (100 mm x 2.1 mm, 1.7 µm particle size), using water and methanol — both acidified with 0.1% (v/v) formic acid — as the mobile phase components. The LC system was coupled to a Vion IMS-QTOF mass spectrometer via an electrospray ionisation (ESI) interface. Each sample was injected twice to acquire data in both positive and negative ESI modes. Moreover, HRMS acquisition was performed in high definition MS^E mode (*m/z* range 100–1000), which combines data independent acquisition (DIA) with ion mobility. Suspect screening analysis was conducted for metabolite identification using information compiled from the literature and online databases.

The proposed methodology enabled the tentative identification of approximately 80 metabolites, primarily betacyanins, betaxanthins, and phenolic compounds. In general, powders exhibited a higher abundance of phytochemicals compared to juices. Particular attention was given to the betacyanin profile, which included betanin, isobetanin, and other metabolites resulting from decarboxylation and/or dehydrogenation degradation processes induced by thermal, light, or humidity conditions. In this regard, the results revealed significant variability among the analysed samples, highlighting the challenges in standardising these types of food products.

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P3-049**VOLATILOMICS FOR THE AUTHENTICATION OF POMEGRANATE JUICE USING GAS CHROMATOGRAPHY COUPLED TO ION MOBILITY SPECTROMETRY****Rocamora-Rivera, Blas; Arroyo-Manzanares, Natalia; Viñas, Pilar.***Department of Analytical Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100 Murcia, Spain, Murcia, Spain.*

Pomegranate (*Punica granatum* L.) is considered a luxury fruit, whose juice has well-known healthy benefits, particularly a high antioxidant activity. Its constantly increasing demand always surpasses the production, resulting in an elevated price. This context makes this juice vulnerable to adulteration. This practice can consist of diluting with cheaper juices like apple and grape juice what allows to increase the profits. Other reported strategy is to add dark juices like black grape or blueberry juices to mask poor-quality juices and to imitate the colour of pomegranate juice. ^[1-3] In order to detect the adulteration and ensure the authenticity of this product, food industry measures many physicochemical parameters with several instruments, which is expensive and time-consuming. Therefore, an analytical method to control the quality with a single measurement is highly desired. Besides, almost all the reported methodologies are based on the determination of polyphenols, anthocyanins or sugars present in the pomegranate juice and, in this context, the volatile organic compounds (VOCs) profile of this food remains underused. With the aim of studying the applicability of these analytes for the quality control of pomegranate juice, a methodology based on the analysis of VOCs profile obtained by headspace gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS) was developed and validated.

Spectra of 176 genuine pomegranate juices and 120 different mixtures of these pure juices with adulterants were analyzed in a non-targeted way. The mixtures were prepared with seven apple juices and three black grape juices (levels from 1 to 40%) and two blueberry juices (levels from 0.1 and 10%). All collected data were used for the construction of chemometric models resulting in the next strategy. Firstly, a model based on orthogonal partial least squares discriminant analysis (OPLS-DA) was built to classify the sample into pure or adulterated pomegranate juice with 100% validation success rate. If the sample is considered adulterated, knowing the illegally added juice could be of interest. For this reason, another OPLS-DA model was developed to distinguish between the three adulterants studied with 87.5% validation success rate. The incorrectly classified juices correspond to low levels of adulterants (0.1 and 0.5%). Finally, three partial least squares (PLS) regressions were constructed to quantify the percentage of adulterant in the juice. This strategy was successfully applied to seven commercial juices. The classification of these samples corresponds with the declared ingredients in the labels in six out of the seven cases.

In this work, the potential of VOCs for the authentication of pomegranate juice was demonstrated with a chemometric model able to detect until 1% of adulteration with apple and black grape juice and until 0.1% with blueberry juice. In addition, the proposed methodology can distinguish between these three adulterants and quantify their levels in the samples. All this information on a sample can be obtained with a single HS-GC-IMS measurement. This technique has many advantages, such as low operation cost, high sensitivity and it is more eco-friendly than GC-Mass Spectrometry because the IMS spectrometer works at atmospheric pressure. ^[4] The excellent results obtained together with the minimal sample treatment, the environmental sustainability provided by IMS and the short analysis time (16 min) make the proposed methodology a powerful tool for use in the quality control of pomegranate juice.

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P3-050**NOVEL APPROACH TO TITRATION ANALYSIS USING CONTINUOUS FLOW**

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Flow analysis techniques offer numerous possibilities for automating analytical methods, including titration analysis. Using flow-based systems, titration can be performed according to traditional procedures or by employing novel concepts of its implementation. These methods differ in how the sample is introduced into the flow system, how it is combined with a titrant solution, and often in how the analyte is determined. Based on these aspects, generally developed titration methods can be grouped into approaches that rely on continuous flow of sample and titrant – propelled at constant or variable flow rates – discrete addition of titrant (or sample) to a continuous sample (or titrant) stream, volumetric fraction manipulation, or a combination of these approaches [1].

Acidity is an important parameter measured during the analysis of raw materials and food products. It is mainly affected by the presence of organic acids, fatty acids, as well as proteins and their decomposition products. Acids in food have different origins; they may occur naturally in products, be added for acidification, stabilisation, or preservation, or form as a result of fermentation and microbial activity. Deviations from the specified acidity levels may indicate undesirable biological and chemical processes or errors in the technological procedures. The most common method for determining total acidity is titration of a sample with a strong base.

The presented research concerns the development of a novel titration procedure using a flow system with spectrophotometric detection. This method is based on continuous, controllable dilution of a sample with a titrant across a broad concentration range. The approach was tested on acid-base titrations, where acids in the sample reacted with sodium hydroxide in the presence of phenolphthalein as an indicator. The study included selecting optimal conditions for flow-mode titration, such as the flow rates of the sample and titrant streams, and developing a way to determine the endpoint of titration. The method was validated for model samples by determining citric and tartaric acids. The results obtained were characterised by very good accuracy and precision. The developed titration procedure takes about 2 min and requires the use of 1 mL of both sample and titrant. The usefulness of the method was confirmed by its application to the determination of acidity of various non-alcoholic beverages and wines, using potentiometric titration as the reference method.

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P3-051**CHEMOMETRIC ANALYSIS OF POLYPHENOLS FOR THE CLASSIFICATION OF DARK CHOCOLATE PRODUCTS**

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Chocolate is the most widely consumed cocoa-derived product, valued for both its sensory qualities and health benefits, primarily due to its high content of bioactive compounds, especially polyphenols [1]. The polyphenolic profile depending on factors such as cocoa bean variety, cultivation practices, geographical origin, post-harvest conditions and processing methods. Due to this variability, polyphenols are useful chemical markers for characterizing and differentiating chocolate products [2]. This study aims to assess the polyphenolic profile of dark chocolate samples to evaluate their potential for distinguishing products based on cocoa origin, cocoa content, variety, and certification status (e.g., organic or fair trade).

A total of 94 dark chocolate samples with varying declared origins, cocoa contents, bean varieties, and certification labels were analyzed. Polyphenols were extracted using ultrasound-assisted extraction with a methanol:water:formic acid (60:39:1) solvent mixture. The extracts were analyzed using high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS). Multivariate analysis using Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were applied to explore patterns and classify samples according to their declared features.

Several polyphenols, including proanthocyanins, 3-flavonols, and phenolic acids, were consistently detected across samples. The chemometric results revealed patterns, and PLS-DA achieved high classification performance, particularly with respect to cacao content and geographical origin. These findings highlight the potential of polyphenolic profiles as reliable biomarkers for the traceability and classification of dark chocolate.

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P3-052**IN-VITRO AND URINARY METABOLIC PROFILING OF THE TWO RUBBER-RELATED COMPOUNDS, N-(1,3-DIMETHYLBUTYL)-N'-PHENYL-P-PHENYLENEDIAMINE (6PPD) AND HEXAMETHOXYMETHYLMELAMINE (HMMM)**

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and hexamethoxymethylmelamine (HMMM) are commonly used in rubber products as tire anti-degradants and cross-linking agents, respectively. However, in recent years, concern about their extensive use has been growing, as both compounds are readily leached from polymeric materials and accumulate in environmental ecosystems ^[1]. Moreover, 6PPD undergoes oxidative degradation to 6PPD quinone (6PPD-Q), a compound known to be highly toxic to some aquatic species ^[2], and dehydrogenation to 4-hydroxyphenylamine (4-HDPA) and N-(4-(1,3-dimethylbutyl)amino)2,5-cyclohexadiene-1-ylidene (6PPD-I) ^[3]. Nonetheless, despite their prevalence and potential hazards, there is limited information on the human health risks associated with these substances, and data on their metabolism remain scarce, hindering comprehensive risk assessment efforts. Thus, in this work, Phase I and II *in-vitro* biotransformation of HMMM, 6PPD and its transformation products, 6PPD-Q and 4-HDPA, was investigated using human liver microsomes (HLMs) and cytosol (HLCYT). Samples were analyzed using liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF) with a suspect screening workflow to identify the potential metabolites.

6PPD *in-vitro* assays confirmed that 6PPD-I is formed exclusively by an abiotic pathway, whereas 6PPD-Q and 4-HDPA may have a dual origin, from both abiotic degradation of 6PPD and liver metabolization. Additionally, direct hydroxylation and glucuronidation reactions led to the identification of two metabolic products: 6PPD-284 and 6PPD-444. Phase II biotransformation of the Phase I metabolite 4-HDPA also resulted in the formation of glucuronide and sulfate conjugates, 6PPD-265 and 6PPD-361. These Phase II metabolites were further confirmed by 4-HDPA *in-vitro* assays. Similarly, *in-vitro* metabolism studies of 6PPD-Q identified four additional metabolites. Consecutive hydroxylation reactions yielded di-, and tri-hydroxylated derivatives. Moreover, Phase II conjugation of the mono- and di-hydroxylated metabolites led to the identification of two sulfation products: 6PPDQ-394 and 6PPDQ-410. Regarding the *in-vitro* metabolism of HMMM, only a single Phase I oxidation product, HMMM-360, was identified. As with 6PPD-Q and 4-HDPA, this product has previously been reported in the literature as an abiotic transformation product ^[4], so future studies should evaluate the chemical exclusivity for use as a biomarker of human exposure to 6PPD and HMMM.

Finally, *in-vivo* analysis using pooled urine samples from a non-occupational exposure population revealed that HMMM was the unique precursor positively identified above blank levels. Moreover, 4-HDPA, 4-HDPA glucuronide (6PPD-361) and the tri-hydroxylated metabolite of 6PPD-Q (6PPDQ-346) were tentatively identified in the pooled urine samples.

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P3-053

LC-MS BASED PROTEOMIC PROFILING OF FECAL SAMPLES IN IBD, IBS AND HEALTHY INDIVIDUALS

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Introduction: Inflammatory Bowel Disease (IBD) and (IBS) are two prevalent gastrointestinal disorders that significantly impact patient's life, yet they differ fundamentally in their underlying mechanisms and clinical management. IBD encompasses Crohn's disease (CD) and ulcerative colitis (UC), which is chronic disease marked by cycles of intestinal inflammation[1]. It affects approximately 2.5-3 million patients in Europe[2] and more than 7 million patients worldwide[3]. Subclinical inflammation often persists during clinical remission, contributing to irreversible damage and complicating disease management.

In contrast, IBS is a disorder of the gut-brain interaction defined by recurrent abdominal discomfort associated with altered bowel habits, without any overt structural or biochemical abnormalities[4]. Despite the differences in etiology and pathology, IBD and IBS can present with overlapping symptoms,[5] which often brings a diagnostic challenge for clinicians. Adding complexity to this challenging situation is the reality that around 40% of IBD patients report abdominal symptoms even without active inflammation[6], resulting in unnecessary diagnostic procedures and treatment interventions. Thus, precise and non-invasive tracking of this concealed inflammation is vital for timely intervention and successful management of the disease over time.

Traditional diagnostic methods, including colonoscopy and fecal calprotectin tests (FC), often involve invasive procedures or suffer from specific issues, respectively. Current diagnostic methods also have limitations in accuracy and invasive damage[7], thus, it is necessary for developing a more precise and non-invasive markers to monitor disease activity. In the study, we aim to find new proteins that can differentiate among IBD patients, IBS patients and healthy individuals for a potential biomarker of further investment of non-invasive disease diagnose and monitoring.

Methods: Fecal samples were homogenized in urea buffer containing protease inhibitors to extract proteins. The homogenates were centrifuged to remove particulate matter and bacteria, and the supernatants were collected for protein quantification and then the samples were then normalized accordingly. Protein extracts were subjected to reduction, alkylation, and enzymatic digestion, typically using Trypsin/LysC, to generate peptides. The resulting peptides were desalted using C18-based solid-phase extraction and dried under vacuum. Peptides were reconstituted in an LC-MS-compatible buffer and injected into a high-resolution LC-MS system equipped with a reverse-phase C18 column. Data were acquired in data-independent acquisition mode, and raw files were processed using established software pipelines for peptide identification and label-free quantification. After the LC-MS analysis, the enzyme-linked immunosorbent assay (ELISA) will be used to measure the protein concentration in the fecal sample for verification[8].

Results: The LC-MS analysis resulted in the identification of over 500 proteins from fecal samples. Principal Component Analysis (PCA) was used for exploratory visualization and revealed clear clustering patterns corresponding to the different groups (IBD, IBS, and healthy controls). The separation in PCA indicated underlying proteomic differences among the groups. To further investigate these differences, ASCA (ANOVA-Simultaneous Component Analysis) was applied. ASCA identified several proteins that significantly contributed to group separation, highlighting potential biomarkers relevant to disease status. A candidate protein ranking list will be created for further ELISA verification. These findings suggest that fecal proteomics can effectively differentiate between IBD, IBS, and healthy individuals, supporting its potential as a non-invasive tool for gut health assessment.

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P3-055

AN INTEGRATED APPROACH FOR SENSITIVE BOTTOM-UP ANALYSIS OF PROTEIN BIOMARKERS: ON-LINE APTAMER AFFINITY SOLID-PHASE EXTRACTION AND IMMOBILIZED ENZYME MICROREACTOR CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Nowadays, it is widely accepted that protein biomarkers can be reliably measured by targeted bottom-up analysis, through the characteristic signatures of surrogate peptide fragments [1]. However, significant off-line sample handling is required to purify, enrich, and enzymatically digest the protein of interest before the analysis by high performance separation techniques, including liquid chromatography and capillary electrophoresis coupled to mass spectrometry (LC-MS and CE-MS, respectively).

To address these limitations, we present a straightforward valve-free approach for the sensitive, reliable, and high-throughput targeted bottom-up analysis of proteins by on-line aptamer affinity solid-phase extraction and immobilized enzyme microreactor capillary electrophoresis-mass spectrometry (AA-SPE-IMER-CE-MS) [2]. The method was developed by analyzing α -synuclein (α -syn), which is a protein biomarker related to Parkinson's disease. Under optimized conditions, on-line purification and preconcentration of α -syn, enzymatic digestion, electrophoretic separation and identification of the tryptic peptides by mass spectrometry was achieved in less than 35 min. The limit of detection was $0.02 \mu\text{g mL}^{-1}$ of digested protein (66.7% of coverage, i.e. 8 out of 12 expected tryptic peptides were detected). This value was 125 and 10 times lower than for independent on-line digestion by IMER-CE-MS ($2.5 \mu\text{g mL}^{-1}$) and on-line preconcentration for intact protein analysis by AA-SPE-CE-MS ($0.2 \mu\text{g mL}^{-1}$) [3]. The repeatability of AA-SPE-IMER-CE-MS was adequate (at $0.5 \mu\text{g mL}^{-1}$, %RSD ranged from 3.7 to 16.9% for peak areas and 3.5 to 7.7% for migration times of the tryptic peptides) and the modified capillary could be reused up to 10 analyses with optimum performance, similarly to IMER-CE-MS. The method was subsequently applied to the analysis of endogenous α -syn from red blood cell lysates. Ten α -syn tryptic peptides were detected (83.3% of coverage), enabling the characterization and localization of post-translational modifications of blood α -syn (i.e. N-terminal acetylation).

In the future, the presented method could be easily adapted to analyze other protein biomarkers or biopharmaceuticals using other specific aptamers and trypsin, as well as other specific proteolytic enzymes to achieve complementary sequence and post-translational modifications coverage. We also foresee the potential to further lower the limit of detection through the use of more sensitive and selective tandem mass spectrometry (MS/MS) methods.

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P3-056

SELECTIVE MEASUREMENT OF BRANCHED-CHAIN AMINO ACIDS USING AMINOACYL-tRNA SYNTHETASES AND APPLICATION TO PAPER-BASED ANALYTICAL DEVICES FOR COLORIMETRIC DETECTION

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Branched-chain amino acids (BCAAs) constitute approximately 40% of the essential amino acids in muscle proteins and serve as an energy source during physical exercise [1]. Among the canonical amino acids, valine (Val), leucine (Leu), and isoleucine (Ile) are BCAAs. BCAAs are vital for maintaining muscle performance, preventing fatigue during exercise, and aiding recovery [1], and their measurement is critical for assessing nutritional status, health, and disease conditions.

High-performance liquid chromatography is an effective method for amino acid analysis. However, it is time-consuming, and the equipment and reagents are expensive. Enzymatic analysis offers a low-cost, rapid alternative for amino acid analysis. Leu dehydrogenase is commercially available; however, it exhibits group selectivity for Val, Leu, and Ile, and it cannot differentiate between them [2]. Therefore, selective analysis of individual BCAAs remains challenging due to the limited selectivity of conventional enzymes.

Our previous research has shown that aminoacyl-tRNA synthetases (aaRSs) can serve as highly specific recognition elements for the 20 canonical amino acids, including Val, Leu, and Ile [3–5]. Each aaRS specifically recognizes its corresponding substrate amino acid.

In this study, we explore the enzymatic detection of Val, Leu, and Ile among 20 amino acids using their corresponding aaRSs as recognition elements. Based on our findings, a paper-based analytical device (PAD) for Val, Leu, and Ile was developed using filtration paper: a novel analytical method for the selective detection of Val, Leu, and Ile was developed using valyl-tRNA synthetase (ValRS), leucyl-tRNA synthetase (LeuRS), and isoleucyl-tRNA synthetase (IleRS), respectively. The binding affinity and selectivity of each aaRS were evaluated using a microplate reader. PADs were fabricated for the detection of these amino acids, assessing their potential as practical tools for clinical diagnostics. To achieve the simplicity and rapid analysis, PADs with straightforward structures were developed.

The calibration curves, and selectivity of each aaRS were assessed using a microplate reader. Trinder's reagent was used as the colorimetric reaction following aaRS reaction. Additionally, Val, Leu, and Ile analysis was conducted on real food samples to evaluate practical applicability. Linear relationships were established for Val (2–75 mM; correlation coefficient: $R^2 = 0.959$), Leu (2–50 mM; $R^2 = 0.984$), and Ile (1–100 mM; $R^2 = 0.982$), with detection limits of 18.5, 7.6, and 8.4 mM, respectively, using the microplate reader. Furthermore, PAD exhibited linear relationships for Val (7–80 mM; $R^2 = 0.978$), Leu (9–80 mM; $R^2 = 0.956$), and Ile (19–80 mM; $R^2 = 0.961$), with detection limits of 2.2, 3.1, and 6.3 mM, respectively. The analysis was completed within 15 min.

Trinder's reagent, a colorimetric reaction reagent, ensures ease of use and safety owing to its neutral pH. Furthermore, it facilitated the development of PAD and analysis of Val, Leu, and Ile. The findings of this study contribute to the advancement of robust biomedical devices precisely for amino acid analysis. High-resolution detection was accomplished using a USB camera and novel RGB value analysis equations.

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P3-057

PORTABLE PCR-BASED GENOSENSING FOR POINT-OF-CARE DETECTION OF NEONATAL STREPTOCOCCUS AGALACTIAE INFECTIONS

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Streptococcus agalactiae (Group B Streptococcus, GBS) is a leading cause of neonatal infections globally, associated with maternal sepsis, stillbirth, and both early- and late-onset neonatal sepsis. Up to 35% of pregnant women may be colonized, with a disproportionately high disease burden in low-resource settings. Current detection relies on culture-based methods that are time-consuming, require specialized infrastructure, and are impractical for rapid clinical use, particularly in newborns, where sample volumes are limited, and turnaround times are critical. This research presents the development of a portable, point-of-care screening platform combining a handheld thermocycler with an electrochemical genosensing system. The assay employs double-tagging endpoint PCR targeting a GBS-specific gene, using a thermostable DNA polymerase. Various bacterial lysis protocols were evaluated, along with the assay's specificity and analytical sensitivity. Importantly, the method has already been applied to the analysis of clinical vaginorectal swab samples, with successful detection of GBS-positive cases, supporting its potential for real-world application. Ongoing work includes expanding validation with different biological matrices (e.g., blood) and assessing the system's performance across broader clinical scenarios. The proposed platform offers a rapid, simple, and accurate alternative for GBS screening, with significant implications for improving maternal and neonatal outcomes—especially in settings where conventional microbiological methods are difficult to implement.

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P3-058

SENSITIVE AND RELIABLE ANALYSIS OF microRNA BIOMARKERS IN SERUM BY ON-LINE SOLID-PHASE EXTRACTION CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY USING SILICON-BASED SORBENTS

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MicroRNAs (miRNAs) are single-stranded, 19-23 nucleotides long, nonprotein-coding RNAs, whose dysregulated expression has been associated with cancer [1]. The methods currently applied for routine analysis of miRNAs are very sensitive (e.g. quantitative polymerase chain reaction, RT-qPCR), but do not allow the direct detection of the target miRNAs or their post-transcriptional modifications, which have been reported to affect miRNA stability and regulation. Therefore, novel sensitive and selective methods are required for the reliable characterization and quantification of miRNAs.

Nowadays, solid-phase extraction (SPE) kits are commercially available for the rapid and efficient purification of miRNAs, exploiting the high affinity of RNAs towards silicon-based sorbents, including silica, silicon carbide, glass, or diatomaceous earth [2]. This study introduces on-line solid-phase extraction capillary electrophoresis-mass spectrometry (SPE-CE-MS) for the purification, preconcentration, separation, and characterization of endogenous miRNAs and their post-transcriptional modifications in serum, addressing current methodological gaps. Two commercially available sorbents of SPE spin-column kits based on a silicon carbide resin (SiC) and a silica membrane (SiO₂mb) were investigated for the preparation of the SPE-CE microcartridges. The small particle size of SiC made necessary the use of a frit in the microcartridge outlet, while SiO₂mb microcartridge was fritless. The microcartridge was integrated in-line near the entrance of the separation capillary and no valves were necessary for the operation. After loading a large volume of sample (~30 µL), the capillary was rinsed to eliminate non-retained molecules and filled with background electrolyte. Then, the analyte was preconcentrated eluting in a small volume of an appropriate solution (i.e. a mixture water:acetonitrile) before the separation and detection by MS.

Under the optimized conditions with standards, the microcartridge lifetime was similar for both sorbents (around 25 analyses), and repeatability was good (<8.0% RSD for migration times and peak areas). The limit of detection (LOD) for SiO₂mb-SPE-CE (1 nmol·L⁻¹) was slightly lower than for SiC-SPE-CE and 100 times lower than by CE-MS (100 nmol·L⁻¹). In contrast, SiC-SPE-CE gave better results than SiO₂mb-SPE-CE for the analysis of endogenous miRNAs in serum samples probably due to better selectivity of SiC for the enrichment of small RNAs [3]. The potential of SiC-SPE-CE-MS to screen for B-cell chronic lymphocytic leukemia (CLL) was demonstrated analyzing serum samples from healthy controls and patients. Cancer miRNA biomarkers, specifically miR-21-5p and a 5'-phosphorylated miRNA with 3'-uridylation (iso-miR-16-5p), were only detected in the CLL patients [4].

The results achieved demonstrate that SPE-CE-MS is as a promising technique for the direct, high-throughput, sensitive and multiplex analysis of miRNAs and their post-transcriptional modifications in complex samples. It could become a unique complement to current indirect methods applied in routine analysis.

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P3-059**DEVELOPMENT OF AN ANALYTICAL METHOD FOR SITAGLIPTIN USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)****Çim Tunçer, Dilhe Nur¹; Özer Ünal, Durişehvar².**

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Diabetes characterized by hyperglycemia is estimated to affect over 180 million people worldwide, with type 2 diabetes accounting for approximately 90-95% of these cases. Type 2 diabetes results from defects in insulin secretion, insulin action, or both [1]. Various pharmacological agents targeting relative insulin deficiency or insulin resistance are available for the treatment of type 2 diabetes. However, these agents have several limitations, including suboptimal control of postprandial hyperglycemia, an increased risk of hypoglycemia, weight gain, gastrointestinal side effects, and edema, among others [2]. Recent research has focused on identifying new therapeutic targets and approaches to correct impaired glucose homeostasis [3]. Sitagliptin is an agent approved for use in patients with type 2 diabetes. Sitagliptin (MK-0431) is an oral, potent, and selective dipeptidyl peptidase-IV (DPP-4) inhibitor developed for the treatment of type 2 diabetes [4].

The chemical stability of pharmaceutical molecules is crucial, as it affects the safety and efficacy of the drug product. European Medicines Agency (EMA) and International Conference on Harmonisation (ICH) guidelines emphasize the necessity of stability testing data to understand how the quality of an active substance and drug product changes over time under the influence of various environmental factors. Degradation studies are required for the development of new active substances and new drug products.

The study developed and validated a method for the quantification of the active pharmaceutical ingredient sitagliptin at nanogram levels using an HPLC device.

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P3-060

DEVELOPMENT OF POLYFUNCTIONALIZED DENDRITIC NANODEVICE AS A LABELING ELEMENT APPLIED IN THROMBIN-SPECIFIC BIOSENSOR

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Early detection, diagnosis and continuous follow-up of cardiovascular diseases could drastically decrease the burden of diseases, improve the quality of life of patients and their families, among other aspects, given that these diseases have positioned themselves as the leading cause of death in many countries [1, 2]. Therefore, an improvement of existing analytical methodologies and the development of innovative technologies that allow highly sensitive and specific detection of biomarkers for these diseases is a priority [3].

On these bases, here we describe the use of PAMAM G-4.5 dendrimer as core nanomaterial to assemble a novel enzyme-free signal enhancer nanodevice for thrombin determination. It was achieved by covalent attachment of specific anti-thrombin aptamers on the PAMAM surface and *in situ* growth of catalytic Pt nanoparticles (PtNP) encapsulated into the dendron cavities. The resulting labeling element was employed to determine thrombin through a sandwich-type biodetection architecture, by using a screen printed carbon electrode nanostructured with Au nanoparticles (AuNP) and functionalized with capture aptamers as transduction element.

For this work, a thrombin-specific amperometric biosensor has been developed, whose novelty focuses on the use of a PAMAM G-4.5 dendrimer to assemble a novel enzyme-free signal-enhancing nanodevice. For analyte determination, specific aptamers were covalently attached to the PAMAM surface and *in situ* growth of catalytic Pt nanoparticles inside the dendrimer cavities. The whole complex acts as a labeling element of a sandwich-type detection system on screen-printed carbon electrodes nanostructured with Au nanoparticles and functionalized with capture aptamers.

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P3-061**A SINGLE-CELL BIOSENSING PLATFORM FOR TETRODOTOXIN DETECTION AND TOXICOLOGICAL CHARACTERISATION**

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Tetrodotoxins (TTXs) are among the most potent marine neurotoxins, with pufferfish being the principal vector. These toxins exert their effects by blocking voltage-gated sodium channels (VGSCs) in excitable cells, leading to serious gastrointestinal and neurological disturbances that may prove fatal. To date, over 30 TTX analogues have been identified, although their individual contributions to toxicity remain largely unknown. In this work, we introduce the use of an automated patch clamp platform as a high-throughput, cell-based biosensing tool for the detection of TTX and its analogues in pufferfish tissue. By recording electrophysiological alterations in individual Neuro-2a cells exposed to fish extracts, the system demonstrated a half maximal inhibitory concentration (IC_{50}) of 6.4 nM for TTX. The assay's sensitivity was not compromised by the presence of matrix components from various tissues of a TTX-free pufferfish at concentrations up to 10 mg/mL. As a result, a limit of detection (LOD) of 0.05 mg TTX equivalents per kg was achieved (well below the Japanese regulatory threshold of 2 mg/kg). The platform was further employed to determine toxicity equivalency factors (TEFs) for five TTX analogues, which were purified from the liver of a *Lagocephalus sceleratus* specimen collected near Crete, Greece. These TEFs were then applied to naturally contaminated samples, and the results obtained via automated patch clamp were compared to LC-MS/MS quantifications. A strong correlation between the two methods confirmed the reliability of the system. Altogether, this automated patch clamp approach offers a rapid, highly sensitive, and robust toxicological screening tool for evaluating TTX contamination in seafood, contributing to improved food safety and consumer protection.

P3-062

MASS SPECTROMETRY-BASED CHARACTERIZATION OF NEUROCHEMICAL PROFILES IN SCN1AB KNOCKOUT ZEBRAFISH LARVAE

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Introduction: SCN1A gene mutations are closely associated with Dravet syndrome, a severe epilepsy characterized by early-onset, treatment-resistant seizures. Although SCN1A knockout models are widely used, the specific neurochemical changes in these models are not well understood. Understanding neurotransmitter alterations in SCN1A-deficient models could reveal biomarkers or therapeutic targets for seizure control.

Methods: This study employed liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) to profile neurochemical alterations in SCN1ab knockout (–/–) zebrafish larvae, focusing on neurotransmitter systems including amino acid, cholinergic, histaminergic, dopaminergic, serotonergic, kynurenergic, and GABAergic pathways. Metabolite levels were statistically compared between knockout and wild-type larvae using t-tests or ANOVA, with $p < 0.05$ considered statistically significant. Additionally, this broad-spectrum LC–MS/MS platform, capable of quantifying 31 neurochemicals, was utilized to assess neurochemical metabolism through the analysis of precursor-to-metabolite ratios.

Results & Discussion: Significant dysregulation was observed, including increased levels of arginine (ARG), betaine (BET), dopamine (DA), norepinephrine (NE), and glutamine (GLU), and decreased levels of 3-methoxytyramine (3-MT) and 5-hydroxyindoleacetic acid (5-HIAA). These results indicate disrupted neurotransmitter metabolism and homeostasis, potentially contributing to the seizure phenotype. SCN1A deficiency induces significant dysregulation across multiple neurotransmitter systems, reflecting altered metabolic activity that may contribute to the behavioral abnormalities and seizure susceptibility observed in Dravet syndrome. These findings highlight the neurochemical consequences of SCN1A loss and support neurochemical profiling as a valuable translational tool for elucidating disease mechanisms and guiding the development of targeted CNS therapies.

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P3-063**FLOW ANALYSIS SYSTEM FOR DETERMINATION OF SERUM LACTATE DEHYDROGENASE FOR THE NEEDS OF ONCOLOGICAL DIAGNOSTICS**

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The routine method for LDH (lactate dehydrogenase) activity determination is to monitor the increase of NADH concentration at 340 nm. There are some inconvenience in taking measurements in the near-UV region, especially in the case of serum samples analysis. In this work, modification of the routine LDH activity assay based on the use of detection of NADH with ferric ions and ferrozine is proposed [1]. A fully-mechanized Multicommutated Flow Analysis (MCFA) system with optoelectronic photometric detector was developed. The linear range of determination offered by this system is 6.0–200.0 U L⁻¹ LDh with estimated detection limit at 0.2 U L⁻¹. To confirm the analytical usefulness of the proposed flow system, the analysis of real human serum samples was carried out. Moreover, the MCFA system modified with respective LDH inhibitor line was utilized in the analysis of serum samples obtained from patients with cancer [2]. The proposed approach can differentiate oncological serum samples from reference ones based on the LDH fractions activity ratio, even when their total LDH activity level is low.

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P3-064

CITRONELLOL-INDUCED NEUROTOXICITY VIA KYNURENERGIC PATHWAY ACTIVATION: A CROSS-SPECIES, MASS SPECTROMETRY STUDY

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Introduction: Citronellol, a naturally derived fragrance compound commonly found in personal care and household products, is generally regarded as safe due to its volatility and environmental degradability. However, its neurotoxicity has not been previously reported. In this study, we present the first evidence of citronellol's neurotoxic potential using a cross-species approach, combining zebrafish, mice, and human brain organoid and BBB organ-chip models. Mass spectrometry-based neurochemical profiling was performed to uncover mechanistic insights into its CNS toxicity.

Methods: Zebrafish larvae were exposed to citronellol (2, 4, and 8 mg/L), mice administered oral doses (345, 690, and 3450 mg/kg), and human brain organoids and BBB-chip systems were treated with 1, 10, and 100 μ M citronellol. Locomotor activity and anxiety-like behavior were assessed in zebrafish, while histological analysis examined neuroinflammation, apoptosis, and BBB integrity. Targeted LC-MS/MS quantified 31 neurochemicals, focusing on the kynurenine pathway, neurosteroids, and oxidative stress markers in zebrafish larvae, mouse's plasma and brain tissues, and organoid samples.

Results & Discussion: Citronellol induced behavioral impairments in zebrafish, with histological evidence of BBB breakdown and neuroinflammatory responses. LC-MS/MS revealed a significant upregulation of kynurenine-to-3-hydroxykynurenine (3-HK) conversion, accompanied by elevated cortisol and reduced levels of neuroprotective steroids including allopregnanolone and progesterone. These neurochemical disruptions were consistently observed across zebrafish, mice, and organoid models. Critically, BBB organ-chip and in vivo analyses confirmed for the first time that citronellol crosses the BBB. This cross-species investigation demonstrates that citronellol exerts neurotoxic effects through kynurenergic pathway dysregulation, oxidative stress, and steroid imbalance. Given its widespread use, these findings raise important concerns about citronellol's potential risks to human health.

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P3-065**SALIVA STUDY OF CORTISOL LEVELS IN COVID-19 RECOVERED PATIENTS****Dziurkowska, Ewelina¹; Guz-Rzeniecka, Grażyna²; Dziurkowski, Maciej².**¹*Department of Analytical Chemistry, Medical University of Gdansk, Gen. J. Hallera 107, 80-416 GDAŃSK, Poland;*²*Hospital for Nervous and Mental Diseases, Skarszewska 7, 83-200 Starogard Gdanski, Poland.*

Cortisol, secreted by the adrenal cortex, is also known as the stress hormone. Cortisol release follows a diurnal rhythm and is controlled directly by adrenocorticotrophic hormone (ACTH) and indirectly by corticotropin-releasing hormone (CRH). Under physiological conditions, healthy adults, in a stressfree situation, secrete between 10 and 20 mg of cortisol on a daily rhythm. Its highest concentration in the blood is observed in the morning, between 8 and 10 am. The toxic effects of cortisol can damage to the central nervous system, which can consequently impair cognitive function. Elevated cortisol levels relative to a control group have also been observed in individuals with psychotic major depression and schizophrenia spectrum disorders. Some studies indicate that hypercortisolemia may lead to psychiatric disorders and that the accompanying stress may lead to psychosis. The determination of cortisol may be indicated, among other things, in psychiatric disorders, where cooperation with the patient may be difficult. The basic diagnostic material is blood, which makes it possible to determine the concentration of the analysed compounds in both free and protein-bound forms. However, blood collection is associated with numerous inconveniences, such as the obligation to use disposable, sterile equipment and the involvement of qualified persons. In the case of cortisol determination, the stress of blood collection can further affect not only the patient's discomfort but also the level of the compound being determined. In addition, for children or elderly people, the traditional determination of the hormone in the blood is difficult to perform and uncomfortable for the patient. Therefore, monitoring of cortisol levels can be performed in saliva, due to the high correlation between blood and salivary levels of the hormone. The correlation that exists between cortisol concentrations in blood and saliva allows saliva to be used as a valuable diagnostic material. In saliva, the content of the active substance is often lower than in blood, so most analytical procedures developed for their determination require the collection of a larger volume (approximately 1 mL) of biological material for study. In the case of patients using psychotropic drugs, obtaining such a volume of saliva may be difficult, due to emerging side effects such as dry mouth. Taking this into account, we aimed to develop a sensitive analytical method to determine cortisol in a small volume of human saliva (200 µL). The isolation of cortisol was performed using SPE columns, thus reducing the use of volatile solvents, and the determination was performed using HPLC with DAD, further reducing the cost of the study. The linearity of the method was determined in the range of 4-500 ng/mL ($R^2 > 0.9986$) and the intra- and inter-day precision expressed as coefficient of variation (CV%) did not exceed 12%. The method was then applied to determine cortisol levels in the saliva of post-COVID-19-recovered patients and healthy volunteers. The determined cortisol levels were 12.24 ± 7.33 ng/mL in the recovered patients and 4.11 ± 1.46 ng/mL in the healthy subjects, respectively. A comparison of the results showed that cortisol levels in the recovered patients and healthy volunteers were statistically significantly different.

P3-066

DEVELOPMENT AND IMPLEMENTATION OF AN EXPERIMENTAL METABOLITE DATABASE FOR CETOF-MS-BASED CHARACTERIZATION OF THE POLAR METABOLOME IN LIVER

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Capillary electrophoresis coupled to mass spectrometry (CE-MS) is a powerful and valuable technique for untargeted metabolite analysis, especially for polar and charged compounds, often complementing techniques such as liquid chromatography. Although CE-MS provides remarkable selectivity and sensitivity, a persistent challenge in untargeted metabolomics lies in the confident annotation of metabolites. This is partly due to the inherent variability of migration time (MT) in CE and, in many CE-TOF-MS systems, the limited access to MS/MS spectra acquisition(1). To improve confidence in metabolite annotation beyond accurate mass alone which is considered low-confidence complementary tools are essential. Relative migration time (RMT) and in-source fragmentation (ISF), which provides structural information, play a key role in increasing annotation reliability(2). In this work, the experimental metabolite database for CE-TOF-MS applications at the Centre for Metabolomics and Bioanalysis (CEMBIO) was expanded. Originally comprising 327 metabolites analyzed in positive ionization mode and 330 in negative mode, it was extended with 142 additional true standards. The new entries integrate RMT values, accurate masses, adducts, and ISF-derived fragment ions, enabling the detection and annotation of 78 metabolites in positive electrospray ionization mode (ESI+) and 40 in negative electrospray ionization mode (ESI-), covering key classes of biologically relevant compounds. For annotation purposes, ISF spectra were compared against theoretical fragmentation predictions and experimental MS/MS spectra from public databases such as HMDB (<https://hmdb.ca>), highlighting the complementarity and potential of ISF data to enhance metabolite identification confidence when integrated with in silico tools. To improve the usability of the database, all relevant information for compound annotation from experimental CE-TOF-MS data is provided in an open-access online repository within the CEU Mass Mediator environment (https://ceumass.eps.uspceu.es/index_cesearch.xhtml), where users can search metabolites based on RMT and ISF, among several criteria. The expanded database was applied to the metabolomic characterization of liver samples, enabling substantial compound coverage using CE-TOF-MS. In ESI+, 609 signals were detected, of which 102 metabolites, 29 fragment ions, and 15 adducts were successfully annotated, leaving 448 signals unassigned. In ESI-, 183 signals were detected, with annotation of 47 metabolites, 8 fragment ions, and 5 adducts, while 119 signals remained unidentified. Metabolic pathway exploration was also conducted, providing biological context to the results and further underscoring the potential of the developed database as a key tool for CE-TOF-MS-based metabolomic studies in complex biological matrices. To functionally interpret the detected compounds, a metabolite set enrichment analysis was performed using the KEGG pathway database. The results revealed that CE-MS is very well suited to evaluate enrichment in amino acid and nucleotide-related pathways, such as arginine and proline metabolism, arginine biosynthesis, and purine metabolism, highlighting the biosynthetic role of the liver under physiological conditions.

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P3-067

DRESS THE FUTURE: NOVEL COMBINED WEARABLE INTEGRATED SYSTEMS

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Measuring physiological markers is a well-known method of determining an individual's emotional state, which is a crucial topic in the field of human interactions. STARGATE (*DreSs The future: novel combined weARable inteGrAted sysTEms*) is the acronym for the two years project, which aims to develop an emotion recognition system based on physiological signals, specifically by realizing a multi-sensor wearable device capable of recognizing emotions. Although the literature has proposed integrated sensors and actuators, most solutions are custom-made (e.g., smartwatches, glasses clips, biosensors embedded in dress fabric, etc.) and lack a common framework that enables the interconnection of multiple devices. Closing this gap and taking advantage of IoT technologies could enable the development of sophisticated interactive personal networks built on integrated wearable devices. These networks could be able to monitor an individual's emotions, determine their stress level and respond appropriately, and integrate interpersonal communication by acting on wearable actuators. This goal necessitates the measurement of particular physical-chemical parameters, their processing to provide emotional state estimations, and the potential production of sensory outputs. In order to achieve this, the project team supports four STEM Research Units (University of Perugia UNIPG), University of Cassino and Southern Lazio (UNICAS), University of Rome Tor Vergata (UNITOV), CNR Unit) that offer the skills required to develop high-tech systems (such as materials, small-sized integrated electronic components, electrochemical sensors data transfer protocols, estimation algorithms, and integrated detection of physical and chemical quantities).; Additionally, the Academy of Fine Arts of Frosinone (ABAF) represents a Research Unit whose expertise will enable the identification of important applications of the frameworks, identifying case studies that will be turned into prototypes. During the first year, UNITOV has developed electrochemical sensors, capable of analyzing sweat and measuring cortisol levels (follow the Oral presentation n° 611 of A. Licheri); at the same time, UNICAS has developed the interface of these sensors to the portable potentiostat, creating a chronoamperometric system. Furthermore, the preliminary results were presented during the Fashion Week (2025) in Milan by ABAF, in collaboration with CNR and UNICAS. To apply cutting-edge estimate algorithms to the parameters that the sensors will generate, UNIPG, the PI of the STARGATE project, concentrated on signal analysis and parametric estimation. In order to achieve this, UNIPG has created a number of algorithms that divide the problem into linear and non-linear subproblems while improving calculation times and accuracy, beginning with the model knowledge. The findings will be utilized to create algorithms for recognizing stress events, potentially utilizing machine learning methods as well.

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P3-068

APPLICATION AND VALIDATION OF A SEC-BASED METHOD FOR THE ISOLATION OF PS-LIPOSONES IN SERUM SAMPLES FOR PHARMACOKINETIC STUDIES IN AUTOIMMUNE DISEASES

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A novel curative therapeutic approach for the treatment of autoimmune diseases is currently under development. These chronic and complex conditions affect approximately 10% of the global population (Angum, F. et al., 2020) and they result from a failure in immune tolerance, where the immune system mistakenly attacks the body's own cells and tissues (Pujol-Autonell, I. et al., 2015). This innovative therapeutic strategy uses phosphatidylserine (PS)-liposomes loaded with the antigen responsible of triggering the autoimmune response. It provides a promising alternative to existing palliative treatments, which often lead to severe side effects. By encapsulating the autoantigen, PS-liposomes aim to induce antigen-specific immune tolerance by mimicking apoptotic cells and efferocytosis. This platform enables the targeting of multiple autoimmune diseases—including type 1 diabetes, rheumatoid arthritis (RA), multiple sclerosis, and myasthenia gravis (MG)— simply by modifying the encapsulated peptide. For the successful commercialization of PS-liposomes, robust bioanalytical methods are essential to isolate and characterize them from biological fluids, thereby supporting preclinical pharmacokinetic and safety studies. However, isolating PS-liposomes from biological matrices remains challenging due to the presence of extracellular vesicles (EVs) and other components with similar sizes and compositions (Boriachek, K. et al., 2019). Despite the availability of bibliographical information about EV isolation, protocols for liposome isolation from biological fluids are scarce.

A Size Exclusion Chromatography (SEC) method was previously developed by our group for the isolation of PS-liposomes for the treatment of MG, obtained from fortified minipig serum samples, achieving good results for both lipid and autoantigen components. In this work, we evaluated the applicability of this methodology to minipig serum fortified with PS-liposomes targeting other conditions, such as RA and Celiac Disease (CD), obtaining similarly favourable results. Additionally, the method was successfully applied to rat and human serum samples fortified with PS-Liposomes for the treatment of MG. Prior to analysing real samples, we evaluated the potential loss of PS-liposomes during the serum obtention process from blood. The results indicated that this loss was not significant. To assess the applicability of the established isolation method for pharmacokinetic studies, it was applied to real samples obtained from rats treated with PS-liposomes for MG. The results confirmed the method's suitability for pharmacokinetic analysis, allowing the detection of liposomal components, phosphatidylcholine (PC) and cholesterol, at 1-, 5-, and 15-minutes post-administration. However, PS was not detected, likely due to its rapid absorption. Finally, the method was validated in terms of repeatability, reproducibility, and sensitivity, with established limits of detection (LOD) and quantification (LOQ) for each component of the MG-PS-liposomes.

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P3-069**SWEAT-BASED PROTOCOLS FOR RELIABLE DETERMINATION OF CREATININE AND CYSTATIN-C IN CHRONIC KIDNEY DISEASE**

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Creatinine and Cystatin-C are key biomarkers used to estimate eGFR, a critical indicator of kidney function and damage. Current eGFR estimation methods rely on blood or urine analysis, which are invasive and require specialized laboratory equipment. While serum creatinine testing is cost-effective and widely used in clinical practice, Cystatin-C testing presents challenges. Cystatin C exists in different proteoforms, whose biological significance is not fully understood, although their distribution is largely independent of renal function. Additionally, variability in Cystatin-C assay results can occur due to the limited detection of structural modifications by antibodies in PENIA and PETIA assays, leading to inconsistent results. Cystatin-C testing is also approximately ten times more expensive than serum creatinine testing. To address these challenges, we propose a non-invasive protocol using micro-extraction by packed sorbent (MEPS) coupled with targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) to simultaneously measure creatinine, intact Cystatin-C, and its proteoforms in sweat, offering a reliable tool for kidney function monitoring. In the EU-funded KERMIT project, we prospectively enrolled 50 nominally healthy volunteers (local bioethics committee approval n.18/2024) to test the effectiveness of pilocarpine and carbachol in stimulating sweat via iontophoresis, a commonly used non-invasive technique. Sweat samples were collected passively using filter paper (120x80x0.13 mm) pretreated with bovine serum albumin (BSA, 0.5 g/L). These samples were used to develop and validate the MEPS-LC MS/MS platform, following IUPAC guidelines, and to determine the concentration of creatinine and Cystatin-C in the volunteers. Both pilocarpine and carbachol effectively stimulated sweat production. After 30 minutes, pilocarpine resulted in an average sweat volume of 294 μ L (range: 22–815 μ L), while carbachol produced a higher average volume of 331 μ L (range: 48–1148 μ L). Carbachol was generally more effective, providing more sustained sweat production, with significant amounts collected even 3 hours after stimulation. The limit of detection for creatinine was 0.03 mg/dL, and for Cystatin-C was 0.2 μ g/L. MS/MS signals showed excellent linearity ($R^2 > 0.995$) across tested concentrations. Recovery from filter paper was consistent at approximately 80% for both intra- and inter-day measurements. Precision remained robust, with intra- and inter-day coefficients of variation below 15%. Calibration curves from working solutions and spiked sweat samples showed no significant difference at the 95% confidence level, indicating no measurable matrix effect. Importantly, we found that preconditioning materials in contact with sweat samples using BSA was essential to prevent secondary interactions, ensuring consistent Cystatin-C measurements. Creatinine and native Cystatin-C concentrations in sweat from healthy volunteers ranged from 0.1–1.5 mg/dL and 0.5–2 μ g/L, respectively. For the first time, we have identified the presence of Cystatin-C and its proteoforms in sweat. Our MEPS-LC-MS/MS platform enables the simultaneous measurement of creatinine and intact Cystatin-C in sweat, offering a non-invasive, reliable, and alternative method for monitoring kidney function and disease.

Lenzi et al. Development of a High-Throughput Liquid Chromatography-Tandem Mass Spectrometry Platform for the Determination of Intact Natriuretic Peptides in Human Plasma. *Talanta* 2024; 275:: 126077

P3-070**TACKLING COMPLEXATION OF DNA G-QUADRUPLEX STRUCTURES WITH SMALL LIGANDS BY CAPILLARY ELECTROPHORESIS WITH SPECTROPHOTOMETRIC DETECTION AND MULTIVARIATE CURVE RESOLUTION****Hatamli, Kanan; Giménez, Estela; Gargallo, Raimundo; Benavente, Fernando.***University of Barcelona, Barcelona, Spain.*

G-quadruplex DNA (G4) structures are stable, four-stranded conformations that form through Hoogsteen hydrogen bonding between four guanine bases in guanine-rich nucleic acid sequences [1]. In recent years, the interaction between G4 structures and small-molecule ligands has received increasing attention due to its biological relevance and potential applications in both analytical and therapeutic fields. This is particularly relevant in analytical applications, as many aptamers adopt a G4 structure. Ligands such as TMPyP4 can stabilize G4 structures by stacking onto the terminal G-tetrads or interacting within grooves, thus modulating their thermal stability and resistance to denaturation [2]. The binding affinity and selectivity of ligands are often influenced by factors such as G4 topology (parallel, antiparallel, or hybrid), ionic environment, and temperature. Studying these interactions requires analytical techniques capable of resolving subtle conformational changes and distinguishing coexisting species in complex mixtures. Among the widely used methods are circular dichroism (CD), absorbance spectroscopy, fluorescence spectroscopy, and gel electrophoresis techniques.

In this study, as an alternative, we applied capillary electrophoresis with ultraviolet absorption spectrophotometric detection (CE-UV) in combination with multivariate curve resolution–alternating least squares (MCR-ALS) to analyze DNA G-Quadruplex formation and complexation with small molecule ligand TMPyP4 using two different DNAs (Pu22T14T23 and QDJ1). CE-UV analyses were conducted using hydroxypropyl cellulose (HPC)-coated capillaries and a 25 mM cacodylic acid with 50 mM KCl (pH 7.4) buffer, which enabled fast and reproducible separations. Meanwhile, the use of MCR-ALS allowed the deconvolution of overlapping peaks in multiwavelength electropherograms, enabling the resolution of individual spectral components corresponding to different species [3]. This combined approach effectively identified and distinguished within the mixtures between free DNA structures, the ligand, and G4 complexes, making the proposed approach an interesting alternative for the study of G4 – ligand complexation. While CE-UV enabled rapid separation of the different species, MCR-ALS was essential to resolve overlapping peaks and improve the identification of individual components in the complex mixtures.

Acknowledgements

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P3-071

OXYSTEROL ANALYSIS IN SINGLE HUMAN GASTRULOIDS

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Human gastruloids are 3D stem cell-derived models that provide valuable platforms for studying early human development and disease mechanism[1]. Oxysterols – oxidized derivatives of cholesterol – are bioactive lipids involved in key signaling pathways and pathological processes[2]. Their structural similarity, low abundance, and the limited sample volumes of single gastruloids present major analytical challenges. However, investigating oxysterol presence in individual gastruloids sheds light on lipid signaling during early embryogenesis[3].

In this study, a previously published method using liquid chromatography – mass spectrometry (LC-MS)[4] with miniaturized sample preparation was employed to determine and quantify oxysterols present in single human gastruloids. This work establishes a sensitive, reproducible, and greener platform for oxysterol analysis in low-volume biological systems, paving the way for developmental metabolomics and proteomics.

The method was optimized using an Experimental Design setup. Key factors influencing derivatization efficiency were optimized, achieving a tenfold reduction in final reaction volume (from 735 μ L to 75 μ L) without compromising detection limits.

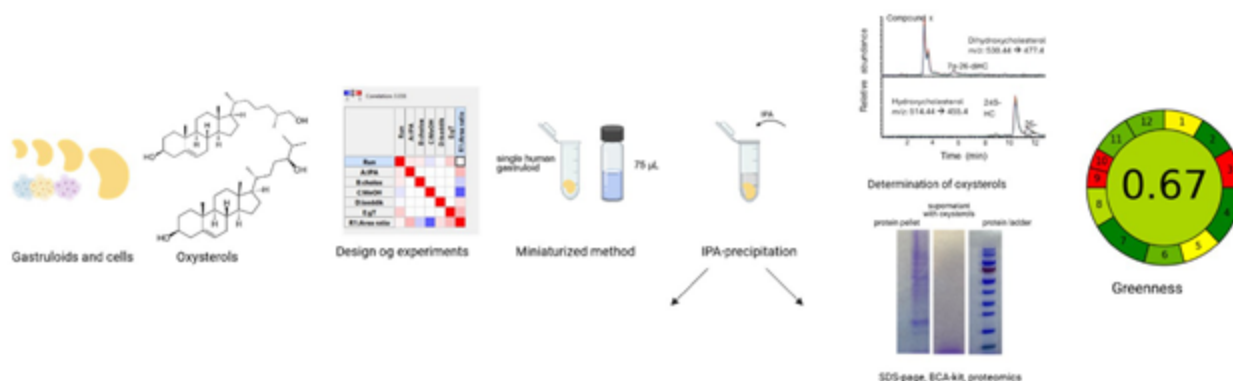
The method was partially validated in terms of linearity ($R^2 > 0.99$), analytical precision ($< 20\%$ RSD), reproducibility (between days and operators), and relative error. All parameters were found to be satisfactory within established analytical standards.

The samples included gastruloids at various stages of development (ranging from the first day after the cells started forming into 3D structures up to day five), as well as the original cells used for aggregation into gastruloids. The aim was to pinpoint when during development the oxysterols emerge in the system and to link that to potential biological functions by inferring possible biological processes and their significance in early embryogenesis.

The miniaturized method was successfully employed to determine and quantify oxysterols present in single human gastruloids. 24S-hydroxycholesterol, 26-hydroxycholesterol, and a dihydroxycholesterol for which no matching standard was available, were detected and quantified in individual gastruloids.

The study also reveals biologically significant heterogeneity among the gastruloids and emphasizes the necessity of single-gastruloid analysis. Hence, it introduces a normalization strategy based on isopropanol (IPA)-induced protein precipitation. This approach enables the correlation of oxysterol concentrations with protein content – measured using a BCA Protein Assay Kit and confirmed by SDS-PAGE gel electrophoresis – as an effective method for separating proteins from lipid-rich supernatants.

Finally, the miniaturized method was assessed using the AGREE metric and showed a 24% improvement in environmental and operator safety compared to the original method.



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P3-072

DEVELOPMENT OF A MICROFLUIDIC PAPER-BASED DEVICE FOR ALUMINIUM QUANTIFICATION IN URINE: POTENTIAL IMPLICATIONS FOR ALZHEIMER'S DISEASE MONITORING

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In this work, the development of a simple and low-cost microfluidic paper-based analytical device (mPAD) for the colorimetric detection and quantification of aluminium in urine samples is presented. Aluminium exposure is an increasing public health concern due to its potential neurotoxic effects and its association with disorders such as Alzheimer's disease and autism spectrum disorder. In this context, it may be highly advantageous to have a simple fast quantification method to evaluate aluminium exposure. The developed device relies on the colorimetric reaction of Al(III) ions through complexation with Chrome Azurol S (CAS) in a paper-based platform. The formed blue-coloured complex is measurable via scanner-based image analysis using a free image-processing software, such as ImageJ. The colour intensity of the product and convert it into pseudo-absorbance values and a linear relationship with the aluminium concentration is established. The microfluidic paper-based device (mPAD) assembly consists of two layers of paper, enabling vertical fluid transport with simple fabrication. The top layer, composed of Whatman Grade 3 filter paper, acts as a physical barrier to mitigate potential interferences from macromolecules in the urine matrix and as protective barrier for the reagents in the bottom layer. The bottom layer, composed of Whatman Grade 1 filter paper, contains the CAS reagent and an acetate buffer solution. Several chemical and physical optimization studies were carried out to improve the performance of the device. One of the major challenges was matrix interferences, particularly phosphate ions, which can form stable complexes with aluminium, hindering the Al-CAS reaction. To address this, AgNO₃ was successfully used as a masking agent, precipitating phosphate and significantly improving the method's selectivity. The mPAD enabled the quantification of aluminium within the concentration range of 0.20 to 2.0 mg/L of Al(III), which covers the levels of interest for exposure monitoring. The limit of detection and limit of quantification were 0.08 and 0.20 mg/L, respectively. The device demonstrated reliable performance for aluminium determination in real urine samples, without requiring complex sample pretreatment. Its simplicity, portability, and selectivity make it a promising tool for rapid and accessible monitoring of aluminium exposure in clinical and environmental settings.

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P3-073

TOWARDS PERFECT MOLECULAR IMPRINTING: UNRAVELING MONOMER-ANALYTE INTERACTIONS

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Molecular imprinting [1] is a powerful technique for designing synthetic polymer matrices with highly specific recognition sites for target molecules, offering broad applications in biosensors, drug delivery, and environmental monitoring. This study presents a detailed computational investigation into the pre-polymerization complex formation of three hazardous food contaminants—Aflatoxin B1, Glyphosate, and Sulfamethoxazole—with 28 functional monomers. By employing a combination of the semi-empirical CREST [2] conformational search method and density functional theory (DFT), this work identifies the most stable conformations and accurately calculates their stabilization energies, providing crucial insights into the molecular interactions governing imprinting efficiency.

Key findings demonstrate that functional monomers containing multiple carboxylic or hydroxyl groups, such as itaconic acid and dopamine, exhibit the highest stabilization energies. In contrast, monomers such as styrene, lacking these functional groups, display significantly weaker interactions. A clustering approach effectively classifies the investigated monomers into two distinct groups based on their stabilization energies, correlating well with their chemical structures and interaction tendencies. Results indicate that templates with highly electronegative functional groups preferentially form stronger interactions with acidic functional monomers, reinforcing the significance of molecular charge distribution in imprinting efficacy. The strongest interactions were observed between the template molecules and functional monomers that formed multiple hydrogen bonds, leading to enhanced stabilization. Conversely, monomers with aromatic or lipophilic structures exhibited significantly lower stabilization energy due to the absence of strong hydrogen bonding capabilities. For each template, the most and least stable complexes were subjected to non-covalent interaction (NCI) [3] analysis, confirming that hydrogen bonding and electrostatic interactions drive the stabilization energy trends observed in the computational results. Aflatoxin B1 formed the most stable pre-polymerization complexes with carboxyl- and hydroxyl-rich monomers, which successfully organized around the polycyclic system, forming an electrostatically driven molecular imprint. Glyphosate exhibited strong interactions primarily through phosphate and carboxyl groups, further reinforced by the monomer's ability to establish hydrogen bonds with all available electronegative centers. Sulfamethoxazole demonstrated high selectivity for monomers capable of interacting with the nitrogen and oxygen atoms in the oxazole and sulfonyl groups, forming specific binding sites within the polymeric matrix. A critical finding of this study is that successful imprinting not only depends on the direct interactions between the template and functional monomers but also on monomer-monomer interactions. NCI analysis confirmed that strong hydrogen bonding and electrostatic attraction define high-stability complexes, while dispersion interactions alone contribute insufficiently to strong binding, leading to weaker pre-polymerization complexes.

The combined CREST/DFT approach provides a reliable and efficient computational workflow for predicting optimal monomer-template interactions, enabling targeted design of molecularly imprinted polymers (MIPs). The insights gained from this study contribute to the advancement of imprinting technology, optimizing monomer selection and polymer design for improved molecular recognition. These findings have significant implications for developing highly selective MIPs for detecting hazardous food contaminants, ultimately improving analytical applications in biosensing, environmental monitoring, and pharmaceutical research. The methodology presented herein establishes a foundation for future *in silico* studies aimed at accelerating the development of molecularly imprinted materials for a wide range of applications.

Acknowledgment

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P3-074**A GREENER IN-VIAL THIN FILM MICROEXTRACTION FOR SEXUAL HORMONES DETERMINATION IN ENVIRONMENTAL WATERS AND BIOFLUIDS**

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Sustainability of sample treatment is a hot topic that can be addressed by miniaturizing techniques and designing more efficient and sustainable sorbents. In such scenario, in-vial microextraction is gaining success as a reliable alternative to other solid-phase extraction setups, as integrates sampling, adsorption and desorption in a single device afore instrumental analysis, clearly ensuring sustainability without compromising analytical performance. Polymeric thin films have proven to be a versatile and efficient materials for microextraction processes[1], but more attention needs to be paid to the preparation steps both in terms of the nature of the polymer and possible co-extractant, and the solvents used during the solubilization process.

In this work, a thin film obtained by polycaprolactone polymer (PCL) was coated, as the extraction phase, on the bottom wall of a 40 mL glass vial. We rationalized the manufacturing steps by dissolving the polymer in a suitable and environmentally friendly solvent (methyl-tetrahydrofuran, derived from renewable biomass) instead of the usual hazardous organic solvents (e.g., chloroform, dichloromethane, tetrahydrofuran), followed by solvent casting process directly inside the vial.

The PCL film-coated vial has been tested, before HPLC separation, for sex hormones, a class of endocrine disrupting compounds that needs to be monitored in both environmental waters and biofluids.

Preliminary tests were done in tap water (15 mL, native pH) to evaluate key extraction parameters, viz. contact time, desorption solvent and conditions. A fast and satisfactory extraction is completed within 30 min, while the desorption of the analytes is faster (only 5 min), by using small volumes of EtOH (0.75 mL) as green solvent, thus avoiding the evaporation step. These results clearly highlight that the lipophilic interactions between the target hormones and the polymer are strong enough to ensure proper extraction of the analytes from the sample without the need for any other extractant, but at the same time, weak enough to guarantee fast and effective desorption. Good analytical performance, relative to accuracy, linearity, matrix effects and limits of quantification, was achieved in raw river water and urban wastewater treatment plant effluent; as well, the greenness of the sample treatment was satisfactory, as indicated by appropriate metric tools (0.66 by AGREEprep and 7.58 by SPMS).

Driven by these results and with the aim of extending the applicability of in vial TF-ME to urine samples, we tested a miniaturized version of the PCL film-coated vials in a 5 mL glass vial adapting the amounts of PCL dispersion to the bottom surface to be coated. In a first series of experiments, extraction tests were done on synthetic urine to confirm the sorption affinity of the target analytes for the sorbent. The sample volume was fixed as the maximum volume of the vial, leaving a small headspace to allow proper mixing of the solution on orbital shaker. Quantitative adsorption was achieved in a shorter extraction time (10 min) without the need for dilution or protein precipitation, thus simplifying sample treatment and/or avoiding any loss of analytes in this last step. For elution, ethanol was again chosen to disrupt analyte-sorbent interactions, further reducing the volume to 0.5 mL. The washing step afore the elution is being studied to clean eluates to be injected into the HPLC system, and to allow the reusability of the PCL film-coated vials, avoiding the clogging of the pores of the film by proteins, aggregates, and macromolecules present in urine.

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P3-075

QUANTIFICATION OF BISPHENOL A IN ENAMELS, SOLVENTS AND INDUSTRIAL PAINTS AT PPB LEVEL: A FULLY AUTOMATED WORKFLOW

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Bisphenol A (BPA) is well known endocrine disruptor: its presence in several artifacts, with particular regard to FCMs, is subject to many EC regulations. Since the production of such FCMs often involves processes like painting and coating, any product used for this purpose must be tested to ensure acceptable levels of BPA.

Industrial enamels, solvents and paints are complex mixtures, whose may require different sample-prep procedures according to their composition.

SRA has developed a validated and effective sample-prep platform able to fully automate the whole workflow, where the unique manual process required is weighing the initial sample aliquote.

P3-076**NEW ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF HINDERED AMINE LIGHT STABILIZERS (HALS) IN DUST****Castro, Gabriela; Pereiro, Pablo; Rodríguez, Isaac.***Institute for Aquatic Environment Research for Global Health (iARCUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain.*

Hindered Amine Light Stabilizers (HALS) are polymer additives widely used in plastic materials to improve their durability by preventing any degradation caused by UV radiation. Due to its properties, the presence of HALS in polymeric materials cover a broad range of applications for both outdoors and indoors [1]. This widespread application together to the fact that these substances are not chemically bound to materials, and are easily released into the environment through volatilization, abrasion, or dissolution, leads to their accumulation in dust and other environmental matrices. The presence of HALS in indoor and outdoor dust raises concerns regarding potential human exposure and environmental persistence, necessitating reliable analytical methods for their detection and quantification.

This poster presents the development and optimization of a novel analytical methodology for the determination of HALS in dust samples. Different sample preparation techniques, including accelerated solvent extraction (ASE) and ultrasonic assisted extraction (UAE), were evaluated to enhance extraction efficiency and reduce matrix interferences. The determination of HALS was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), using a triple quadrupole mass analyzer (QqQ), ensuring high sensitivity and selectivity. The proposed methodology was validated in terms of linearity, sensitivity, precision, and recovery. Finally, the optimized methodology was applied to real dust samples collected from various indoor environments to assess the occurrence and distribution of HALS. The results provide valuable insights into HALS contamination in dust and contribute to a better understanding of human exposure pathways.

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P3-077**NANOFIBROUS POLYAMIDE-BASED SORBENTS: A NOVEL APPROACH FOR EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM RIVER WATER****Czyż, Ewelina; Šatinský, Dalibor.***Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic, Hradec Králové, Czech Republic.*

Polymer-based nanofibers have become a hot trend in new extraction sorbent development [1,2]. These sorbents are rather hydrophobic with higher affinity toward lipophilic analytes. In this study various polyamide (PA) homopolymers and copolymers nanofibers with different alkyl chain lengths have been investigated as novel sorbent materials for on-line solid phase extraction of polycyclic aromatic hydrocarbons from river water. PA4/6 copolymer nanofibers showed the highest retention of tested analytes and comparable performance with commercial C18 sorbent. Developed on-line solid phase extraction method coupled with ultra-high performance liquid chromatography with fluorescence detection showed excellent analytical performance, with linearity ranging from 0.01 – 0.5 $\mu\text{g L}^{-1}$ for acenaphthene, anthracene, pyrene, chrysene, and 0.05 – 0.3 $\mu\text{g L}^{-1}$ for benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(c,d)pyrene and benzo(g,h,i)perylene, achieving determination coefficients (R^2) between 0.9989 – 0.9999. The limits of detection and quantification were 0.003–0.01 and 0.01–0.05 $\mu\text{g L}^{-1}$, respectively. The recoveries were between 92% and 105%, with RSD 1.5 – 6.7 %. The total time for the extraction and analysis of nine analytes was 14 minutes and was done automatically. That saves the time and limits the operator error. The polyamide-based sorbent requires no modifications and can be inexpensively prepared as homogenous mats by electrospinning process. It can be cut and manually packed into the column, allowing for easy column replacement, when needed, with inter-columns repeatability of RSD < 13%. In addition, due to the excellent resistance of polyamide nanofibers to HPLC conditions, a single extraction column can be reused for the preconcentration of multiple samples.

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P3-078**EXTRACTION AND CHARACTERIZATION OF BIOACTIVE CARBOHYDRATES FROM *Palmaria palmata* AND *Saccharina latissima***

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Algae polysaccharides are reported to have several bioactive properties such as anti-inflammatory, antihyperlipidemic, antiobesity, immunomodulatory, antidiabetic, antioxidant, anticancer or immunomodulatory [1, 2]. As a result, the demand for extracts enriched in these bioactive compounds is steadily increasing within both the food and cosmetic industries. Red algae such as *Palmaria palmata* is known to contain carragenans (sulphated galactans) and xylans [3], while brown algae such as *Saccharina latissima* is constituted by alginates [with (1 → 4) linked β-D-mannuronic acid and α-L-guluronic acid], cellulose and fucoidans (fucose-rich sulphated polysaccharides) [4].

The aim of this work was the optimization of a new extraction method of polysaccharides from *P. palmata* and *S. latissima*. For this purpose, the efficiency of different solvents and extraction techniques such as solid-liquid extraction (SLE), ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) and pressurized liquid extraction (PLE), was evaluated. A previous extraction using ethanol to remove lipids, chlorophylls and carotenoids was also considered. Fifteen minutes was enough to eliminate most of the interference with the least loss of carbohydrates. Water was the best solvent for carbohydrate extraction from *S. latissima*, while water with 0.3M citric acid was selected for *P. palmata*. In a second step a Box-Behnken experimental design to optimize the extraction conditions (time, temperature and biomass:solvent ratio) of the most effective technique was performed. Those conditions which provided the maximum yield of total sugar and sulfate content and the highest total phenolic antioxidant activity were selected.

Characterization of obtained extracts was done by different analytical methods. GC-MS analyses of carbohydrates, before and after trifluoroacetic acid hydrolysis, and subsequent derivatization, allowed the identification of their monomeric composition, while size exclusion chromatography (SEC) with light scattering detection (ELSD) provided information about their molecular weight distribution. Other complementary techniques such as infrared spectroscopy was used to get more information about polysaccharide structures. Antioxidant activity of extracted polysaccharides was also studied by three different methodologies (DPPH, ABTS and TPC), while the anti-aging activity was evaluated by the anti-tyrosinase assays. Extracts enriched in these polysaccharides offer high potential for new applications in the nutraceutical and cosmetic industries.

Acknowledgements

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P3-079**DBS – NEW PERSPECTIVES AND CHALLENGES IN ANTI-DOPING ANALYSES**

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The blood sampling method known as Dried Blood Spots (DBS), based on the technique of collecting whole capillary blood samples, is becoming increasingly important in anti-doping control fields. This method involves obtaining a small amount of capillary blood and applying it to the specialized absorbent devices, which, depending on the manufacturer, can be either cellulose- or polymer-based. After collecting and drying the blood spots, samples can be easily stored and transported to the laboratory.

The use of DBS in anti-doping control offers numerous advantages compared to traditional matrices used in anti-doping tests (i.e., venous blood and urine). The first and highly significant aspect is that DBS collection is less invasive than traditional venous blood sampling, increasing athletes' comfort, which could lead to increased acceptability of testing for anti-doping purposes. Moreover, DBS analysis could be complementary in interpreting results and assessing whether anti-doping rules have been violated as it examines substances at different times of their presence in the body. The other group of factors – simplification of sample collection, transport, and storage procedures could reduce the costs of individual tests. Additionally, the sample collections do not require specialized medical personnel, which could reduce logistical challenges and costs. Finally, a crucial aspect of this approach is that it aligns with the concept of green chemistry, as laboratories aim to reduce the consumption of organic solvents and minimize laboratory waste, thereby significantly lowering the environmental impact. The World Anti-Doping Agency (WADA) aims to implement DBS samples into routine testing, mandatory for all anti-doping laboratories. For this purpose, in 2021, WADA published new Technical Documents with requirements for the laboratories, updated in 2023 [1].

When combined with analytical techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), DBS enables increasingly effective detection of prohibited substances, including e.g. anabolic steroids, diuretics, steroid esters, and peptides widely used for doping purposes. The application of the LC-MS/MS technique in DBS sample analysis ensures high sensitivity and selectivity, allowing for the simultaneous detection of multiple prohibited substances with minimal sample volume used.

In this study, the screening method for detecting testosterone esters in the DBS sample was presented, emphasizing the importance of proper solvent selection and the optimization of the conditions of extractions and derivatizations (i.e., temperature and time). Two commercially available DBS sampling kits that differ in their absorbent devices type were tested: Tasso - M20 (polymer-based absorbent) and Lockcon with AHLSTROM cards (cellulose-based absorbent). Additionally, various liquid chromatography conditions were compared, including types of chromatographic columns and mobile phases. The detection was conducted by means of ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

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P3-080

ADVANCING IN ANTIBIOTIC ANALYSIS: A NOVEL μ SPEed MICROEXTRACTION COMBINED WITH UHPLC-PDA FOR MULTI-RESIDUE ANALYSIS IN ENVIRONMENTAL SAMPLE

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The overuse of antibiotics in animal farming, driven by the increasing demand for animal-based products, has raised concerns regarding the presence of antibiotic residues in food and the environment [1]. Residues of commonly used antibiotics in the environment, such as sulfonamides, tetracyclines, and penicillins, can contribute to antibiotic resistance, disturb gut bacteria, and cause allergic reactions or toxicity [2,3]. To address these issues, it is crucial to apply stricter regulations and use analytical methodologies that allow the detection of low concentrations that may exist in the environment. Although traditional extraction techniques such as Solid-Liquid Extraction (SLE), Solid-Phase Extraction (SPE), and Liquid-Liquid Extraction (LLE) have been used to extract and pre-concentrate veterinary antibiotic residues prior to their analysis, there is a growing need for more sustainable and efficient extraction methods based on the miniaturization of these techniques, following green chemical principles [4].

In this work, an analytical methodology was developed based on a semi-automated micro-solid phase extraction (μ SPEed) combined with UHPLC-PDA analysis for the determination of six antibiotics — tetracycline, chlortetracycline, oxytetracycline, doxycycline, sulfamethoxazole, and trimethoprim — in wastewater samples. The extraction process was optimized by assessing different sorbent materials (butyl silica (C4), octyl silica (C8), octadecyl silica (C18-RPS), octadecyl silica hydrophilic ODS (C18-ODS), unmodified silica (Sil), aminopropyl silane sorbent (WAX), polyfluoroalkyl substances (PFAS), porous PS/DVB normal phase (PS/DVB), porous PS/DVB reversed phase (PS/DVB-RP), PS/DVB anionic exchange (PS/DVB-SAX), PS/DVB cationic exchange (PS/DVB-SCX), and porous graphitic carbon (μ CARB)), sample volumes (500 and 800 μ L), sample loading cycles (3-5), pH conditions (2.5, 4.0, 7.0, and 8.5), and elution solvents (methanol, acetonitrile, and acidic methanol (with 5% and 1% formic acid)) to achieve maximum efficiency. The best performance was obtained using a PS/DVB-RP sorbent with three 250 μ L loading cycles and two consecutive elutions with 50 μ L of acidified methanol (0.1% formic acid). The developed methodology enabled a rapid analysis with a total chromatographic run time of just six min. The validation results confirmed a good linearity, precision, and satisfactory recovery. Detection limits ranged from 0.30 to 1.23 μ g L⁻¹ and quantification limits from 0.92 to 3.73 μ g L⁻¹. The developed μ SPEed/UHPLC-DAD methodology proved to be effective for the analysis of wastewater samples, offering a reliable alternative for controlling antibiotics in environmental samples.

Acknowledgements

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P3-081**DETERMINATION OF BISPHENOLS IN HONEY AND BEE POLLEN BY USING MOLECULARLY IMPRINTED POLYMERS**

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The presence of bisphenols (BPs) in agriculture and food, more specifically in bee products, is a concerning issue due to the risks to bee populations and human health. Investigating this contamination in bee matrices is vital for comprehending its ecological and toxicological effects and subsequently formulating effective strategies to mitigate the risks. For this reason, the development of methods for detecting and quantifying these compounds is necessary.

This study aimed to develop and optimize two solid-phase extraction (SPE) methods using molecularly imprinted polymers (MIPs) for the analysis of BPs in both honey and bee pollen. This approach, known as molecularly imprinted solid-phase extraction (MISPE), was applied to improve the selective isolation and concentration of fifteen BPs. Five different polymers were tested, including one non-imprinted polymer (NIP) and four MIPs synthesized using bisphenols A, B, C and E (BPA, BPB, BPC and BPE) as template molecules. The extraction efficiency of these polymers was evaluated through qualitative and quantitative chromatographic analysis using high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD), and a comparative assessment with ultra-high-performance liquid chromatography with the same detection (UHPLC-DAD). Key SPE parameters—conditioning, sample loading, washing, and elution—were optimized, considering any necessary pretreatment of the matrices before MISPE application. As a result, two efficient methods that enable the isolation, identification, and quantification of fifteen BP species in honey and bee pollen were successfully developed, achieving satisfactory recovery rates (between 79 and 97%) for most analytes.

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P3-082**STIMULANTS: CHANGES IN TRENDS OF USE BY THE ATHLETES IN POLAND**

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Stimulants (also known as central nervous system stimulants or psychostimulants) are a class of substances whose primary purpose is to increase alertness. They can be administered for various reasons, such as enhancing attention, physical performance, and cognition. They can affect multiple organism functions, including arousal, attention, learning, memory, and emotion. Effects range from mild stimulation to euphoria, depending on the specific drug, dose, route of administration, and inter-individual characteristics. They have been used to treat various conditions, such as narcolepsy, attention deficit hyperactivity disorder (ADHD), obesity, depression, and fatigue.

Unfortunately, the use and especially the abuse of various stimulants have potential health risks and side effects, such as addiction, tolerance, withdrawal, psychosis, anxiety, insomnia, cardiovascular problems, and neurotoxicity. It can also lead to overdose, dependence, crime, violence, and, in the worst-case scenario, death. Therefore, the use of stimulants is regulated by laws and requires medical supervision and prescription in some cases^{1,2}.

Nevertheless, the positive effects of stimulants on the human body also create temptation for athletes. They are looking for a way to achieve better results in the shortest possible time. For this reason, stimulants are one of the groups of substances (S6) on the 'List of Prohibited Substances and Methods' created, updated, and published yearly by the World Anti-Doping Agency (WADA). Stimulants belong to a so-called open group, meaning that the group covers not only substances, namely enlisted, but also those with a similar structure or biological effects. Stimulants are also banned in competition for all categories of sports^{3,4}.

In the Polish Anti-doping Laboratory, all urine samples taken from athletes during competitions are subjected to anti-doping tests for the use of stimulants. According to WADA technical documents, a positive result for the presence of stimulants in urine is when its concentration exceeds 50 ng/mL (with an exception for a specified compound like ephedrine (10 µg/mL), pseudoephedrine (150 µg/mL), cathine (5 µg/mL), methylephedrine (10 µg/mL). For this purpose, methods for identifying these compounds have been developed using analytical systems such as GC/MS and LC/MS/MS. GC/MS system has been used as a primary system for stimulant detection and identification from 2009 until the end of 2020. From then on, all the analyses concerning the detection and identification of the stimulants have been conducted on the LC/MS/MS system.

For the last 16 years, 41641 urine samples collected during competition have been analyzed for the presence of the stimulants. The presence of prohibited substances was confirmed in 261 urine samples, representing 0,63% of all samples analyzed. In these samples, 279 stimulants were confirmed at concentrations exceeding the minimum required performance levels. The suspicious substances have been detected in 193 male urine samples and 69 female urine samples, which allows us to conclude that men reach for stimulants more often than women. The most frequently detected substances over the last 16 years have been stimulants such as 1,3-DMAA (72 samples), amphetamine (29 samples), cocaine (25 samples), 1,4-DMAA (20 samples) and oxilofrine (19 samples).

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P3-083**DEVELOPMENT OF 3D-PRINTED EXTRACTION DEVICES MODIFIED WITH METAL-ORGANIC FRAMEWORKS FOR THE ANALYSIS OF MARINE TOXINS**

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The detection of emerging contaminants (ECs) in marine ecosystems has become a critical environmental and economic concern due to their impact on biodiversity, food chain, and the health of both humans and wildlife. Among these pollutants, marine biotoxins such as domoic acid (DA) pose significant threats, particularly through their accumulation in seafood products. Consequently, the development of efficient and reliable extraction platforms with efficient sorbents able to preconcentrate target ECs even at trace levels is highly desirable. The utilization of novel extraction devices manufactured using 3D printing has experienced significant increase in the field of sample preparation, since they offer some advantages such as versatility in materials, cost-effectiveness in printing, flexibility in shape design, and adaptability to specific applications. Moreover, 3D-printed extraction devices can be readily post-functionalized to immobilize diverse materials, thereby enhancing their performance. In this context, metal-organic frameworks (MOFs) have demonstrated to be effective sorbents, owing to their promising properties such as high surface area, tunable pore size, and modifiable functionality.

This work proposes the development of sustainable 3D-printed extraction platforms modified with MOFs for the extraction of DA from marine organisms. First, different MOFs were assayed using dispersive solid-phase extraction, being the MOF that offered the best extraction performance selected for the modification of the 3D-printed device. After incorporation of the selected MOF into the 3D-printed devices, some extraction parameters (including loading and elution solvents, time of extraction and elution, among others) were optimized. Finally, the MOF-modified 3D-printed device was applied to the isolation and determination of DA in different marine organisms.

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P3-084**A COMPARATIVE SAMPLE TREATMENT OF URBAN POLLUTANTS IN WATERS WITH SOLID PHASE EXTRACTION AND VACUUM -ASSISTED EVAPORATION**

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Access to good water quality is a fundamental for life and societal development, as well as preservation of natural ecosystems. However, its availability is increasingly compromised due to factors such as climate change, population growth, and pollution. According with European legislation (Water Frame Directive 2000/60/EC & Groundwater Directive 2006/118/EC) the quality of water bodies is influenced among others, on its chemical status. Organic micropollutants of anthropic origin such as pharmaceuticals and personal care products, industrial compounds, agrochemicals, and their transformation products represent a threat for water resources. Conducting a monitoring study of urban organic contaminants is crucial for establishment of environmental quality standards and evaluation of remediation strategies.

One of the most commonly extraction and preconcentration technique for urban pollutants analysis is solid-phase extraction (SPE). This technique isolates and concentrates contaminants present in water using cartridges containing polymeric sorbents designed to retain specific chemical compounds. However, the effectiveness of this method is highly dependent on the affinity between each compound and the sorbent material, which can introduce analytical limitations and affect the recovery of certain contaminants. To overcome these limitations, vacuum-assisted evaporation (VAE) has been proposed as an alternative. To date, few studies used VAE to preconcentrate organic pollutants in water, but have shown good recoveries for polar and non-polar compounds.^{1,2} However, it also has certain drawbacks, such as a lower preconcentration capacity compared to SPE, and the potential to precipitate some compounds or irreversibly volatilization of volatile compounds.² VAE is based on sample concentration through controlled evaporation at temperatures below 100°C, is simpler and also more sustainable than SPE. Additionally, it allows working with small sample volumes. In this work, water samples of 20 mL were evaporated using BUCHI syncore plus system to 0.5mL, achieving a preconcentration factor of 40. This study compared both methods, SPE and VAE, to evaluate their effectiveness in detecting and quantifying urban pollutants in water. Approximately 150 compounds from different chemical families and different properties (logP: -1.5 to 5) were analyzed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Specifically, an HSS T3 Premier column and a Q-Exactive Orbitrap mass spectrometer was used.

The objective of this study was to determine which method provides better recovery rates for contaminants and which method is more suitable for multiresidue analysis. In a preliminary study, 68% of the compounds were better extracted using VAE compared to SPE with OASIS HLB cartridges.³ These results will contribute to improving monitoring strategies on water quality control, thereby aiding in the protection of water resources and eventually public health.

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P3-085**DETERMINATION OF PHARMACOLOGICALLY ACTIVE DYES IN AQUACULTURE SAMPLES BY USING AN EFFICIENT SAMPLE PREPARATION PROCEDURE AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY****Mitrowska, Kamila; Sielska, Katarzyna.***National Veterinary Research Institute (PIWet), Department of Chemical Research of Food and Feed, Pulawy, Poland.*

Because of the low concentration of pharmacologically active dye residues in aquaculture product samples and the complexity of the matrix, an efficient sample preparation procedure is essential for extracting, cleaning up and preconcentrating the dyes from aquaculture sample matrices. The objective of this study was to develop a simple and efficient sample preparation procedure followed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for the determination of 25 pharmacologically active dyes from different chemical classes (malachite green, leuco malachite green, crystal violet, leuco crystal violet, brilliant green, leuco brilliant green, ethyl violet, methyl violet 2B, pararosaniline, Victoria blue B, Victoria blue R, Victoria pure blue BO, methylene blue, azure A, azure B, azure C, thionine, new methylene blue, Nile blue A, acriflavine, proflavine, rhodamine B, rhodamine 6G, safranin O, Janus green B) at residue levels in aquaculture products such as fish, shrimps and mules. The developed method used acetonitrile and ammonium acetate buffer to extract the pharmacologically active dyes from aquaculture products. Next, the p-QuEChERS procedure, using a salt mixture of potassium phosphates for phase partition, was applied, followed by the addition of Z-Sep+ sorbent for further acetonitrile extract clean-up. The extract was evaporated to dryness and then reconstituted with a mobile phase. The concentration of the dyes was analysed by UPLC-MS/MS using a pentafluorophenyl (F5) analytical column and mobile phases of ammonium acetate buffer and acetonitrile/methanol. The method has been validated according to the Commission Implementing Regulation (EU) 2021/808 and showed low decision limits ($< 0.5 \mu\text{g/kg}$), good trueness (from -50 % to +20 %), repeatability and within-laboratory reproducibility (CV $< 25\%$) for all dyes quantified in a wide variety of aquaculture products. Applying the p-QuEChERS procedure with clean-up using Z-Sep+ sorbent resulted in significantly lower matrix effect and higher recovery of analytes from aquaculture product extracts. The developed method was successfully applied to quantify and confirm residues of a wide range of pharmacologically active dyes at low concentrations ($< 0.5 \mu\text{g/kg}$) in aquatic products and was found to be suitable for routine monitoring.

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P3-086**STABILITY OF ARSENIC SPECIES IN PLANT AND FUNGI MATERIAL UNDER VARYING STORAGE CONDITIONS**

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Arsenic is highly toxic for all organisms, including plants and fungi. Due to fact that toxicity, biological availability and transport mechanisms of arsenic highly depend on its chemical form, total concentration determination is insufficient in assessing the real impact of arsenic [1]. The extraction process is crucial step in speciation studies because it directly influences accuracy and reliability of results. The primary challenge is the development of an effective extraction method that prevents contamination, loss and more importantly species transformation [2]. Ensuring the stability of trace element chemical species during sample handling and storage is essential for obtaining accurate results in speciation analysis [3].

The aim of the study was to investigate effect of storage conditions of extracts on selected arsenic compounds (arsenobetaine, dimethylarsinic acid, monomethylarsonic acid, As(III) and As(V)) in plant material samples and fungi. The determination of the arsenic species was performed by HPLC-ICP MS and total arsenic content by ICP MS.

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P3-087**SUPRAMOLECULAR SOLVENTS-LC-MS/MS-BASED METHOD FOR ASSESSING DERMAL EXPOSURE TO CONTAMINANTS OF EMERGING CONCERN THROUGH THE USE OF ELECTRONIC DEVICES****Pulido-Zurera, Alejandro¹; Caballero-Casero, Noelia²; Rubio, Soledad³.**¹PhD, Cordoba, Spain; ²Dr, Cordoba, Spain; ³Dr, Córdoba, Spain.

Keywords: contaminants of emerging concern, supramolecular solvents, electronic devices, dermal contact.

Contaminants of emerging concern (CECs), such as flame retardants, plasticizers, and bisphenols, are widely used in the manufacturing of electronic devices and other consumer products. It has been demonstrated that these compounds can be released from electronic devices and accumulate in household dust, creating an inhalation exposure route for the population (1,2). However, other potential exposure pathways, such as dermal absorption, remain largely unexplored. The interaction of hydrophobic and hydrophilic contaminants with the skin surface is governed by complex adsorption mechanisms, through which CECs can transfer to the epidermis and penetrate through the pores. To assess this exposure route, sterile gauze pads were used to collect CECs deposited on the palms of workers handling electronic devices.

To detect and quantify flame retardants, plasticizers, and bisphenols in the gauze pads, a sample treatment method based on supramolecular solvents (SUPRAS) was developed. These solvents are nanostructured liquids formed through the spontaneous self-assembly of amphiphiles. SUPRAS exhibit outstanding extraction properties, enabling all-in-one extractions by providing a variety of polarity microenvironments and multiple binding sites. This study evaluated the ability of alkanol- and alkanediol-based SUPRASs to extract CECs covering a wide polarity range.

The developed method was simple: 50 mg of powdered sample was weighed, and 300 microliters of SUPRAS were used for extraction. The determination and quantification of target analytes were performed using liquid chromatography tandem mass spectrometry. SUPRAS based on alkanols (1-octanol and 1-decanol), which form inverse hexagonal nanostructures (3), yielded better results compared to those based on alkanediols (1,2-octanediol and 1,2-decanediol), which form sponge-like structures (4). Specifically, 1-octanol SUPRAS efficiently extracted flame retardants, plasticizers, and bisphenols from the gauze pads, achieving recoveries between 70% and 135% with intra-day precision of 10–19% RSD for all target compounds.

The developed SUPRAS-based all-in-one extraction approach was applied to 45 gauze pad samples collected from volunteers whose jobs involved the use of electronic devices. The concentrations of CECs found in the samples were used for a preliminary assessment of dermal exposure risk. The results highlight the importance of identifying all potential exposure pathways for workers. Additionally, the use of SUPRAS proved to be an effective tool for the extraction and quantification of CECs, emphasising their potential in environmental pollution studies and occupational safety. Further research is essential to understand the associated risks better and develop more effective protective measures for workers exposed to these substances.

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P3-088**USE OF SPE FOR THE ISOLATION OF PSYCHOACTIVE SUBSTANCES FROM HUMAN SALIVA**

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The determination of drug levels in the body allows for the observation of the drug's absorption rate and indirectly helps determine the health status of the patient. In addition, monitoring the concentrations of the active ingredient and its metabolites provide an opportunity to determine how well drug metabolism is proceeding [1].

The primary biological material in which psychoactive substances are determined is blood. However, the inconvenience of sampling, such as the possibility of infection, the presence of qualified medical staff, the use of sterile instrumentation, or the stress of the procedure and the possibility of falsification of the test result, for example in the case of cortisol determination, are reasons why other alternative biological matrices are being considered. One of the alternatives is saliva. Using saliva for drug level determination has considerable advantages: sample collection is noninvasive, can be performed solely by the patient, and can be carried out at home [2]. As saliva sampling is non-invasive, monitoring the concentration of analytes in saliva is recommended for children, the elderly and those who may not cooperate with the physician.

As a test material saliva has several disadvantages. Sampling is not sterile, which means that the compounds to be determined may be degraded by microorganisms [2]; the concentration of the test substance(s) is usually lower than in blood and depends on many factors, including the molecular weight of the analyte, lipid solubility, pKa of the compound, the degree of binding to blood proteins or salivary flow; the concentration of a given substance is an individual feature of the tested person; saliva contains only the unbound forms of substances, i.e., active forms. The use of saliva in the analysis of active compounds is not common. Therefore, there are no uniform procedures to determine how to sample saliva, store it and determine the substance being tested. In addition, the preparation of the sample requires appropriate treatment if it has to be analyzed chromatographically [3]. The SPE method is one of the most often chosen for the isolation of analytes from blood, because of its selectivity and high extraction recovery. Solid-phase extraction enables the isolation of compounds with different structures and physicochemical properties. The commercial availability of many types of sorbents allows for the appropriate selectivity of the extraction process. In addition, it is also possible to control and modify each stage of the SPE.

Saliva, as an easily accessible research material, can be a good source of information about the medications administered. However, the lack of adequate procedures for sample collection and isolation of the compounds contained in saliva make it necessary to develop new procedures for the determination of psychoactive compounds in this biological material. Our research focuses on developing new methods for determining these substances in the smallest possible volume of saliva. Typically, we collect 200 µL for testing, which also allows us to take a sample from people with dry mouth. Among the compounds tested were those with neuroleptic (amisulpride, aripiprazole, olanzapine, quetiapine, clozapine and risperidone), antidepressant (citalopram, venlafaxine), and anti-epileptic (lamotrigine, carbamazepine) effects. In addition, we have also developed a method for the determination of caffeine, which is the most commonly used CNS stimulant. In each of our methods, we used SPE extraction to isolate the compounds, in which we selected a suitable sorbent and optimised the extraction procedure to ensure the best possible yield. We also used the developed methods to determine the concentration of the compounds under study in saliva samples from users [4-6].

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P3-089**ELECTROMEMBRANE EXTRACTION OF OXICAMS USING DEEP EUTECTIC SOLVENTS AS LIQUID SUPPORTED MEMBRANE****Rubio, Iván¹; Vidal, Lorena¹; Canals, Antonio².**

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Oxicams represent a subgroup of non-steroidal anti-inflammatory drugs (NSAIDs), which function by inhibiting cyclooxygenase (COX) enzymes involved in prostaglandin synthesis, that plays a role in inflammation, pain, and fever. A notable oxicam example is meloxicam, which has been formulated to reduce the gastrointestinal side effects that are commonly observed in traditional NSAIDs such as ibuprofen and diclofenac. Meloxicam's COX-2 inhibition properties have been the subject of extensive research, providing a valuable framework for the development of NSAIDs with reduced side effects¹. Their widespread use without a prescription means they can be consumed in large quantities. Therefore, the analysis of oxicam derivatives in water and urine enables the evaluation of its environmental impact, the control of its therapeutic use, the prevention of abuse, and the protection of public health. The primary concern pertains to its persistence and the possibility of toxicity in the aquatic environment, in addition to its misuse or uncontrolled use in humans.

In this study, we examine the potential of hydrophobic deep eutectic solvents (DESs) as supported liquid membranes (SLMs) for electromembrane extraction (EME). The non-ionic DES components tested in this study include 6-methylcoumarin and thymol, among others². The current literature does not include SLMs for the extraction of these analytes. Therefore, EME of five basic drugs belonging to the oxicam family (i.e., tenoxicam, isoxicam, lornoxicam, droxicam and meloxicam) has been evaluated from water and urine, with 2-nitrophenyl octyl ether and DES components as SLM and combined with a liquid chromatograph equipped with a diode array detector (LC-DAD), to test the potential for analytical applications. In light of the present findings, further research into the use of DESs in EME is recommended.

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P3-090**DETERMINATION OF WATER- AND FAT-SOLUBLE VITAMINS IN SEAWATER SAMPLES BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY AFTER SIMULTANEOUS EXTRACTION**

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The marine environment is a unique source of bioactives and organisms with unique properties with exceptional medical, nutritional, industrial, and biotechnological applications. Marine therapy is a therapeutic approach that uses ocean and aquatic resources to assist in the improvement of the physical and mental well-being of individuals [1]. The therapeutic use of sea salt dates to the earliest civilizations, largely because seawater contains nearly all the elements found in nature [2].

Among the different nutrients found in seawater, vitamins have been recently identified in the marine environment as crucial regulators of the phytoplankton species [3]. However, vitamins are present at trace levels, and, therefore, analytical methods for vitamin determination need to be developed due to the scarce studies available due to the challenging salty matrix. In view of all these difficulties, solid-phase extraction (SPE) is a highly consolidated technique for the extraction of vitamins in different complex matrix samples. This conventional extraction technique (i.e., SPE) is the most suitable for extracting vitamins from seawater samples because it allows large sample volumes of seawater (e.g., 0.5-1 L) to be treated, removing the saline matrix and therefore allowing trace amounts of vitamins to be detected.

Therefore, in this research, two analytical methods using the same sample preparation procedure, to simultaneously extract water- and fat-soluble vitamins, were successfully developed to determine 8 water-soluble vitamins and 4 fat-soluble vitamins in seawater samples [4]. The extraction procedure was optimized and the SPE cartridge (Strata C18-E, 55 µm, 70A10g/60 mL, Gigatubes, Phenomenex, USA) was initially conditioned by running 40 mL methanol followed by 40 mL ultrapure water through the cartridge. One-liter seawater sample, set at pH 6.5, was transferred to the SPE cartridge at 3 mL min⁻¹ employing a peristaltic pump with Tygon tubing. Then, the cartridge was rinsed with 40 mL ultrapure water, in order to remove the salt retained in the cartridge. Afterwards, 40 mL of methanol was used for elution, and the solvent was dried at room temperature under a stream of nitrogen gas. Finally, samples were reconstituted in 1 g of methanol. For water-soluble vitamin determination, 0.5 g of reconstituted sample was used and the rest of the reconstituted sample (i.e., 0.5 g) was used to determine fat-soluble vitamins by liquid chromatography/triple quadrupole mass spectrometry (LC/MS QQQ) in positive mode. The method was validated and seawater samples (filtered and unfiltered) were successfully analyzed and some vitamins quantified. In conclusion, it is important to emphasize that quantifying vitamins in seawater samples provides valuable information for understanding the marine environment better, as well as for consumers of seawater-based food supplements [5].

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P3-091**ELEMENTAL ANALYSIS OF ESSENTIAL OILS COMBINING INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY AND LIQUID PHASE MICROEXTRACTION WITH MAGNETIC EUTECTIC MIXTURES**

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The unique properties of magnetic solvents, particularly their magnetism, make them ideal candidates as extractant solvents for the development of high-efficiency and high-speed microextraction procedures. This fact has recently opened a very active and promising field of research. In the present work, a method has been developed in which dispersive liquid-liquid microextraction (DLLME) is used to determine Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn in oleic matrix samples at trace levels by inductively coupled plasma optical emission spectrometry (ICP OES), using a magnetic deep eutectic solvent (MDES) as extractant. For this purpose, a hydrophilic MDESs was synthesized, using ethylene glycol (EG) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in 2:1 molar ratio. Subsequently, the MDES was characterized by using differential scanning calorimetry (DSC) and magnetometry.

Afterwards, the amount of the sample, the volume of MDES added for extraction, and the extraction time were optimized through multivariate analysis in two stages; the first consisted of a full factorial design and the second was a central composite design (CCD). The optimal values obtained were 6.98 g of solution mass, 151 μL of MDES and 1 min of extraction time. In addition, the analytical figures of merit of the developed methodology were evaluated, obtaining limits of quantification lower than 5 $\mu\text{g kg}^{-1}$. Likewise, a matrix certified reference material (MCRM) was analyzed in order to validate the developed method.

Finally, the optimized method was applied to analyze eucalyptus, lavender, and tea tree essential oils, and the trueness was assessed by recovery test, achieving recovery values close to 100 %.

Acknowledgements

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P3-092**USE OF A FERROFLUID AS AN EXTRACTANT PHASE IN THE ELEMENTAL ANALYSIS OF WASTEWATER BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY****Zapater, Cristina; Castillo-Adrover, Natalia; Abellán-Martín, Sergio J.; Aguirre, Miguel Ángel; Canals, Antonio.***Department of Analytical Chemistry, Nutrition and Food Science, and University Institute of Materials, University of Alicante, Alicante, Spain.*

Water pollution constitutes a critical global challenge, primarily driven by rapid population growth and technological advancements. To promote the treatment and reuse of this resource, wastewater undergoes treatment processes aimed at removing its contaminants. Among the most common pollutants found in wastewater are heavy metals, which are highly toxic and exhibit low biodegradability. In recent years, deep eutectic solvents (DESs) have emerged as a safe sustainable, and cost-effective alternative in microextraction processes [1]. However, the necessity for centrifugation to recover the extractant phase remains a limitation. In this context, the use of ferrofluids, separable by an external magnetic field, presents a promising solution to overcome this issue [2].

This study aims to develop a new experimental procedure focused on the analysis of Cd, Cr, Cu, Mn, Ni, Pb, and Zn in wastewater samples. This approach combines vortex-assisted dispersive liquid-liquid microextraction (VA-DLLME), using a ferrofluid composed of a deep eutectic solvent (DES) (Thymol:Coumarin, molar ratio 2:1) and commercial magnetic toner particles, followed by inductively coupled plasma optical emission spectrometry (ICP OES) detection. Due to incompatibility between the ferrofluid and the ICP OES sample introduction system, a back extraction step with HCl was implemented to enhance the analytical process.

To optimize the procedure prior to the analysis of real wastewater samples, a two-stage multivariate analysis was employed. The initial stage utilized a Plackett-Burman design to identify significant factors affecting extraction efficiency, leading to the identification of the sample amount and HCl concentration as critical variables. In the subsequent central composite design stage, optimal conditions of 8.1 g for sample mass and 130 mg for HCl were determined.

Under optimized conditions, analytical figures of merit were evaluated, yielding limits of detection below $10 \mu\text{g kg}^{-1}$ for all analytes evaluated and demonstrating repeatability below 10% in all cases. Furthermore, trueness was assessed through certified reference materials and recovery tests, achieving recovery rates between 91 and 109 %, indicating negligible matrix effects.

Acknowledgements

The authors are grateful for the financial support received from the Valencia Regional Government (Spain) (CIPROM/2021/062, INVEST/2022/70) and the Spanish Ministry of Science and Innovation (PID2021- 126155OB-I00). Authors also wish to thank the Spanish Network of Excellence in Sample Preparation (RED2022-134079-T), which was granted by the Spanish Ministry of Science and Innovation, and the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society

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P3-093**STRATEGIES FOR THE (NANO)ENABLING AND ELECTROANALYTICAL ENHANCEMENT OF SCREEN-PRINTED CARBON ELECTRODES****Díaz-Cruz, José Manuel; Huang, Jing; Bastos-Arrieta, Julio; Serrano, Núria.***Universitat de Barcelona, Barcelona, Spain.*

Screen-printed carbon electrodes (SPCEs) have emerged as versatile and cost-effective analytical devices suitable for a broad range of electrochemical applications, from environmental monitoring to clinical diagnostics. However, their performance can be limited by relatively low sensitivity and selectivity. In recent years, numerous modification strategies have been investigated to overcome these drawbacks. In particular, physical and chemical surface modifications incorporating functional coatings (e.g., nanoparticles, carbon nanotubes, conductive polymers, among others), employing specific surface treatments, and optimizing electrode morphology have shown remarkable potential to improve electron transfer kinetics and enhance electroanalytical sensitivity.

In this study, we present a systematic investigation into different physical and chemical (nano)coatings and functionalization approaches that aim the enhancement of the electrocatalytic properties and analytical throughput of commercially available SPCEs. On one hand, physical modification strategies include drop-casting, spin coating, layer-by-layer and dip coating. On the other hand, chemical activation using alkaline treatment has also been addressed. Finally, combined physical and chemical modification has been carried out.

The effectiveness of the surface modification was evaluated with morphological characterization by means of contact angle measurements and atomic force microscopy (AFM). AFM measurements provided quantitative assessment of surface roughness of the (nano)enabled – activated SPCEs surface compared to unmodified SPCEs. Additionally, electrochemical characterization, by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), provided further confirmation and details of the different surfaces including electroactive area and resistivity. Electroanalytical measurements, based on differential pulse voltammetry (DPV), show the differential response toward model analytes like paracetamol, salbutamol, uric acid and dopamine.

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P3-094**MINIMALISTIC POINT-OF-CARE SALIVA BIOSENSOR FOR NON-INVASIVE GLUCOSE MONITORING IN DIABETES TYPE 2****Rebollo-Calderón, Beatriz; Guerrero, Elena; Grinyte, Ruta; Martin, Daniel; Cabot, Joan Marc.***Leitat Technological Center, Terrassa, Spain.*

Diabetes type 2 already affects over 415 million adults and its prevalence and burden to the healthcare system is only expected to increase [1]. Consequently, a reliable and frequent control of the glucose levels of the patients is essential for the clinical management of diabetes type 2. However, current approaches for self-monitoring rely on finger-pricking for blood analysis or the use of needles for the continuous analysis of the interstitial fluid. The main drawback of the former is the pain caused by the finger-pricking, which in some cases may be necessary up to 10 times a day depending on the patient. Regarding the latter, they tend to be less painful and more convenient but still present other types of shortcomings. Their application to the skin can cause bleeding, bruising, skin irritation and have a higher risk of biofouling and a decreased precision during the first 24h - 48h after placement (which is required every 10 to 14 days). Moreover, some of these devices may still require an analysis by finger-pricking for calibration or be only suitable for adjuvant use (which means that finger-pricking is still necessary for clinical decisions). Furthermore, these types of devices are very expensive and mostly aimed at type 1 diabetes patients and its use it's not widespread in type 2 patients.

Here we present a non-invasive, glucose sensor aimed at the point-of-care (POC) analysis of saliva samples. This sensor constitutes a more convenient and painless alternative to other invasive traditional glucometers, which would improve the frequency and adherence to controls by the patients.

Due to the low concentration of glucose in saliva in comparison to blood, we have developed a highly sensitive saliva glucometer. The biosensor is single use and low cost. The electrode consists of a screen-printed carbon paste and is functionalized by the addition of two enzymes, as well as an electron transfer mediator.

The associated electronic components have been designed and fabricated to achieve a self-operated and low energy device, which can carry out the amperometric detection with the use of a smartphone by NFC connection. A custom-made APP is under development for the data acquisition and visualization and management of the glucometer. Additionally, a housing which defines the placement of the smartphone and contains all the electronic components has been designed and 3D printed; as well as supports for the flexible electrodes to facilitate their handling.

The proposed biosensor displays good stability up to 24 days when stored protected from the light, under vacuum and at 4°C. It also shows a satisfactory repeatability (RSD < 10%) between electrodes, allowing for batch calibration of the sensors. Furthermore, calibrations done with glucose standard solutions in commercial artificial saliva reveal a lineal range from 1.05 to 8.05 mg/dL glucose, which is suitable for the determination of saliva glucose in diabetic patients.

We have already validated this saliva glucometer with healthy volunteers, with promising results. Pre-clinical studies with diabetic patients are currently undergoing and will continue for the rest of the year 2025, to evaluate the sensitivity, reliability, robustness of the biosensor. Moreover, both healthcare professionals and patients will provide feedback on its user friendliness level.

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P3-095

DEVELOPMENT OF AN ELECTROCHEMICAL SYSTEM FOR THE DETECTION OF CITRIC ACID IN POWDER

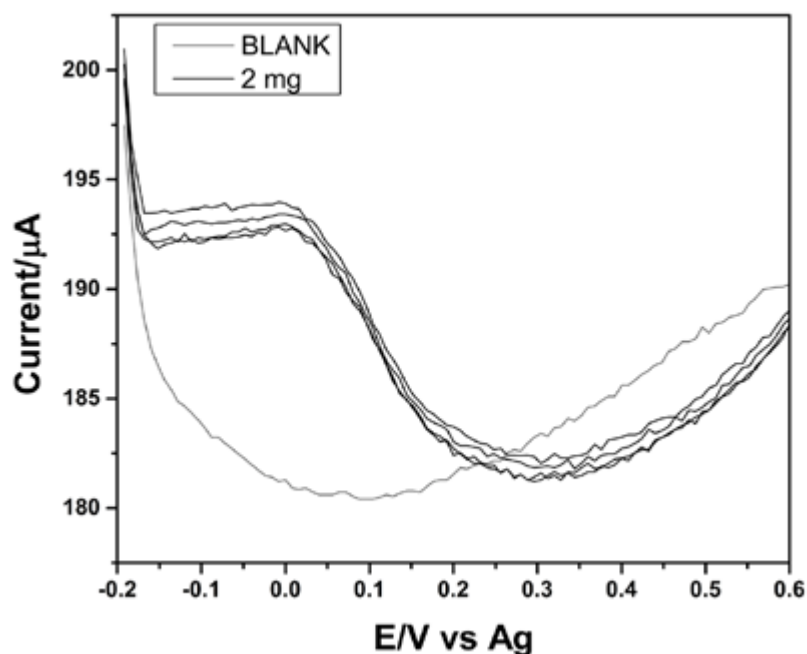
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Solid-phase electrochemical detection has gained relevance as a rapid and direct alternative for the analysis of real samples without the need for prior pretreatment. Recent research has developed portable, miniaturized devices that allow voltammetric fingerprinting of complex powders, for example matrices adulterated with cocaine and other solid analytes. Kokkinos et al. and De Jong et al. have demonstrated that it is possible to detect compounds of forensic or food interest directly in the solid state, paving the way for more efficient and applicable in situ methodologies.

In line with these advances, this project focuses on the direct solid-phase detection of citric acid as a model analyte for the development of a functional electrochemical system.

To this end, screen-printed electrodes modified with carbon-based nanomaterials bonded with ionic liquid were used, complemented by a conductive gel that facilitates the closure of the solid-state electrochemical circuit. The signal obtained using pulse voltammetry techniques allowed obtaining a current signal of the analyte without the need for prior solubilization. These preliminary results confirm the feasibility of the approach and suggest that this methodology could be extrapolated to the detection of other compounds in complex solid matrices, with potential applications in the fields of safety, quality control, and the food industry.



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P3-096**STUDY OF THE MECHANISM AND KINETICS OF THE ELECTROREDUCTION PROCESS OF In(III) IONS IN SYSTEMS WITH ORGANIC SOLVENTS****Nosal - Wiercińska, Agnieszka¹; Pawlak, Alicja¹; Bazan - Wozniak, Aleksandra²; Pietrzak, Robert².**¹Maria Curie - Skłodowska University in Lublin, Lublin, Poland; ²Adam Mickiewicz University in Poznań, Poznań, Poland.

The interest in using metals as materials for anti-corrosion coatings goes back many years. Elements such as germanium, gallium, indium and tin have attracted particular attention. Indium metal is extracted mainly from zinc or tin ores containing this element and then subjected to purification processes [1]. As early as the 1980s and 1990s, indium plating was used to create diffusion bearing alloys, in which thin films of indium were applied to metals non-ferrous metals or their alloys, such as Pb-Cu or Cd-Ag-Cu, and then onto steel or aluminum bearing substrates [2].

Nowadays, indium's anti-corrosion properties are still used in industry [3], but its applications go beyond protecting materials from corrosion. Indium plays a key role in the production of transparent conductors (ITO) used in touch screens and displays, as well as in chemical and physical vapor deposition (CVD, PVD) technologies, where its compounds are used in advanced semiconductors for LEDs and lasers [4].

In addition, indium is used in flexible electronic circuits, where the liquid metal fills microchannels in the elastomer, enabling the creation of modern, flexible circuits [5]. A crucial process for obtaining metallic coatings in both elementary and more advanced applications is electrodeposition, which allows precise deposition of metal layers onto various surfaces. In the case of indium, this process is mainly based on the electroreduction of In(III) ions, and knowledge of the kinetics and mechanism of this process can play a fundamental role in shaping the properties of the indium coatings obtained.

The research presented in this study focused on analyzing the In(III) ions electroreduction from mixed aqueous-organic supporting electrolyte solutions.

Such electrochemical techniques as square-wave voltammetry (SWV), cyclic voltammetry (CV) and direct-current (DC) polarography or electrochemical impedance spectroscopy (EIS) were used, allowing a more thorough understanding of the process and assessing the influence of organic substances on the kinetics and mechanism of electroreduction of metal ions [6].

The results of these investigations can be helpful in evaluating the conditions affecting the morphology and properties of the resulting indium coatings, which may be important for optimizing electrodeposition conditions in modern industrial applications.

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P3-097

RESOLUTION OF OVERLAPPING OPTICAL SIGNALS: DUAL SENSING VS CHEMOMETRICS

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The development of portable and reliable chemical sensors has become crucial in the past years as the need of fast and on-site analysis arises. In this context, the use of UV-vis dip probes is quite extended because they allow the measurement of optical absorbances in liquid samples without the need of optical cuvettes and they are able to acquire an entire spectrum in less than one second. However, the large abundance of optically active substances makes signal overlapping a common issue that often prevents the full implementation of UV-vis dip probes in on-site analysis, even in the case of relatively simple chemical systems.

In this work, two strategies for the resolution of optical signals without the need of sample treatment are compared: (i) dual sensing based on the combination of optical and electrochemical signals; and (ii) data treatment of optical signal by means of chemometrics. For this purpose, two different systems were evaluated. The first one comprised pyrocatechol (CC) and isonicotinamide (INAM) as an example of a system containing only one electroactive specie, and the second one contained CC and isoniazide (INH) as an example of a chemical system with two electroactive compounds. Both strategies proved successful in the resolution of the optical overlapping in static conditions (i.e., non-stirred solutions), with slopes in the range of 0.88 – 1.08 and R^2 values higher than 0.989 for the representation of predicted vs expected concentration values for both chemical systems. Nevertheless, only chemometrics was able to resolve both systems in dynamic conditions (slopes in the range of 0.873 -1.10 and R^2 values higher than 0.99), as the increased transport of INH significantly affected electrochemical measurements. Dual sensing was also successful in the resolution of CC and INAM (slopes in the range of 1.014 -1.035 and R^2 values higher than 0.99), proving even better than chemometric analysis, particularly in the region of lower CC concentrations.

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P3-098

ELECTROCHEMICAL QUANTIFICATION OF IBUPROFEN IN COATED TABLETS USING BUCKYPAPER BASED MULTI-WALLED CARBON NANOTUBE-MODIFIED ELECTRODES

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Pharmaceutical analysis is crucial for ensuring the quality, safety, and efficacy of pharmaceutical products at various stages, including synthesis, quality control, and post-marketing surveillance. Among the available techniques, electrochemical analysis stands out due to its high sensitivity, specificity, rapidity, low cost, and minimal reagent consumption. Advances in nanotechnology and modified electrodes have further enhanced this field.

In this study, ibuprofen (IBF) was analyzed using a batch injection analysis system (BIAS) in combination with screen-printed electrodes (SPE) modified with carbon nanotube buckypaper (BP). The use of CNTs as a flexible and adhesive film enables a simple and reliable electrochemical platform [1]. A key application of these modified electrodes is the electrochemical determination of drugs for quality control purposes [2].

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) widely used to relieve pain, fever, and inflammation, including muscle aches, headaches, arthritis, menstrual cramps, toothaches, and other inflammatory conditions. In this study, BPs improved the electrochemical response of the system by providing a reproducible and user-friendly platform, significantly reducing analysis time compared to other analytical techniques [3].

Ibuprofen was quantified in coated tablets with a declared dose of 400 mg using amperometry in a BIAS cell equipped with modified screen-printed electrodes. Each measurement was carried out by injecting 10 μ L of the analyte at a potential of 1.0 V (vs. Ag as a pseudo-reference electrode, using a SPE CNT110 Dropsens). All analyses were conducted in a phosphate buffer solution at pH 7.4. A calibration curve was obtained using IBF solutions prepared from a stock solution with concentrations ranging from 0.3 mM to 1 mM, diluted in pH 7.4 phosphate buffer (Fig. 1).

Electrochemical characterization revealed the irreversible electro-oxidation of IBF at potentials between 1.0 V and 1.2 V (vs. Ag/AgCl). According to the literature, the electrochemical oxidation occurs via a one-electron (1 $\bar{e}$) and one-proton (1 H^+) transfer, leading to radical cation formation. This process has been described as a pH-independent mechanism [4-6].

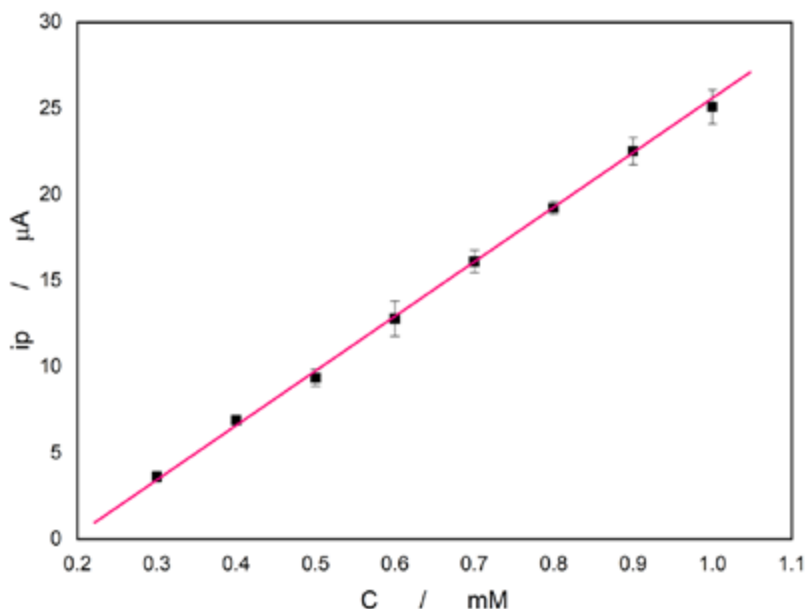
Additionally, a standard addition curve was constructed using concentrations between 0.25 mM and 0.45 mM of the added standard. All measurements were performed in triplicate.

The amperometric calibration curve followed the equation:

$$I_p(\mu A) = 30.9 (\mu A/mM) * C_{IBF} - 5.54 (\mu A)$$

with an adjusted R^2 value of 0.999. The confidence intervals for the slope and intercept included one and zero, respectively. The limit of detection (LOD) was calculated as 0.0398 mM, while the limit of quantification (LOQ) was 0.313 mM. Repeatability ($n = 10$) yielded a coefficient of variation (CV) of 5.48%.

A recovery percentage of 100.3% was obtained relative to the declared IBF content in the pharmaceutical sample, with a CV of 6.27%.



Using the standard addition method, the recovery obtained for the pharmaceutical samples was 106.7%. The slope of the standard addition curve was similar to that of the calibration curve, indicating comparable sensitivity:

$$I_p(\mu A) = 29.8 (\mu A/mM) * C_{IBF} + 6.08 (\mu A).$$

Our results demonstrate that in the electrochemical determination of IBF using CNT-modified electrodes under the described conditions, filtration of insoluble excipients from coated tablets was not required, confirming the absence of matrix effects. The implementation of BIAS improved the analytical sensitivity of the method and reduced the total response time.

Conclusion. This study confirms that it is possible to calculate the acceptance value for the unit dosage uniformity test by weight variation, allowing for the accurate identification and quantification of ibuprofen in coated tablets. These findings support the use of electrochemical techniques with multi-walled carbon nanotube-modified electrodes coupled to BIAS as a viable approach for pharmaceutical quality control.

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P3-099**ELECTROANALYTICAL METHOD DEVELOPMENT FOR THE TYROSINE KINASE INHIBITOR LENVATINIB USING A $\text{Ti}_3\text{C}_2\text{T}_x$ -MXene-Based MOLECULARLY IMPRINTED POLYMER MODIFIED ELECTRODE****Gülcan, Mehmet¹; Talay Pinar, Pinar².**

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In this study, an MXene-based molecularly imprinted polymer (MX/GCE-MIP) sensor was developed for the first electrochemical determination of the tyrosine kinase inhibitor lenvatinib. The sensor integrates the high surface area, electrical conductivity, and chemical stability of MXene with the molecular recognition ability of MIP, enabling the selective and sensitive detection of lenvatinib. The sensor's performance was evaluated using square wave voltammetry (SWV) across varying lenvatinib concentrations. The results demonstrated a wide linear dynamic range (5.0–70 nM) and a low limit of detection (LOD) of 1.2 nM. Selectivity studies were conducted in the presence of potentially interfering molecules such as dopamine, uric acid, ascorbic acid, and dabrafenib, which share structural similarities with lenvatinib. The findings confirmed the high specific binding affinity of the MIP sensor. Key analytical parameters, including repeatability (3.20%), reproducibility (3.65%), and long-term stability (4.1%), were assessed, and the low relative standard deviation (RSD) values obtained demonstrated the sensor's reliability. Furthermore, the MX/GCE-MIP sensor was successfully applied to the determination of lenvatinib in synthetic serum and urine samples, yielding recovery rates of 103.6% and 112.4%, respectively. These results highlight the sensor's high accuracy and efficiency in complex biological matrices.

Keywords: MXene, Molecularly Imprinted Polymer, Lenvatinib, Synthetic Urine, Synthetic Serum.

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P3-100

DESIGNING ELECTROCHEMICAL SENSORS USING METAL-ORGANIC FRAMEWORKS: TAILORED STRUCTURES AND RESPONSE CHARACTERISTICS

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Metal-organic frameworks (MOFs) have gained significant interest due to their potential applications in catalysis, batteries, separation processes, and gas sensing.¹ These materials provide an innovative approach to the modular design of functional porous substances. By modifying the arrangement of inorganic nodes—composed of metal atoms or metal-oxo clusters—and organic linkers, typically polycarboxylates, a diverse range of three-dimensional frameworks can be synthesized. These frameworks exhibit a variety of topologies, textural properties, pore accessibility, and chemical characteristics on their internal surfaces.²

Metal-organic frameworks made from rare-earth (RE) elements—such as scandium, yttrium, and the lanthanide series—are an intriguing category known for their unique structural and functional properties. The coordination chemistry of RE metals is diverse, with only minor energetic differences among various coordination numbers and geometries, which are primarily influenced by the steric effects of ligands. Unlike d-block metals, RE metals display distinct electronic properties due to their 4f electron configurations, which are shielded from the external environment by the 5s and 5p orbitals. This shielding results in unique electronic and magnetic characteristics that are largely unaffected by coordinating ligands. By carefully adjusting the RE metal nodes and organic linkers, researchers can create RE-MOFs with complex structures and varied topologies, providing many opportunities for the development of functional materials.

In this study, we present the synthesis of various rare earth metal-organic frameworks (RE-MOFs) featuring different rare earth metals—specifically lanthanum, samarium, gadolinium, and dysprosium—along with benzene-1,3,5-tribenzoate (BTB) as the organic linker. The synthesis process commenced with the preparation of the organic linker, followed by the hydrothermal synthesis of the MOFs. The subsequent analysis focused on the influence of the metal on the structural and morphological characteristics of the synthesized MOF materials, employing techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). Furthermore, the study examined the electrochemical properties of the resultant MOFs, exploring their potential applications for the development of electrochemical sensors through cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

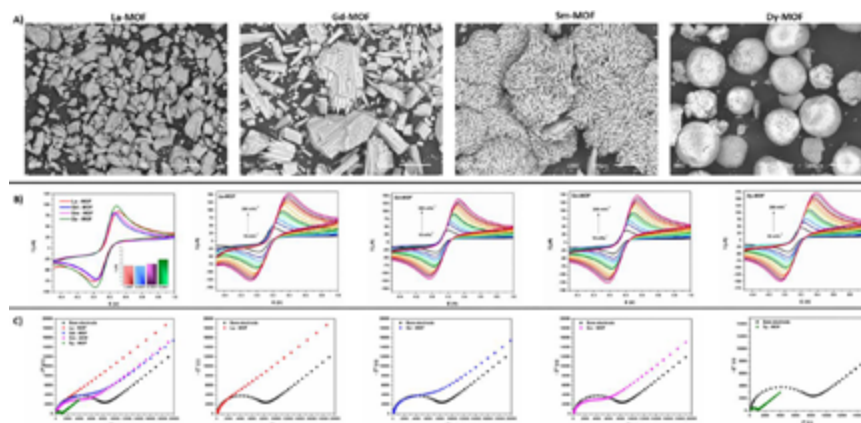


Figure 1. Morphological and electrochemical characterization of obtained RE-MOF materials A) SEM images of RE-MOFs; B) CV studies of electrodes modified with RE-MOFs; C) EIS studies of electrodes modified with RE-MOFs.

These findings provide valuable insights into the application of MOFs in electrochemical sensor development and highlight the efficacy of different rare earth metals in the synthesis of electrochemically advantageous MOFs. This research was supported by the Science Fund of the Republic of Serbia, grant No. 11077, Development of MIP functionalized MOF-based electrochemical Sensor for food Safety monitoring – MiMoSenSa.

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P3-101

SPECIFIC DETECTION OF PROSTATE-SPECIFIC ANTIGEN BY SANDWICH-TYPE ELECTROCHEMICAL APTASENSOR WITH SUPRAMOLECULAR ARCHITECTURE

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The design and development of surface-based biosensors is challenging as non-specific binding is a complex and persistent problem that adversely affects their performance. Electrochemical biosensors specifically have aroused great interest for the design and development of point-of-care diagnostic devices due to their diverse properties [1]. In this regard, those based on the immobilization of a capture probe on an electrode surface to enhance probe-surface interactions and avoid unwanted phenomena have resulted in highly complex and heterogeneous electrode surfaces [2]. Therefore, it is important to design and develop new methods to modify the electrode surface in a controllable manner in order to eliminate weakly nonspecific adsorptions.

This work is based on the use of an adamantane-modified aptamer as an affinity bioreceptor and a perthiolated β -cyclodextrin covalently bound to a gold-modified electrode surface. As a proof of concept, we have developed a sandwich-type amperometric aptasensor to detect prostate-specific antigen using the proposed strategy. For this purpose, the surface of screen-printed carbon electrodes is modified with reduced graphene oxide and fern-like gold nanoparticles, which were modified with CD for subsequent anchoring of an aptamer-adamantane conjugate. The analytical signal is obtained after recognition of the analyte by a second aptamer-peroxidase complex as a labeling element [3].

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P3-102**LASER-INDUCED BISMUTH-DOPED GRAPHENE SENSORS FOR SIMULTANEOUS DETECTION OF Zn, Pb, AND Cd IN PHYTOREMEDIATION ASSESSMENT**

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Heavy metals (HMs) are among the main environmental pollutants affecting ecosystems and human health [1]. They accumulate in soil and water bodies due to industrial activity, mining, and agricultural runoff. Once present, they persist and interfere with biological systems by damaging membranes, disrupting enzymes, and altering genetic material [2]. Phytoremediation, using metal-accumulating plants, offers a sustainable and low-cost strategy to reduce HM contamination [3]. However, assessing its effectiveness still relies on centralized laboratory techniques. Electrochemical sensors are a promising alternative: they are accurate, portable, and robust. Yet, complex sensing material fabrication continues to limit their scalability and practical use for in-field environmental monitoring.

In this work, we explore bismuth-doped laser-induced graphene (Bi-LIG) as a sensing platform for the simultaneous detection of Zn, Pb, and Cd at trace levels using square wave anodic stripping voltammetry (SWASV). Bi-LIG is made through a single-step laser process using a polyimide film coated with a bismuth-PVA hydrogel ink. The fabrication is fast, low-cost, and scalable. The resulting nanostructured sensing film combines the mechanical flexibility and porosity of LIG with the electrocatalytic properties of bismuth nanoparticles, making it a strong candidate for in-field sensing [4].

The morphochemical features of the graphene were evaluated via Raman spectroscopy, while SEM-EDS and XPS were used to study the composition and distribution of the laser-formed bismuth nanoparticles. Electrochemical characterization was performed using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Eventually, the sensors' figures of merit for the simultaneous detection of Zn, Pb, and Cd were assessed, obtaining captivating results. Analyses for the determination of these compounds in real samples concerning phytoremediation are currently on course. Summing up, herein, a one-step strategy to produce bismuth-doped graphenic sensors able to detect simultaneously heavy metals is proposed, resulting in a scalable and field-deployable alternative to detect these compounds in the agricultural/environmental framework.

Acknowledgments

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P3-103

ELECTROCHEMICAL DETERMINATION OF SURFACE pK_a IN 2-THIOBARBITURIC ACID-MODIFIED GOLD ELECTRODES: A NOVEL PLATFORM FOR BIOSENSOR DESIGN BASED ON H-BOND INTERACTIONS

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Biomolecule immobilization on functionalized metallic surfaces constitutes a cornerstone of contemporary biosensor engineering and molecular device fabrication. While electrostatic attachment mechanisms are extensively employed for protein immobilization, they frequently induce deleterious conformational changes that compromise biomolecular functionality. Hydrogen bond-mediated adsorption emerges as a sophisticated alternative strategy, exploiting the fundamental role of hydrogen bonding networks in native biomolecular recognition processes.

2-Thiobarbituric acid (TBA) represents an exceptional molecular platform for achieving controlled biomolecule immobilization, featuring a bifunctional architecture that combines robust surface anchoring capabilities with tunable interfacial chemistry. This molecule possesses thiol moieties enabling chemisorption onto gold surfaces via established sulfur-nitrogen chelate metal coordination chemistry, while simultaneously presenting functionalities that exhibit extreme pK_a values (1.874 and 10.16). This remarkable pH stability window facilitates predominantly non-electrostatic intermolecular interactions, particularly hydrogen bonding networks that preserve native biomolecular conformations. However, ionizable adsorbed thiols undergo substantial pK_a perturbations upon surface immobilization due to interfacial dielectric effects, necessitating comprehensive surface acid-base characterization.

The voltammetric behavior of the hexacyanoferrate(II/III) redox couple in acidic media exhibited a characteristic post-wave peak during cathodic potential excursions, attributed to electrostatic adsorption of anionic species onto protonated surface-bound TBA. This electrochemical signature was systematically exploited to determine the surface pK_a of immobilized TBA. Experiments employed polished planar gold electrodes modified through TBA adsorption (30 min) until achieving stationary coverage, as verified by quartz crystal microbalance (QCM) and electrochemical impedance spectroscopy (EIS). Modified electrodes were subjected to cyclic voltammetric analysis across an extended sweep rate range ($0.005 - 5.0 \text{ V s}^{-1}$) in solutions of varying pH (0.1 M KCl, 1 mM hexacyanoferrate(II/III)), with systematic monitoring of post-wave peak potentials.

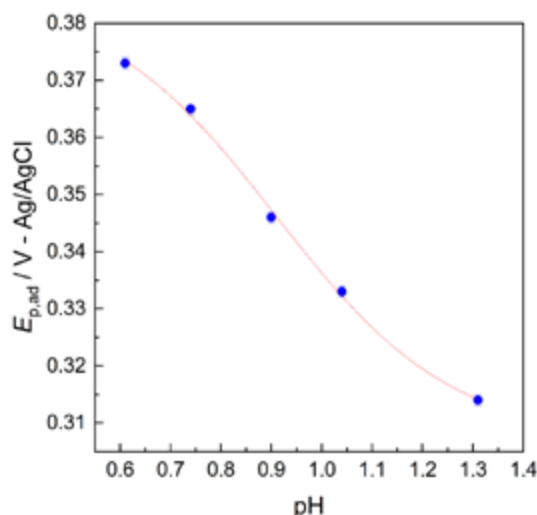
A comprehensive electrochemical model was developed by integrating the Nernst equation for soluble redox species with the Laviron formalism for surface-confined processes. This theoretical treatment established the relationship between post-wave peak potential and solution pH, exhibiting the classical sigmoidal behavior characteristic of acid-base transitions.

Experimental data demonstrated excellent agreement with theoretical predictions, yielding a surface pK_a value of 0.91 ± 0.02 for immobilized TBA (Figure 1). This represents a significant shift from the solution-phase pK_a (1.874), confirming substantial interfacial perturbation of acid-base equilibria upon surface immobilization. As other ionisable SAMs, the pK_a value in the adsorbed state is lower than the value observed in solution.

This investigation presents a systematic electrochemical characterization of surface acid-base properties for an exposed carbonyl functionality in adsorbed thiol derivatives. The developed methodology establishes an analytical platform for pH-controlled biomolecule immobilization via hydrogen bonding interactions and provides a robust tool for surface pK_a determination of advanced materials under extreme pH conditions. These findings have significant implications for rational design of biosensor interfaces and molecular recognition platforms.

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P3-104**EVALUATION OF SODIUM TAUROCHOLATE MICELLIZATION PROCESS IN SIMULATED INTESTINAL FLUIDS THROUGH COMPLEMENTARY ANALYTICAL TECHNIQUES****Amézqueta, Susana; Fuguet, Elisabet; Ràfols, Clara.***Universitat de Barcelona, Barcelona, Spain.*

Bile salts, such as sodium taurocholate (NaTc), play a pivotal role in enhancing the bioavailability of drugs due to their unique ability to form micelles. These micelles are aggregates that can encapsulate a wide range of drugs, significantly improving their solubility in biological fluids. The process of micellization and the types of micelles that are formed are influenced not only by the surfactant itself but also by various environmental factors, including the solution medium used, temperature, and the presence of other compounds [1]. Therefore, it is crucial to ensure the presence of micelles during solubility assays to accurately assess drug solubility and bioavailability.

In this study, the critical micelle concentration (CMC) of NaTc was evaluated using three distinct techniques: conductometry, fluorescence, and calorimetry. These evaluations were conducted in water as well as under various biorelevant conditions, including intestinal biorelevant aqueous buffers, FaSSIF-v2 (Fasted State Simulated Intestinal Fluid version 2), and FeSSIF-v2 (Fed State Simulated Intestinal Fluid version 2) [2]. Each of these techniques offers unique insights into the micellization process.

Conductometry, which measures the electrical conductivity of a solution, was found to be inadequate for determining the CMC under biorelevant conditions due to the high ion strength of the solution media (> 100 mM). On the other hand, fluorescence and calorimetry provided complementary information that was more reliable for CMC determination.

Fluorescence spectrometry involves the use of fluorescent markers that suffer a modification in their fluorescence properties when micelles are formed [3]. This method is sensitive, low-sample consuming, and can provide detailed information about the micellization process. Calorimetry, which measures the heat changes associated with micelle formation, offers another layer of insight. It provides information about the thermodynamics of micellization, including the enthalpy and entropy changes that occur during the process.

The CMC experiments conducted in this study showed that ion strength and the presence of mixed micelles influence the micellization process. The presence of ions in the solution stabilizes the micelles and promotes their formation at lower concentrations of surfactant. On its behalf, mixed micelles are formed by the combination of NaTc with other components such as phosphatidylcholine and lipids. These mixed micelles are formed at low NaTc concentrations and, therefore, can enhance the solubility of drugs even further.

The findings of this study have important implications for drug formulation and delivery. By understanding the factors that influence micellization, researchers can better design drug formulations that maximize bioavailability. Additionally, the use of biorelevant conditions in CMC experiments ensures that the results are more applicable to real-world scenarios, where drugs are administered in complex biological environments.

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Figure 1. Experimental data fitted to a generalized sigmoidal function

P3-105**OVERVIEW OF ANALYTICAL METHODS FOR ETHYLENE GLYCOL AND DIETHYLENE GLYCOL AND THEIR EVALUATION IN COMPLEX MATRICES****Cho, Hyunsu.***Analytical Research and Verification Lab, Amorepacific R&I Center, Yongin-si, South Korea.*

Since the tragic accident in The Gambia in 2022, which the WHO (World Health Organization) attributed to ethylene glycol (EG) and diethylene glycol (DEG) contamination in cough syrup, the FDA (U.S. Food and Drug Administration) has announced a new guideline to manage the risk from EG and DEG [1] [2].

According to the guideline and several previous studies, EG and DEG possess similar organoleptic characteristics to glycerin, propylene glycol, and sorbitol, such as sweetness and viscosity, making them easy to adulterate in place of appropriate materials [3]. Due to their continuous hazard, verifying the presence of EG and DEG in related pharmaceutical products has become increasingly important.

This study investigates and compares analytical methods in global standards, including those from China, the EU, India, Japan, South Korea, the United States, etc. According to FDA warning letters, 79 cases have been documented involving insufficient testing of EG and DEG in glycerin, propylene glycol, or sorbitol. Notably, the issue has expanded beyond syrup-based medications, which were the initial focus of concern regarding EG/DEG, to a wide variety of products across different industries, such as topical ointments, wet tissues, mouthwashes, sunscreens, toothpastes, hand soaps, and hand sanitizers. Moving forward, it will become necessary to implement EG/DEG analytical methods for all forms of pharmaceuticals. This study particularly focuses on medicinal cosmetic raw materials composed of multiple compounds.

By applying the EG/DEG analytical method in the USP glycerin monograph, a screening test for EG/DEG was conducted in pharmaceutical cosmetic raw materials analyzed by GC-FID to evaluate the detection levels (0.1%). Typically, most analytical methods adhering to global standards are based on analyzing a single substance. However, most raw materials in practical cases are not single substances, but complex mixtures composed of multiple substances. This study demonstrates the detection of EG/DEG in such complex matrices and provides a reference for industries that require such regulatory oversight.

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P3-106**IDENTIFICATION OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1) USED IN TYPE 2 DIABETES THERAPY AS A POTENTIAL DOPING FACTOR**

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According to the data published by WHO, the number of people living with diabetes rose from 200 million in 1990 to 830 million in 2022, and the majority of them did not take medication for their diabetes [1]. In accordance with the Polish Diabetics Association and clinical guidelines for the treatment of diabetes patients from 2014, it is recommended to use a particular group of drugs: metformin, sulphonylureas, α-glucosidase inhibitor, thiazolidinediones (pioglitazone), SGLT-2 inhibitors (Sodium-glucose transport proteins), drugs incretin – dipeptidyl peptidase 4 (DPP-4) or glucagon-like peptide – 1 receptor agonists (GLP-1) [2]. Due to the effect, medications classified in group GLP-1 agonists are also used in the treatment of overweight. As a result, there is a noticeable social problem of abuse of these preparations for more than just medical purposes. Considering the usefulness of GLP-1 analogues as a weight-reducing agents, there is a high probability of athletes abusing this kind of substance for doping purposes. To achieve a needed result, athletes could take this medication in doses exceeding therapeutic levels, which could cause side effects, including those adversely affecting health and posing a threat to life.

According to the current regulations of the World Anti-Doping Agency (WADA), GLP-1 agonists and its analogs are not prohibited in sports. However, in 2024, WADA decided to add semaglutide to the list of the 2024 Monitoring Program for both In-competition and Out-of-competition use [3].

This study presented the identification of compounds classified as GLP-1 analogs. The detection was conducted by means of ultra-performance liquid chromatography-tandem mass spectrometry

(UPLC-MS/MS). Three different sample preparation methods were compared: method I – based on enzymatic hydrolysis with liquid-liquid extraction, method II – Dilute and Shoot approach, and method III – Solid Phase Extraction. Therefore, different chromatographic columns were tested. Additionally, real case samples collected from diabetic patients were performed.

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P3-107

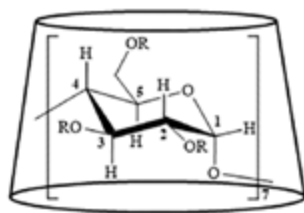
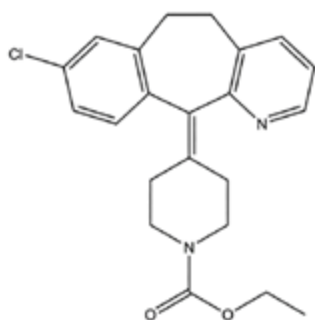
EFFECT OF β -CYCLODEXTRIN AND ITS DERIVATIVES ON LORATADINE STABILITY

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Loratadine (LOR) is a second-generation antihistaminic, classified as a Class II drug according to the Biopharmaceutical Classification System due to its low solubility and high permeability [1]. Cyclodextrins (CDs), cyclic oligosaccharides, are often used to enhance the solubility of such drugs by inclusion complexation. However, this may alter the chemical stability of drugs [2].

This work aimed to study the effect of β -CD and its derivatives, hydroxypropyl (HP- β -CD), randomly methylated (RM- β -CD) and sulfobutylether sodium salt (SBE- β -CD), on LOR chemical stability. Forced degradation studies were performed at hydrolytic (acidic and basic) and oxidative conditions, both at room temperature and 80 °C. Samples were analyzed after 1, 2, 5 and 8 hours of degradation. For quantification of loratadine, the UHPLC-DAD method was developed and validated. Chromatographic separation of LOR and its degradation products was achieved by gradient elution on a Zorbax Eclipse Plus C18 column (1.8 μ m, 2.1 $\times$ 30 mm) using mobile phases consisting of 0.1% formic acid in water (A) and acetonitrile (B). The flow rate was set at 0.2 mL min⁻¹, and the column temperature was 40 °C. The wavelength for quantification was set at 278 nm. The developed method was linear in the range (5 – 50) μ g mL⁻¹, with limits of detection and quantification at 0.61 μ g mL⁻¹ and 1.86 μ g mL⁻¹, respectively. According to the results, significant degradation wasn't observed at room temperature. When degradation was performed at 80 °C, LOR was stable in acidic media, while the highest degradation was achieved under basic conditions, where approximately 70% of LOR was degraded. CDs had no significant effect on the degradation of LOR, except in oxidative conditions, where β -CD caused the most significant increase in LOR degradation, resulting in 30% of LOR degradation compared to 15% degradation of pure LOR.



β -CD	R = -H
HP- β -CD	R = -H or -CH ₂ CH(OH)CH ₃ ; DS = 4.5
RM- β -CD	R = -H or -CH ₃ ; DS = 12
SBE- β -CD	R = -H or -(CH ₂) ₄ SO ₃ Na; DS = 6.5

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P3-108

EVALUATION OF THE BINDING BETWEEN HUMAN SERUM ALBUMIN AND DRUGS USING FLUORESCENCE SPECTROSCOPY

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The binding between drugs and plasma proteins plays an essential role in modulating the effective drug concentration at pharmacological binding sites in drug development studies due to the high concentration of proteins in plasma and the tendency of many drugs to bind to them [1]. On one side, protein binding is essential in carrying drugs to tissues and organs in the human body and in maintaining an almost constant concentration of the drug in plasma by releasing the drug gradually from the complex formed with the protein. On the other side, high binding may have disadvantages as it demands higher doses to reach the effective concentration, in addition to having limited distribution to the action site and restricted elimination from the body [2]. The importance of studying the binding between drugs and plasma proteins comes from the contribution of this binding in the pharmacological activity and the adverse effects or drug safety problems that appear if this binding is strong enough [3].

Human serum albumin (HSA) is the most predominant protein in plasma. It is a monomeric macromolecule composed of three homologous helical domains (I, II, and III), each of which consists of two subdomains termed A and B. HSA demonstrates a high capacity to ligand binding and serves as a depot and a transporter for numerous endogenous and exogenous compounds. The binding sites of HSA are placed mainly in two pockets, subdomain IIA (site 1) and subdomain IIIA (site 2). Studies also reported the presence of a new secondary site at the border between subdomains IIA and IIB (IIA-IIB) [4,5].

In this study, fluorescence spectroscopy has been used to evaluate the interaction between HSA and five drugs representing different pharmacological categories: Nicardipine, Nizatidine, Trazodone, Maprotiline, and Imipramine. All the investigated drugs contain amine groups, with Nicardipine and Nizatidine additionally having nitro groups. The experiments have been conducted under physiological conditions (phosphate buffer saline at pH 7.4 and ion strength =150 mM).

The reason for using fluorescence spectrometry technique in studying drug-albumin interactions is that albumin has three fluorescent amino acids, tryptophan, tyrosin, and phenylalanine. The fluorescence quenching phenomenon occurs when a decay in the fluorescence intensity of a sample happens due to molecular interactions such as ground-state complex formation, which can be demonstrated through the Stern-Volmer equation. However, this model is valid as long as the drug's own fluorescence, if exists, doesn't cause interference with albumin's fluorescence [6]. Proving the creation of a ground-state complex formation allows the calculation of the process parameters, which are the binding constant and the stoichiometry, by applying the double logarithmic Stern-Volmer equation [7].

Although some drugs displayed fluorescence, they did not interfere significantly with the HSA spectra as their maximum intensities were observed at wavelengths different from those of HSA, which enables using the same model for calculating their binding parameters. Thus, the findings of this study show that Maprotiline doesn't have a significant binding with HSA, whereas the other four drugs exhibit weak interactions.

According to the calculated binding constants, the bound drugs can be ranked in ascending order as: Nicardipine, Imipramine, Trazodone, and Nizatidine. It is important to mention that the fluorescence measurements show low binding affinities for the studied drugs. Therefore, further investigation using other complementary techniques is recommended to obtain a deep understanding of their binding behavior.

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P3-109**GENTAMICIN DETERMINATION USING SILVER NANOPARTICLES AND SMARTPHONE-BASED DETECTION****Kubacki, Karol¹; Paluch, Justyna²; Kozak, Joanna².**

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Gentamicin is an aminoglycoside antibiotic used to treat severe bacterial infections. Due to its narrow therapeutic index, it is necessary to monitor the concentration of this antibiotic in the blood to ensure the effectiveness of the therapy and avoid side effects such as nephrotoxicity and ototoxicity. In addition, since the concentration of antibiotics (including aminoglycosides) in the ecosystem is constantly increasing, resulting in increased drug resistance of bacteria, it remains important to monitor the antibiotic content in the environment and food [1].

In recent years, there has been significant interest in using nanomaterials in analysis, including silver nanoparticles. They are also used in methods dedicated to the determination of antibiotics [2]. Modern chemical analysis increasingly uses smartphones as easily accessible and portable detection systems. For this purpose, smartphones are usually adapted to spectrophotometric, fluorimetric, and chemiluminescent methods. Images from the smartphone camera, processed using various software, can be described using colour spaces (e.g. RGB, CMYK, HSL, HSV). The value of the analytical signal can be obtained, for example, using one of the channels representing the colour component in the selected colour representation.

The research concerned the development of a method for the determination of gentamicin using silver nanoparticles and smartphone-based detection, including the selection of reagents required for nanoparticle synthesis, their concentrations, reaction conditions, and time, as well as the concentration of nanoparticles used for antibiotic determination. Gallic acid was selected for the synthesis of silver nanoparticles, which has been described as a natural reducing and stabilising agent for the spectrophotometric determination of streptomycin [2].

Analytical parameters such as linear range, detection limit, quantification limit, precision, and accuracy were determined for the developed smartphone-based detection method and spectrophotometric method. The influence of potential interferents was investigated. The proposed method was verified by determining gentamicin in synthetic samples and applied to the determination of gentamicin in medicinal products. The obtained results were compared with the results of spectrophotometric determinations. The method shows potential for use with other types of samples.

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P3-110**IDENTIFICATION OF ETODOLAC REACTIVE METABOLITES FORMATION AND PROTEIN BINDING BY HIGH-RESOLUTION MASS SPECTROMETRY****Liu, Kwang-Hyeon; Kim, Eui-Hyeon.***BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu, South Korea.*

The occurrence of drug-induced liver injury (DILI) is associated with the covalent binding of drugs or their reactive metabolites to proteins. When drugs are metabolized to electrophilic, reactive metabolites, they can bind to nucleophilic proteins and cause DILI. Liver damage caused by etodolac, one of the non-steroidal anti-inflammatory drugs that selectively inhibits cyclooxygenase-2 (COX-2), also has been reported. In a previous study, we identified three reactive metabolites of etodolac in human liver microsomal incubations by glutathione trapping using liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS). Based on these results, we evaluated protein binding of etodolac reactive metabolites. Etodolac was incubated with glutathione S-transferase P1 (GSTP1) or human serum albumin in human liver microsomes in the presence of NADPH generating system. After microsomal incubation, enzymatic digestion with trypsin or pepsin, and clean-up using solid phase extraction were performed. Etodolac adducts with these proteins were identified using high-resolution mass spectrometry. In addition, adducts between hydroxyetodolac and these proteins were also identified based on isotope pattern and product ion scan mass spectrum. Etodolac and hydroxyetodolac were formed covalent bond with cysteine residue of these proteins. The generation and characterization of etodolac-protein adducts may help in understanding drug-induced liver injury and serve as a potential tool to assess the risk of covalent binding of drugs to proteins.

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P3-111**ENANTIOMERIC SEPARATION OF TIANEPTINE BY CYCLODEXTRIN-ELECTROKINETIC CHROMATOGRAPHY USING DEEP EUTECTIC SOLVENTS AS SUSTAINABLE ADDITIVES****Martínez-Olivares, José Agustín; Marina, María Luisa; Castro-Puyana, María.***Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares (Madrid), Spain., Spain.***Keywords:** *chiral electrokinetic chromatography, cyclodextrins, deep eutectic solvents, tianeptine.*

Chirality is a critical factor in the development of pharmaceuticals. Despite having the same molecular formula, two enantiomers can exhibit differential interactions with biological systems. In numerous instances, merely one of the enantiomers exhibits the desired therapeutic activity, while the other may be inactive or even induce adverse effects. The separation and enantiomeric analysis of pharmaceuticals are, therefore, paramount to ensure their safety, efficacy, and quality. In the field of chiral separations, Electrokinetic Chromatography (EKC), is a highly effective analytical tool, enabling the separation of enantiomers with high resolution, efficiency, and minimum sample requirement [1].

To enhance the efficiency of chiral separations by EKC, a range of additives have been evaluated. Among these, Deep Eutectic Solvents (DESSs) have emerged as one of the latest trends. These compounds, which are formed by combining a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD), exhibit high stability, low volatility, tunable solubility, and ease of preparation. Of particular interest is the fact that these substances are often derived from naturally occurring materials, a characteristic that renders them both cost-effective and low in toxicity, as well as highly biodegradable. While the use of DESs in EKC is still in its early stages, some works have substantiated their effectiveness in enhancing stereoselective separations in combination with chiral selectors such as cyclodextrins (CDs) [2,3].

In this context, this work aimed to develop an enantioselective methodology by EKC based on the combination of CDs with DESs for the separation of tianeptine, an antidepressant drug that is commercialized as racemate. For this purpose, the potential of different neutral and negatively charged CDs as chiral selectors was evaluated at two pH values (3.0 and 7.0). Among all the tested CDs, sulphated- α -CD at a concentration of 1.5 % (w/v) enabled the separation of tianeptine enantiomers with a resolution value of 1.8 in 10 min. Subsequently, a series of ready-made DESs, as well as their individual components, were assessed in conjunction with the selected CD. The results obtained demonstrated that the combined use of sulphated- α -CD and tetrabutylammonium bromide (TBABr) enhanced the enantioselectivity of the method. The analytical characteristics of the developed method were evaluated and found to be adequate for the determination of tianeptine in pharmaceutical formulations showing its potential to achieve their adequate quality control.

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P3-112**PHARMACOLOGICAL POTENTIAL AND CHEMICAL PROFILE OF *TAGETES ERECTA* L. "TIGER EYE" EXTRACTS USING VARIOUS EXTRACTION METHODS****Meirbekov, Nurkanat; Alimzhanova, Mereke.***KazNU named after al-Farabi, Almaty, Kazakhstan.*

The *Tagetes erecta* L. cultivar known as "Tiger Eye" is not only an ornamental plant with vivid coloration but also a promising source of natural therapeutic compounds. It is of particular interest as a reservoir of biologically active constituents exhibiting high pharmacological potential. This study aims to comprehensively evaluate the chemical composition and biological activity of extracts derived from this cultivar using four innovative extraction techniques: subcritical CO₂ extraction, ultrasound-assisted extraction (UAE), Soxhlet extraction, and hot reflux extraction.

The primary goal of the research is the isolation, identification, and comparative analysis of key groups of natural compounds such as terpenoids, essential oils, fatty acids, phenolic compounds, and others. Special attention is given to evaluating the efficiency of each extraction method, its impact on compound yield, their stability, and subsequent pharmacological activity. The chemical profiles of the extracts are analyzed using high-precision gas chromatography-mass spectrometry (GC-MS), allowing for the determination of composition, concentration, and the potential biological relevance of the identified compounds.

In parallel with chemical analysis, the antimicrobial activity of the "Tiger Eye" extracts is examined against several pathogenic microorganisms, including *Plasmopara viticola*, *Botrytis cinerea*, and *Colletotrichum* spp.

The results show that extracts obtained through ultrasound-assisted extraction contain notable amounts of terpenoids and esters (germacrene D – 4.57%, 9,12-octadecadienoic acid, ethyl ester – 8.03%), while CO₂ extraction provides high purity of terpenoids (caryophyllene – 10.18%, phytol – 5.29%) and thiophenes (α -terthienyl – 5.08%). Soxhlet extraction revealed the highest fatty acid content (hexadecanoic acid – 8.09%, 9,12-octadecadienoic acid – 7.37%), whereas hot reflux extraction showed the greatest concentration of sterols (stigmasterol – 30.67%, γ -sitosterol – 18.65%, campesterol – 17.65%). These differences directly influence the antifungal activity level of each extract type, making it possible to tailor the extraction method according to the intended application.

The research findings are particularly significant for the pharmaceutical field. The identified compounds—especially terpenoids, sterols, and thiophenes—demonstrate pronounced antifungal activity. This opens the possibility for developing targeted herbal formulations, including ointments, tinctures, inhalable forms, and capsules based on standardized *Tiger Eye* extracts. Thus, *Tagetes erecta* L. "Tiger Eye" may serve as a valuable source of novel active substances for the development of modern plant-based medications, offering safe and eco-friendly alternatives to synthetic counterparts.

The diversity and high concentration of bioactive compounds such as caryophyllene (10.18%), phytol (5.29%), α -terthienyl (5.08%), hexadecanoic acid (8.09%), 9,12-octadecadienoic acid (7.37%), stigmasterol (30.67%), β -amyryl (15.46%), α -farnesene (1.32%), and others in the extracts make this cultivar a promising raw material for the pharmaceutical, cosmetic, and agricultural industries. The data obtained lay the foundation for further applied research and commercial utilization of *Tagetes erecta* L. extracts in the development of new medications, biopesticides, and natural cosmetic products.

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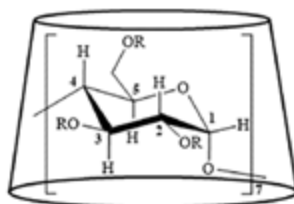
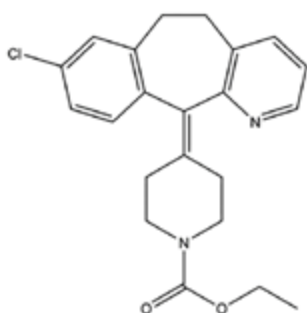
DISSOLUTION RATE OF LORATADINE IN THE PRESENCE OF β -CYCLODEXTRIN AND ITS DERIVATIVES

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Loratadine (LOR) is a second-generation antihistaminic, often used for the treatment of allergy symptoms. It is a weak base with pKa values in the range 4.85 – 6.00. According to the Biopharmaceutical Classification System, it is a Class II drug, characterized by low solubility and high permeability [1]. Cyclodextrins (CDs) are cyclic oligosaccharides known for their ability to form inclusion complexes with various molecules, affecting their physico-chemical properties, such as solubility and stability [1, 2]. Therefore, they are of great interest to the pharmaceutical industry in developing new drug formulations with improved properties. In our previous work, we studied the solubility of LOR in the presence of β -cyclodextrin (β -CD) and its derivatives, hydroxypropyl (HP- β -CD), randomly methylated (RM- β -CD) and sulfobutylether sodium salt β -CD (SBE- β -CD). It has been demonstrated that all CDs significantly enhance the solubility of LOR in water and biorelevant media [1]. Therefore, solid-state complexes of LOR and CDs were prepared and characterized.

In this work, we present the results of *in vitro* dissolution studies of LOR and its binary systems with CDs. Complexes in the solid state were prepared by grinding and spray drying in a 1:1 and 1:2 stoichiometric ratio. *In vitro* dissolution studies were performed in simulated intestinal media (SIM, pH 6.8) at 37 °C. Aliquots of the samples were analyzed after 5, 10, 15, 30, 45, and 60 minutes using a previously validated UV-Vis spectrophotometric method. Due to the low solubility of pure LOR at pH 6.8, liquid chromatography was used for its quantification. The obtained *in vitro* dissolution profiles were characterized by calculating the dissolution efficiency (DE) and dissolution enhancement factor (DEF). Additionally, the model-independent similarity factor f_2 was calculated to compare the *in vitro* dissolution profiles. Results indicate a low dissolution efficiency of pure LOR; however, the addition of CDs significantly increases it, especially in the case of RM- β -CD. The dissolution efficiency of spray-dried samples was in most cases similar to ground samples, except for LOR: β -CD 1:2 and LOR:HP- β -CD 1:1, where dissolution efficiency was greater (LOR: β -CD 1:2) or lower (LOR:HP- β -CD 1:1) than of the ground sample. Based on the similarity factor of pure and ground LOR (f_2 = 99.62), it was concluded that grinding alone does not affect the *in vitro* dissolution profile of LOR. Therefore, the observed enhancement in dissolution in the tested binary samples is related to the efficient CD complexation of the drug achieved in the solid state.



β -CD	R = -H
HP- β -CD	R = -H or -CH ₂ CH(OH)CH ₃ ; DS = 4.5
RM- β -CD	R = -H or -CH ₃ ; DS = 12
SBE- β -CD	R = -H or -(CH ₂) ₄ SO ₃ Na; DS = 6.5

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P3-114**EVALUATING TWO DERIVATIZATION TECHNIQUES FOR TRACE-LEVEL NITRITE QUANTIFICATION VIA LC-MS/MS****Rojas, Oriol; Gotor, Gemma; Nonell, Santi.***IQS, Barcelona, Spain.*

Nitrosamines are chemical compounds that have recently garnered significant attention in the pharmaceutical industry due to their carcinogenic potential. Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have raised alarms about the presence of nitrosamines in pharmaceutical products, leading to recalls of contaminated medications and the introduction of stringent guidelines for acceptable limits.

Nitrosamines can form as unintended impurities during the manufacturing process of pharmaceuticals, particularly when nitrites and amines—both commonly present in raw materials or excipients—interact under acidic conditions. These conditions are often encountered during formulation, storage, or even within the body after administration. Given that even trace amounts of nitrosamines can pose health risks with long-term exposure, it is crucial to accurately detect and quantify their precursors, especially nitrites, in pharmaceutical products.

In response to this challenge, the present study focuses on the evaluation of two derivatization methods for nitrite detection, utilizing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This highly sensitive and selective analytical technique allows for the detection of extremely low concentrations of chemical compounds in complex matrices, such as pharmaceutical formulations. The two derivatization approaches compared in this study are the traditional Griess method⁽¹⁾⁽²⁾ and the 2,3-diaminonaphthalene (DAN) method⁽³⁾.

The Griess method, long established in analytical chemistry, involves a multi-step reaction process that converts nitrite into a colored azo compound, which can then be measured. While this method has proven effective over time, it has notable drawbacks in modern analytical contexts. The multi-step nature of the procedure increases the risk of contamination, introduces variability, and often results in elevated blank signals. These factors can compromise the accuracy of measurements, particularly at the low concentrations required for compliance with regulatory standards.

In contrast, the DAN method provides a more streamlined and efficient alternative. It involves a single-step reaction in which nitrite reacts with 2,3-diaminonaphthalene to form a fluorescent naphthotriazole derivative. This derivative can be detected with high sensitivity using LC-MS/MS, allowing for lower limits of quantification (QL) and reduced background interference. Because the DAN method is simpler and requires fewer reagents and handling steps, it significantly reduces the likelihood of contamination.

The results of this study indicate that the DAN method offers superior analytical performance compared to the Griess method. Specifically, it demonstrates greater sensitivity, allowing for the detection of lower nitrite concentrations, and improved specificity, enabling more accurate differentiation from other potential interferences. These advantages position the DAN method as a highly promising candidate for routine quality control and safety testing in the pharmaceutical industry.

As regulatory scrutiny continues to increase regarding nitrosamine contamination, the development and implementation of more reliable nitrite detection methods like the DAN approach will be critical in ensuring the safety and integrity of pharmaceutical products.

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P3-115**ADDICTIVE FENTANYL AND ITS POSSIBLE ALTERNATIVE**

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Fentanyl belongs to a group of compounds commonly known as synthetic opiates and, in general, has been used as an addition to anesthesia as well as to relieve post operation pain [1-2]. Fentanyl is a potent narcotic analgesic with a rapid onset and short duration of action [3]. The chemical structure of all compounds from the fentanyl group is based on piperidine, which also occurs in morphine [4]. These compounds bind to specific receptors called opioid receptors. Fentanyl owes its popularity to the fact that its compound acts similarly to morphine and heroin. The only difference is that fentanyl acts 75-125 times more strongly than heroin, and also the effects occur much more rapidly. The use of fentanyl, as a very strong pain medication with a narcotic effect, causes that the mechanism of the addiction to it, develop a little faster than, for example, in the case of morphine. Fentanyl is a pure agonist, i.e. it has a high affinity to the μ receptors and low affinity to the δ and κ receptors. Therefore, it has very strong analgesic activity. Fentanyl and its analogues are available as pharmaceutical preparations mainly in the form of injection fluids, but also nasal sprays, lollipops, and products for buccal and sublingual administrations. Fentanyl is well absorbed through the skin and thus it can be applied in the form of transdermal patches. The applied doses depend on the route of administration [2].

Fentanyl can be also abused by athletes to relieve pain to be able to compete in spite of previous injuries. In the World Anti-Doping Agency (WADA) List of Prohibited Substances and Methods, fentanyl and its derivatives are listed in the group S7 – narcotics, and hence its use by athletes during competition is banned for concentration values above 1 ng/mL in urine [5]. Fentanyl as synthetic primary μ -opioid receptor agonist with a potent narcotic and analgesic properties is frequently used as analgesic drug during colonoscopy [6]. Administration of fentanyl during endoscopic procedure can also result in a positive doping test. The exertion profile of fentanyl after its application for medical reasons will be presented.

The alternative, in such case, would be to use a pain reliever which does not lead to addiction or overdose. Suzetrigine, a new pain relief drug, which was approved for medical use can serve as such an alternative to opioids, e.g. fentanyl [7].

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P3-116**PREPARATION AND CHARACTERIZATION OF CHROMOLITH WP 300 EPOXY HPLC COLUMN WITH IMMOBILIZED MALT1****Marković, Olivera¹; Delgado, Anna²; Llinàs, Antonio³; Ràfols, Clara²; Fuguet, Elisabet⁴.**

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In recent years, there has been increasing interest in the preparation and characterization of biomimetic HPLC systems that are able to estimate specific biological properties relevant to drug discovery. The estimation of biological properties can be achieved by measuring retention in HPLC systems. One of the major advantages of using HPLC technology for this purpose is that different stationary phases mimicking biological systems can be used. Mucosa-associated lymphoid tissue lymphoma translocation protein-1 (MALT1) is a human paracaspase which has central role in the immune response^{1,2}. In addition, MALT1 is responsible for the cleavage of various proteins and has a scaffolding function. Consequently, small molecule MALT1 inhibitors could be promising candidates for clinical use in allergic inflammation or autoimmune diseases. The aim of this work was to prepare and characterize a Chromolith WP 300 Epoxy HPLC column with immobilized MALT1. The immobilization of MALT1 was done using the epoxy method. First, the column was equilibrated with an immobilization solution. Immobilization was then performed using a dynamic process in which the MALT1 solution was circulated through the column for 24 hours. Finally, the column was washed to quench the remaining epoxide functions. The prepared column was tested for reproducibility, organic solvent content compatibility, and column linear range. Furthermore, MALT1 inhibitors with known activities towards the protein¹ were injected and correlation between activity and retention of the compounds was investigated. In addition, the column was evaluated using the Abraham solvation parameter model. These results can be used as the basis for further optimization of columns with immobilized MALT1. The preparation and characterization of new biomimetic HPLC column with immobilizing proteins associated to specific pathologies may have a major impact on drug discovery due to the numerous potential applications.

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P3-117**CHARACTERIZATION AND PROFILE OF OLIGONUCLEOTIDES AND THEIR LIPID-CONJUGATIONS BY HYDROPHILIC INTERACTION CHROMATOGRAPHY****Bui, Quang Dong¹; Deschrijver, Tiny²; Noten, Bart²; Eeltink, Sebastiaan¹.***¹Vrije Universiteit Brussel, Ixelles, Belgium; ²Johnson and Johnson Innovative Medicine, Beerse, Belgium.*

Therapeutic oligonucleotides (ONs) are single or double-stranded chemical-modified polymers of nucleotides that show great promise as a treatment for genetic-related diseases in humans. The main structure of ONs comprises a hydrophilic negatively-charged ribose-phosphate backbone with distinct Watson-Crick bases attached to the ribose units. Furthermore, the conjugation of fatty acids to therapeutic ONs plays a crucial role in enhancing tissue targeting. Analytical characterization of oligonucleotide drugs and related products is extremely challenging due to the inherent structural complexity (primary and higher-order structures) and the presence of many, structurally closely related impurities, resulting in a dynamic concentration range over multiple orders of magnitude

Hydrophilic interaction chromatography (HILIC) has been recognized as a suitable chromatographic technique for the analysis of ONs due to their hydrophilic and negatively charged ribose-phosphate backbone. The retention mechanism of ONs on HILIC stationary phases involves hydrophilic partitioning, electrostatic interactions, and donor-acceptor hydrogen bonding. Therefore, HILIC coupled with mass spectrometry (HILIC-MS) has been widely used for characterizing various biomolecules, including proteins, glycopeptides, oligonucleotides, and small molecules such as glycans and saccharides.

This study aims to evaluate the effects of mobile phase additives and organic modifiers on chromatographic resolution and selectivity. First, different additives were assessed for their impact on selectivity between the main product and closely related structural impurities, including deaminated and arabinosyl variants, shortmers, and phosphodiester (PO) impurities. The use of ion-pairing agents resulted in breakthrough peaks for ONs under different gradient conditions, whereas ammonium salts did not exhibit this issue. The PO impurity was successfully separated from the main product at high temperatures. While the n-1 impurity was difficult to resolve in an acetonitrile-based eluent, the use of a protic solvent such as methanol (MeOH) altered selectivity but resulted in lower UV signal intensity. Additionally, mass spectral complexity and signal intensity were compared to determine the optimal method. In the second phase of the study, various lipid-conjugated ONs were characterized using HILIC. The separation of main products from their shortmers was optimized to enhance resolving power. Moreover, small interfering RNA (siRNA) maintained its higher-order structure under HILIC conditions, even at elevated temperatures.

P3-118**NITROSAMINE FORMATION IN LIDOCAINE ACTIVE PHARMACEUTICAL INGREDIENTS AND DRUG PRODUCTS**

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N-nitrosamine compounds have long been known for their carcinogenic properties, but they came into the focus of the pharmaceutical industry in 2018 when N-nitrosodimethylamine was detected in the active ingredient Valsartan. Since then, there has been a growing literature on the formation of N-nitrosamines in pharmaceutical products. Lidocaine, a local anaesthetic, has been used for more than 70 years in a wide variety of preparations like injections, creams, and sprays. In the lidocaine active pharmaceutical ingredient (API) and in its formulations, two potential nitrosamine impurities were identified: the highly carcinogenic low molecular weight nitrosamine, N-nitrosodiethylamine (NDEA), and the drug substance-related N-nitroso-desethyl lidocaine. The acceptable intake (AI) of NDEA is 26.5 ng/day, while for N-nitroso-desethyl lidocaine it is 100 ng/day, established by the European Medicines Agency. Diethylamine, the precursor of NDEA, is added in the synthesis route and can be present as a residue in the API, while desethyl lidocaine, the precursor of N-nitroso-desethyl lidocaine, is formed by the N-dealkylation of lidocaine, and it is a reported impurity by the pharmaceutical authorities.

In this work, we have investigated the possible routes of nitrosamine precursor formation in the hydrochloride monohydrate and in the base form of the API, moreover, in two formulations, in an ointment, and in an injection. Temperature stress experiments were carried out at 25 °C, 50 °C and 75 °C, relevant to the formulation process of the drug products, and the DEA and desethyl lidocaine content of the samples were measured by GC-FID and HPLC-MS. Interestingly, it was found that the lidocaine active ingredient degraded to diethylamine both in the base and salt form of the API, indicating another source of secondary amine precursor, which has not been confirmed yet. The formation of DEA was significantly higher in the base form of the API at the processing temperature of the ointment. Both diethylamine and desethyl lidocaine were detected at ppm levels during the degradation of the active substances, and the excipients contained nitrite at trace levels, so the formation of nitrosamines is feasible in the drug products. We investigated the nitrosamine formation in the drug product in stressed samples (ointment: 70°C/3 hours; injection: 125°C/15 minutes), and the NDEA and N-nitroso-desethyl lidocaine contents were measured by GC-MS and HPLC-MS. The formation of nitrosamines in the drug formulations was found to be not only time, temperature, and nitrite dependent but was also different in the base and salt form of the API and in the studied semi-solid and liquid pharmaceutical formulations. The experimental results were supported by theoretical chemical calculations.

These experiments have proven to be a useful tool for predicting nitrosamine formation in lidocaine pharmaceutical formulations and can be used as a basis for making recommendations to reduce nitrosamine concentrations.

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P3-119**OPTICAL PHOTOTHERMAL INFRARED MICROSCOPY: A MULTIMODAL APPROACH TO SUBMICRON PHARMACEUTICAL ANALYSIS****Siesler, Heinz Wilhelm¹; Unger, Miriam².**

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Optical Photothermal InfraRed (O-PTIR) microspectroscopy is a powerful and innovative technique that is reshaping the analytical landscape in pharmaceutical and biopharmaceutical research. As the demand for high-resolution, chemically specific analysis of complex systems grows, O-PTIR emerges as a solution that addresses the critical limitations of conventional vibrational spectroscopic methods, particularly Raman microscopy and Fourier-transform infrared (FT-IR) imaging.

Traditional Raman microscopy, while capable of identifying particles down to approx. 1 micrometer, often suffers from strong fluorescence interference, limited sensitivity for certain chemical species, and slow acquisition times. Similarly, FT-IR, although robust for chemical identification, typically operates with spatial resolutions limited to approx. 10 micrometer and is adversely affected by Mie scattering and spectral distortions when analyzing small or heterogeneous samples. These limitations make it challenging to analyze sub-visible particles or resolve fine chemical heterogeneities in systems like amorphous solid dispersions (ASDs).

O-PTIR overcomes these barriers by leveraging a photothermal-based detection mechanism, where a pulsed, tunable mid-infrared laser induces localized heating in the sample, and a visible probe laser detects the resulting refractive index changes. This enables the acquisition of infrared spectra with spatial resolutions well below the diffraction limit of conventional IR, reaching down to sub-500 nm. Importantly, O-PTIR operates in reflection mode yet delivers spectral quality comparable to FT-IR transmission or attenuated total reflectance (ATR) modes, while requiring little to no sample preparation.

A key advantage of O-PTIR is its ability to perform simultaneous Raman and IR spectroscopy on the exact same spot, providing complementary molecular information without spatial offset. This dual-modality capability, combined with recent integration of widefield epi-fluorescence imaging, allows for co-located fluorescence, Raman, and IR analysis, enabling comprehensive chemical, structural, and morphological characterization of samples, including those with autofluorescent properties or fluorescent labels.

In pharmaceutical applications, these unique features have demonstrated significant utility. In biopharmaceuticals, O-PTIR enables high-resolution analysis of sub-visible particles such as protein aggregates and process-related contaminants, which are critical for drug safety, efficacy, and immunogenic risk assessment. In formulation science, O-PTIR facilitates detailed characterization of ASDs (amorphous solid dispersions), allowing direct visualization of drug-polymer miscibility, molecular interactions, and phase separation at the sub-micron scale. This capability is especially valuable in assessing homogeneity across both powder and finished tablet forms, informing stability and bioavailability predictions.

Overall, O-PTIR spectroscopy bridges a critical analytical gap, offering unmatched spatial resolution, chemical specificity, and multimodal imaging capabilities. It significantly advances the field of particulate and formulation analysis by enabling a deeper understanding of material behavior at the sub-microscale, ultimately supporting the development of safer, more effective pharmaceutical products.

P3-120**DETERMINATION OF N-NITROSO NORTRIPTYLINE IN AMITRIPTYLINE AND NORTRIPTYLINE PRODUCTS BY LC-MS/MS**

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Amitriptyline and Nortriptyline are tricyclic antidepressant medications that belong to a class of drugs known as non-selective monoamine reuptake inhibitors (NSMRIs). They are commonly prescribed to treat depression and anxiety disorder. N-Nitrosonortriptyline is a nitrosamine impurity found in Amitriptyline and Nortriptyline products. It is a potent carcinogen compound. The maximum allowable concentration in product with respect to the Active Pharmaceutical Ingredient (API) of N-Nitrosonortriptyline in Amitriptyline and Nortriptyline drug product is 0.06 µg/g (0.06 ppm) and 0.18 µg/g (0.18 ppm) respectively. However, there was no method available to determine trace amount of N-Nitrosonortriptyline in Amitriptyline and Nortriptyline products. Hence the lab developed a highly sensitive LC-MS/MS method to accurately quantify N-Nitrosonortriptyline in Amitriptyline and Nortriptyline products to ensure product safety and protect public health.

P3-121**IN VITRO RELEASE METHOD DEVELOPMENT FOR ANTIGENIC PEPTIDE-LOADED PHOSPHATIDYLSERINE-LIPOSOMES INTENDED FOR AUTOIMMUNE DISEASES**

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Autoimmune diseases involve an altered immune response against self-antigens. Current treatments primarily consist of palliative, anti-inflammatory and immunosuppressive drugs. Ahead Therapeutics is developing an antigen-specific therapy using phosphatidylserine-liposomes (PS-liposomes), which bio-mimic apoptotic cells to induce immunotolerance to the encapsulated antigen through macrophage phagocytosis (1, 2). To ensure treatment's efficacy, the antigen must remain encapsulated until phagocytosis occurs. To assess the stability of Myasthenia Gravis (MG) PS-liposome formulations under physiological conditions (37°C, mild agitation), two release testing methods were compared: a dialysis-based method using a 100 kDa MWCO Float-A-Lyzer® device, and sample-and-separate approach (3).

In both methods, antigen concentration was diluted to levels similar to those found in plasma, and sampled over a 48 h period. In the dialysis setup, antigen diffusion was directly quantified by liquid chromatography with ultraviolet detection (HPLC-UV). In the sample-and-separate approach, PS-liposomes were first preconcentrated using Centriscart® centrifugal filters or Nanotrap® Extracellular Vesicle particles, then lysed through liquid-liquid extraction. The encapsulated antigen in the aqueous phase was analysed by HPLC-UV, while the lipids in the organic phase were analysed liquid chromatography coupled to mass spectrometry (HPLC-MS). The most effective methodology was subsequently applied to a Multiple Sclerosis formulation. In addition, a stability study with MG formulation was performed at low temperatures to estimate the unintended antigen loss during storage (leakage rate).

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P3-122**FROM NATURE TO REACTOR: ANALYTICAL ADAPTATION IN THE SYNTHESIS OF NATURALLY DERIVED APIs**

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There are active pharmaceutical ingredients (APIs) that have traditionally been obtained through natural extraction and have begun to be produced via chemical synthesis to ensure purity, scalability, and supply control. The synthetic route introduces significantly greater analytical complexity. While a natural product can typically be characterized using a single chromatographic method, the synthetic counterpart requires a more sophisticated analytical approach to monitor reaction impurities, residual precursors, and stereoisomers.

This work presents an analytical adaptation strategy applied to the synthesis process of a commercially relevant API, based on three pillars: (1) the comparison and selection of complementary chromatographic methods, (2) the verification of specific methods for impurity control, and (3) the calculation of response factors for impurities not specified in pharmacopeias.

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P3-123**PREPARATION AND CHARACTERIZATION OF LIPOSOME SYSTEMS TO EMULATE DRUG DISTRIBUTION IN BIOLOGICAL MEMBRANES****Arshad, Samia¹; Vidal, Paula¹; Fuguet, Elisabet²; Rosés, Martí¹.**

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Understanding the affinity of bioactive compounds for biological membranes is essential in drug development, as it influences key pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET). Traditional methods for evaluating membrane affinity often rely on biological systems, which can be costly, time-consuming, and ethically challenging. This study proposes an alternative approach using synthetic liposomes with defined compositions to mimic different types of biological membranes.

Drug interactions with these liposomes are analyzed using liposome electrokinetic chromatography (LEKC), where the liposomes act as a chromatographic pseudostationary phase. The systems are characterized using the Abraham solvation model, which relates the retention factor (*k*) to solute descriptors associated with polarity and polarizability, hydrogen bonding, and molecular volume:

$$\log k = c + eE + sS + aA + bB + vV$$

The coefficients obtained from the linear correlation provide insight into the nature of solute–liposome interactions. Two different liposomal systems were evaluated in this work: the first consists of 49% phosphatidylcholine (PC), 8% cholesterol (Ch), and 43% palmitic acid (PA); the second contains 21% PC, 29% phosphatidylethanolamine (PE), 8% phosphatidylinositol (PI), and 42% PA. Both linear correlations indicate that hydrogen-bond basicity and molecular volume are the main solute descriptors governing partition into these systems. However, the two systems differ in polarizability, which is higher in the liposomes containing PE and PI.

Comparison using Euclidean distance and principal component analysis (PCA) between the studied systems and various *in vitro* biological partition models suggests that both liposomal systems are good estimators of blood–lung drug distribution. However, to accurately emulate partitioning into other membranes—such as the blood–brain barrier or the blood–liver interface—the liposome composition should be adjusted, particularly by fine-tuning the acidity and basicity of the systems.

P3-124

A PROTEOMICS-DRIVEN APPROACH TO DRUG REPOSITIONING IN PEDIATRIC NEUROLOGICAL DISORDERS

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Pharma-HUB is a research initiative funded by Piano di Sviluppo e Coesione Del Ministero della Salute 2014-2020 aimed at establishing a pharmaceutical HUB for drug repositioning. This project focuses on identifying alternative clinical applications for existing drugs, targeting rare pediatric neurological diseases, in particular Ataxia-Telangiectasia (A-T).

To achieve this goal, two cellular models were developed: Neural Progenitor Cells (NPCs), ideal for studying neurodegenerative diseases, and Urine Stem Cells (USCs), which are easily accessible and can be differentiated into neural cells, providing a non-invasive and practical model for pediatric neurological disorders [1, 2].

The two cell models were characterized by comparing wild-type (WT) vs ATM knockout (KO) samples through a proteomic approach.

The Proteomic analyses were performed using a method previously established by our research group [3], utilizing a micro-LC system (Eksigent Technologies, Dublin, CA, USA) coupled with a 5600+ TripleTOF mass spectrometer (AB Sciex, Concord, ON, Canada) in Data Dependent Acquisition (DDA) mode. Identified proteins were analyzed through multivariate statistical approaches. Specifically, a Partial Least Squares Discriminant Analysis (PLS-DA) classification model was applied to distinguish control and knockout samples. To enhance predictive accuracy, a backward elimination strategy was implemented, iteratively removing variables with minimal predictive power (assessed via cross-validation) using a Monte Carlo approach. This method identified 364 differentially expressed proteins between wild-type (WT) and knockout (KO) samples for the NPCs and 394 for the USCs, achieving perfect classification in both fitting and cross-validation. Further refinement reduced the two models respectively to 28 and 164 proteins (VIP score > 1), still maintaining perfect classification performance.

Proteins were categorized into up- and down-regulated groups, followed by bioinformatics analyses using multiple platforms, including DAVID, ClueGo, STRING, and Ingenuity Pathway Analysis to determine their functional significance. In the USC samples, the analysis revealed key biological processes related to mitochondrial function, oxidative phosphorylation (OxPhos), proton transport, protein synthesis and folding, transport between the ER and Golgi, and mechanisms involved in cell adhesion, particularly cadherins. Additionally, pathways linked to neurodegeneration were identified. Similarly, in the NPC samples, important biological processes involved mitochondrial function, OxPhos, RNA processing, actin cytoskeleton organization, chromatin remodeling, and pathways associated with neurodegeneration. Notably, five proteins - ARFG2_HUMAN, SAR1B_HUMAN, ATPO_HUMAN, NHRF2_HUMAN, and FKBP2_HUMAN - were consistently identified across all analyses in both USC and NPC models, suggesting their potential involvement in fundamental disease mechanisms.

These findings highlight Pharma-HUB's potential to accelerate drug repositioning for rare pediatric neurological disorders by integrating advanced cellular modeling, high-throughput proteomics, and robust computational analysis. The successful identification of disease-specific biomarkers lays the foundation for targeted therapeutic strategies, significantly reducing drug development costs and timelines while addressing urgent clinical needs.

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P3-125

QUANTIFICATION OF CIPROFLOXACIN IN PHARMACEUTICAL PRODUCTS FROM VARIOUS BRANDS USING FT-NIR: A COMPARATIVE INVESTIGATION OF PLS AND MCR-ALS

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This study aims to quantify ciprofloxacin in commercial tablets with varying excipient compositions using Fourier Transform Near-Infrared Spectroscopy (FT-NIR) and chemometric models: Partial Least Squares (PLS) and Multivariate Curve Resolution - Alternating Least Squares (MCR-ALS).

Matrix variation, arising from differences in excipient compositions among the tablets, can impact quantification accuracy. We discuss this phenomenon, emphasizing potential issues introduced by varying certain excipients and its importance in reliable ciprofloxacin quantification.

We evaluated the performance of PLS and MCR-ALS models independently on two sets of tablets, each containing the same drug substance but different excipients. The statistical results revealed promising results with PLS prediction error of 0.38% w/w of the first set and 0.47% w/w of the second set, while MCR-ALS achieved prediction errors of 0.67% w/w of the first set and 1.76% w/w of the second set.

To address the challenge of matrix variation, we developed single models for PLS and MCR-ALS using a dataset combining both first and second sets. The PLS single model demonstrated a prediction error of 4.3% w/w and a relative error of 6.41% w/w, while the MCR-ALS single model showed a prediction error of 1.88% w/w and a relative error of 1.29% w/w.

We then assessed the performance of the single PLS and MCR-ALS models developed based on the combination of the first and the second set in quantifying ciprofloxacin in various commercial tablet brands containing new excipients. The PLS model achieved a prediction error ranging between 6.2% w/w and 8.39% w/w, with relative errors varied between 8.53% w/w and 12.82% w/w. On the other hand, the MCR-ALS model had a prediction error between 1.11% w/w and 2.66% w/w, and the relative errors ranging from 0.8% to 1.74% w/w.

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P3-126**DEVELOPMENT OF A METABOLOMIC LC-QTOF METHOD FOR BIOACTIVE COMPOUND PRODUCTION CONTROL IN PLANT CELL CULTURES**

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The demand for sustainable and environmentally friendly production processes is driving the search for innovative biotechnological solutions. One promising approach is the use of bioengineering to develop plant cells capable of producing bioactive compounds in controlled bioreactor environments. These compounds have potential applications in diverse industries, including pharmaceuticals, cosmetics, agriculture, and industrial biotechnology, aligning with the principles of green chemistry. However, a key challenge in this process is understanding and controlling the metabolites generated by plant cells to optimize the production of high-value bioactive molecules.

In this study, we have developed an analytical method based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF) for the identification of metabolites produced by various bioengineered plant cell lines. The method employs reverse-phase chromatography to efficiently separate the metabolites, utilizing a solvent gradient to enhance compound resolution and detection. Electrospray ionization (ESI) is applied in both positive and negative modes to ensure comprehensive ionization of a broad range of metabolites, thereby increasing the method's analytical coverage.

For data analysis, we use MS-DIAL software to identify the detected compounds, classifying them into different confidence levels based on spectral matching and retention time criteria. Furthermore, statistical analyses are applied to assess the significance of the identified metabolites, highlighting key compounds that may play crucial roles in biosynthetic pathways. This metabolomic approach not only provides deeper insights into the biosynthesis of active molecules but also serves as a robust control strategy for targeted compound production, contributing to the advancement of sustainable bioprocesses.

P3-127**MULTIMODAL STUDY OF α -PiHP TOXICITY ON HEPATIC CELLS**

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The synthetic cathinone α -pyrrolidinoisohexiophenone (α -PiHP) is one of the most found new psychoactive substances (NPS) in European Union seizures [1]. This drug poses significant public health challenges due to its toxicity and frequent involvement in overdoses. Despite these concerns, the metabolic and toxicological profile of α -PiHP remains poorly understood. Therefore, it is essential to characterize its effects at the cellular and molecular levels to gain insight into its metabolism and potential toxic mechanisms. Here we integrate live-cell SR-FTIR (synchrotron radiation Fourier transform infrared spectroscopy), Raman spectroscopy and LC-HRMS/MS (both cell media and intracellular content) together with multivariate statistical analysis for the study of the metabolic effects of α -PiHP in hepatic cells. Different approaches were considered: live HepG2 cells for SR-FTIR, fixed HepG2 cells for Raman and upcyte human hepatocytes for LC-HRMS/MS. Vibrational spectroscopy techniques provided information about the macromolecular changes at the protein, lipid, and nucleic acid levels, whereas LC-HRMS/MS provided insights into key metabolites altered by α -PiHP. This multimodal approach allowed us to obtain an extended picture and collect different information with each of the three analytical methodologies and cellular approaches. Functional and pathway analysis suggested common metabolic alterations with other NPS, indicating potential shared toxicity mechanisms. In summary, these findings contribute to a more comprehensive understanding of α -PiHP's hepatotoxicity, offering valuable data for clinical toxicology. The insights gained may assist in the development of better risk assessment frameworks and therapeutic interventions for α -PiHP intoxications.

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P3-128**IDENTIFICATION OF MARKERS FOR HONEY AUTHENTICATION USING HIGH-RESOLUTION MASS SPECTROMETRY-BASED METABOLOMICS AND DATA FUSION****Navarro-Herrera, Alba María; Rivera-Pérez, Araceli; Garrido Frenich, Antonia.**

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Honey is a natural food widely valued for its sweet taste and organoleptic and medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial effects. One of the most important qualities of honey is its flavor and aroma, which are mainly determined by its floral source and the presence of specific volatile compounds. Based on its botanical origin, honey is classified as monofloral when it is predominantly derived from a single plant species, or multifloral when it is composed of nectar from multiple floral sources. Due to the significant impact of floral origin on honey's nutritional composition, sensory properties, and economic value, the development of precise and reliable analytical methods for verifying the botanical origin of honey is essential to ensure product quality and prevent fraud. This study applied an untargeted metabolomics approach using gas chromatography coupled with high-resolution mass spectrometry (GC-Orbitrap-HRMS) to differentiate between various botanical origins of honey marketed in Spain, including eucalyptus, rosemary, orange blossom, and multifloral honey. A novel salt-assisted liquid-liquid extraction (SALLE) was performed for metabolite profiling, followed by multivariate statistical analysis using principal component analysis (PCA) and supervised orthogonal partial least squares discriminant analysis (OPLS-DA). OPLS-DA models were fully validated and established for botanical discrimination of honey samples, achieving excellent predictive ability for further samples ($R^2Y > 0.8$, $Q^2 > 0.7$) and strong classification performance rates between 94% and 100%. As a result, seven different honey metabolites were identified, meeting established selection criteria and classified as level 2 markers according to the Metabolomics Standards Initiative (MSI), indicating putatively annotated compounds or probable structures. These compounds, not previously reported in the literature, were highlighted in pairwise honey comparisons: orange blossom vs. eucalyptus, rosemary vs. eucalyptus, and rosemary vs. orange blossom; multifloral vs. monofloral honey (eucalyptus, rosemary and orange blossom). Key markers, namely dehydrovomifoliol, 3-oxo- α -ionone, 1,2-dihydro-1,1,6-trimethylnaphthalene, caffeine, and 4-ketoisophorone, were identified as characteristic markers of monofloral honey (eucalyptus, rosemary, and orange blossom honey). Dehydrovomifoliol, 3-oxo- α -ionone, and 1,2-dihydro-1,1,6-trimethylnaphthalene were found in higher concentrations in eucalyptus honey, suggesting their potential as markers to differentiate this botanical origin. Additionally, the study identified caffeine and 4-ketoisophorone as significant markers for distinguishing orange blossom honey. For the discrimination between monofloral and multifloral samples, dehydrovomifoliol, 4-ketoisophorone, 3-oxo- α -ionone, 1,2-dihydro-1,1,6-trimethylnaphthalene, 3-oxo-7,8-dihydro- α -ionone, and 4-methyleneisophorone were identified. These markers up-accumulated in most cases in monofloral samples (except dehydrovomifoliol and 3-oxo- α -ionone) indicated the greater organoleptic complexity of this honey type. Furthermore, advanced omics techniques, i.e. the mid-level data fusion approach, were successfully applied, integrating these results with those previously obtained by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-Q-Orbitrap-HRMS fingerprinting) to improve the discrimination power, demonstrating the complementary nature of both techniques. Reducing data dimensionality, coupled with marker integration, enhances model performance while preserving analytical reliability. This study demonstrates the potential of an untargeted metabolomics approach based on an innovative combination of SALLE with GC-Orbitrap-HRMS fingerprinting and multivariate data analysis for honey authentication based on its botanical origin. It further highlights the significant influence of botanical origin on honey's metabolomic profile, underscoring the potential of GC-Orbitrap-HRMS to identify novel markers for the precise authentication and differentiation of commercial honey varieties. Acknowledgments: This research was funded by "PPITUAL, Junta de Andalucía-FEDER 2021-2027. Programa: 54.A" (P_FORT_GRUPOS_2023/43 and CPUENTE2023/21).

P3-129**TRACING HONEY FINGERPRINT: A POWERFUL UHPLC-Q-ORBITRAP-HIGH-RESOLUTION MASS SPECTROMETRY METABOLOMICS STRATEGY FOR BOTANICAL ORIGIN AUTHENTICATION****Rivera-Pérez, Araceli; Navarro-Herrera, Alba María; Garrido Frenich, Antonia.***University of Almería, Almería, Spain.*

Honey authentication based on botanical origin is essential to ensure product traceability, protect consumers, and prevent fraudulent labeling. The chemical composition of honey is influenced by floral sources, environmental conditions, and processing factors, making botanical differentiation a challenging task. This study applied an untargeted metabolomics approach using ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap-HRMS) to characterize and differentiate honey samples based on their botanical origin. The research focused on three monofloral honey types (eucalyptus, rosemary, and orange blossom) and multifloral honey, all commercialized in Spanish markets. A total of 40 honey samples (10 per botanical origin) were analyzed using a robust workflow that included sample preparation, chromatographic separation, high-resolution mass spectrometry acquisition, and multivariate data analysis. Unsupervised principal component analysis (PCA) and hierarchical clustering analysis (HCA) indicated significant metabolomic variability, particularly within multifloral samples. Supervised orthogonal partial least squares discriminant analysis (OPLS-DA) demonstrated excellent discriminatory performance, with high goodness-of-fit ($R^2Y = 0.929\text{--}0.981$) and predictive ability ($Q^2 = 0.868\text{--}0.952$). These models allowed for the identification of specific botanical markers that differentiate between honey types, with correct classification rates reaching 100%. A total of 15 key metabolites were identified as significant markers for honey classification, including amino acids, Amadori compounds, and sugars. L-proline was highlighted as a marker distinguishing eucalyptus honey, with significantly higher concentrations compared to rosemary and orange blossom honey. Additionally, N-(1-deoxy-1-fructosyl)phenylalanine and L-pyroglutamic acid were up-regulated in eucalyptus honey, suggesting their role as potential indicators of botanical origin and honey aging. Rosemary honey was characterized by elevated levels of L-phenylalanine and raffinose, reinforcing its distinct metabolomic profile. Orange blossom honey, on the other hand, exhibited intermediate characteristics between eucalyptus and rosemary samples but showed lower levels of Amadori compounds, suggesting a lower degree of Maillard reaction products. The differentiation between multifloral and monofloral honey revealed four significant markers: trigonelline, L-isoleucine, N-(1-deoxy-1-fructosyl)isoleucine, and N-(1-deoxy-1-fructosyl)phenylalanine. Trigonelline, an alkaloid commonly found in herbs and spices, was notably higher in multifloral samples, likely reflecting the diverse nectar sources. Similarly, Amadori compounds (fructosyl amino acids) were more abundant in multifloral honey, indicating a greater availability of reducing sugars and amino acids, which could enhance the Maillard reaction. The higher levels of these compounds in multifloral honey suggest that this type of honey undergoes a more intense transformation process during storage, potentially impacting its sensory and physicochemical properties. The application of UHPLC-Q-Orbitrap-HRMS fingerprinting combined with advanced chemometric analysis proved to be an effective strategy for honey authentication. The proposed methodology enables reliable discrimination of honey types based on their botanical origin, providing valuable insights for food traceability, quality control, and fraud prevention. In conclusion, this research represents the first comprehensive metabolomics characterization of eucalyptus, rosemary, orange blossom, and multifloral honey in the Spanish market. The identification of 15 specific metabolomic markers enhances the understanding of honey's fingerprint and provides a powerful tool for authentication. These findings offer a promising approach for future studies aiming to extend this methodology to other monofloral honey varieties, investigate the role of seasonal variations in metabolite composition, and explore the impact of storage conditions and processing techniques on honey's chemical profile. The results obtained in this study contribute to improving honey classification strategies, reinforcing its value as a natural and high-quality food product. Acknowledgements: This research was funded by "PPITUAL, Junta de Andalucía-FEDER 2021-2027. Programa: 54.A". Grant reference: CPUENTE2023/21.

P3-130

LARGE-SCALE NON-TARGETED LC-HRMS/MS WORKFLOW FOR THE PROFILING OF OXYLIPINS AND CO-EXTRACTED METABOLITES IN HUMAN URINE: APPLICATION TO 491 SAMPLES USING MZMINE AND MOLECULAR NETWORKING

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Oxylipins, encompassing enzymatic and non-enzymatic oxidized derivatives of polyunsaturated fatty acids (PUFAs), are key mediators of oxidative stress and inflammation [1]. However, their untargeted analysis in human urine is rarely addressed due to their low abundance, isomeric complexity, and the lack of standardized spectral libraries and commercial standards.

We here present an untargeted LC-HRMS/MS workflow tailored to oxylipin profiling in large-scale human cohorts. Building upon the NEO-MSMS spectral library, previously developed from in vitro oxidations and authentic standards via MS/MS molecular networking [2], we analyzed urine samples from 491 volunteers from the BIOMOUVAL study, a demographically representative cohort of the Valencian Community.

Urinary extracts were prepared using solid-phase extraction (SPE) in 96-well format and analyzed by LC-HRMS/MS (Orbitrap ID-X, DDA mode). Data was processed using mzmine on the LluísVives 2 HPC cluster at the University of Valencia, with feature-based molecular networking (FBMN) performed in GNPS2. Annotation leveraged both public MS2 repositories and the NEO-MSMS library.

Robust quality assurance was ensured using pooled QC samples, retention time monitoring, and evaluation of potential batch effects. Thanks to its untargeted design, the workflow allowed not only the detection of urinary oxylipins and their metabolites (e.g., partial β -oxidation products and glucuronides), but also a wide range of co-extracted compounds. These included dietary polyphenols, xenobiotics, and uncharacterized small molecules of potential exposomic interest, all retained by the same SPE protocol.

To interpret molecular diversity, multivariate analysis (PCA, PERMANOVA, hierarchical clustering) were applied incorporating the recent advances in FBMN post-processing [3]. Furthermore, the network structure was further interrogated through topological data analysis (TDA) [4] uncovering distinct molecular families and exposure-related subnetworks.

Importantly, the study integrates an extensive metadata comprising over 200 variables related to lifestyle factors (diet, habits, physical activity), health status, and sociodemographic information. This rich contextual information enhances the potential of the dataset for integrative molecular epidemiology and exposomic research.

This is, to our knowledge, the first study to apply a fully untargeted, FBMN-based oxylipin profiling strategy to human urine at this scale. Preliminary findings reveal population-level molecular signatures associated with diet and lifestyle and demonstrate the feasibility of using topological and chemometric tools to extract biologically meaningful patterns from highly complex molecular networks.

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P3-131

IN VITRO INVESTIGATION OF HUMAN MELANOMA CELLS VOLATILOME USING HEADSPACE GC/MS

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The study of Volatile Organic Compounds (VOCs) released by biological systems is known as *volatilome*. Changes in the volatilome profile may reflect alterations in various metabolic processes, providing valuable insights into the metabolic pathways [1]. VOCs can be detected in various biological specimens, including the headspace of cell cultures, which serve as a valuable model for studying the unaltered metabolic fingerprint of cells, as well as their responses to drugs and treatments [2]. Given this background, the aim of this study is twofold: 1) to investigate the volatilome profile in the headspace of A375 human melanoma cell line, and 2) to identify changes in this profile induced by the pharmacological treatment with thapsigargin (Tg), a well-known pro-apoptotic agent [3].

The samples, divided in two batches, were analyzed using Solid Phase MicroExtraction – Gas Chromatography/Mass Spectrometry (SPME-GC/MS). The resulting data underwent a pre-processing strategy, starting with peak detection and integration, deconvolution and alignment. Afterward, the workflow included: Probabilistic Quotient Normalization (PQN) and log₁₀ transformation [4]. Exploratory multivariate analysis via Principal Component Analysis (PCA) was performed on the autoscaled data, to reduce dimensionality and uncover patterns and clusters within the data. To remove the principal components associated with unwanted systematic variance (e.g. batch effect), External Parameter Orthogonalization (EPO) [5] was applied. ANOVA Simultaneous Component Analysis (ASCA) [6] confirmed the batch effect removal and the significant impact of Tg treatment on the cells. As a final step, class modelling (Soft Independent Modelling of Class Analogy - SIMCA) [7] and classification approaches were employed to evaluate the significant variables. Univariate analysis allowed to evaluate the trends of the selected variables also compared to the cell-free culture medium, with the aim of better understand their relationship with the Tg treatment and the normal cancer cell metabolism.

Compounds such as 2-ethyl-1-hexanol, n-octane and 3-methyl-heptane were found to be more abundant in untreated cells compared to treated ones. According to the literature, the presence of alcohols is linked to the metabolism of alkanes, which results from fatty acid oxidation driven by increased oxidative stress in tumour cells [8].

The findings of this study indicate that volatilome analysis using headspace SPME-GC/MS may be a useful approach for monitoring cellular responses to treatment, offering insights that could support biomarker evaluation and help in understanding the mechanisms behind drug action and resistance.

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P3-132**BREATH-BASED DIAGNOSTICS WITH VOCORDER: A CLINICAL STUDY PROTOCOL****Giannoukos, Stamatios.***ETH Zürich, Zürich, Switzerland.*

The survival rate of severe illnesses and acute diseases depends on adequate and timely diagnosis and treatment. Unfortunately, many conditions only present noticeable symptoms during advanced stages, when it may be too late to treat. Furthermore, current diagnostic methods (e.g., biopsies) are intrusive, costly, and time-consuming to process in the lab, requiring patients to wait before treatment can start. To alleviate costs and waiting times for disease diagnostics, breath analysis can be applied at the point of care and is a promising technique and field of research. The European Union-funded project 'VOCORDER' aims to develop an infrared device capable of conducting rapid breath analysis tests on patients for continuous diagnostic and health monitoring, based on the volatile organic compound (VOC) profiles and metabolite biomarkers of patients' breath. We present the study protocol for the VOCORDER project, which consists of two phases. Phase 1 is a baseline study aimed at detecting and identifying breath biomarkers for the early diagnosis of lung, stomach/colon, and breast cancers, as well as kidney insufficiency and acute infections (pneumonia). This phase employs ambient mass spectrometry (MS) and gas chromatography coupled with MS (GC-MS) to analyze breath samples from 120 healthy participants and 175 patients (35 per disease group). Phase 2 focuses on validating the newly developed portable breath analyzer in a cohort of 120 healthy controls and 100 patients with the above diseases. Breath samples were obtained in 1 L gas sampling bags at MITERA Hospital in Athens, Greece, and analyzed in the breath analysis laboratory at ETH Zürich, Switzerland, using secondary electrospray ionization high-resolution MS (SESI-HR-MS). The study protocol and preliminary breath metabolomic VOC data from patients with the targeted diseases are presented and discussed.

P3-133

IMPROVING UNTARGETED METABOLOMIC ANALYSIS IN HIV-INFECTED PLASMA THROUGH MINIATURIZED REVERSED-PHASE CHROMATOGRAPHY

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Miniaturized high-performance reversed-phase chromatography (RP) offers significant advantages for metabolomics and small-protein analysis, not only due to reduced sample dilution, enabled by smaller column volumes, but also due to improved electrospray ionization efficiency at low flow rates, which enhances the detection and quantification of trace components [1,2]. This is particularly necessary in studies that analyse differences between healthy and infected cells at ppt levels to aid in disease prediction and treatment, such as in the case of HIV. Using precise microfabrication technologies to manufacture miniaturized separation devices can combine the benefits of increased sensitivity, improved separation efficiency, resolution, and reproducibility. Miniaturization improves efficiency by virtually eliminating Eddy dispersion, increases resolution by enabling the use of very long columns (up to 110 cm), and improves reproducibility due to the high precision of the manufacturing process of the columns that are used, ensuring near-identical column performance [3–5]. The instrumentation utilized includes a capillary liquid chromatography (LC) system paired with a high-resolution Q Exactive Orbitrap Mass Spectrometer (MS). The miniaturization is achieved through the use of ThermoFisher's micro Pillar Array Columns (μPAC).

In this study, the performance of miniaturized reversed-phase chromatography was evaluated using five control cells and five HIV-infected cells. The results demonstrated outstanding peak resolution and minimal peak broadening, facilitating the detection of subtle biochemical changes associated with HIV infection. These differences were further analysed using Compound Discoverer, which generated principal component analysis (PCA) plots and other statistical models to visualize metabolic shifts between control and infected cells. While multiple biomarkers related to HIV infection have already been identified, the majority of these are proteins. In contrast, metabolomic alterations remain largely unexplored, making this type of analysis especially relevant for uncovering new insights into the metabolic impact of HIV infection.

The improved analytical capabilities of miniaturized RP chromatography present a valuable approach for studying the molecular alterations induced by HIV. By optimizing separation performance with minimal sample input, this method contributes to a more comprehensive understanding of infection mechanisms and holds potential for broader applications in bioanalytical research.

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P3-134**FROM DATA TO DIAGNOSIS: MULTIOMICS AS THE FOUNDATION OF FUTURE MEDICINE****Bulska, Ewa¹; Dushna, Olha²; Gawor, Andrzej²; Ruszczynska, Anna².**

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Modern medicine is rapidly evolving, driven by the advancement of omics sciences such as genomics, proteomics, metabolomics, and metallomics. Integrating data across different biological levels enables a comprehensive understanding of disease mechanisms and a deeper insight into molecular processes underlying health and pathology. Supported by advanced bioinformatics and computational tools, omics technologies are becoming the cornerstone of innovative diagnostic and therapeutic strategies. Large-scale biological data analysis, powered by artificial intelligence algorithms, allows for the identification of biomarkers, risk modelling, and the development of personalised therapeutic approaches.

This presentation will highlight selected examples of omics-based approaches in biomedical research. One focus is the safety assessment of fluorinated drugs, whose metabolites may trigger adverse biological effects. The application of proteomics and metabolomics facilitates the identification of early toxicity biomarkers and the evaluation of risks associated with chronic exposure to such compounds. Another research area discussed is the impact of selenium-enriched diets. Selenium, a trace element with antioxidant and immunomodulatory properties, influences numerous biological processes. By integrating multiomic analyses, it is possible to monitor metabolic changes and explore selenium's interactions with other elements and biomolecules, providing insight into its role in physiological regulation. The third example addresses psoriasis, a chronic inflammatory skin disease with complex genetic and immunological origins. Combining proteomic data with elemental signature analysis enables detailed molecular characterisation of pathological lesions, the identification of potential therapeutic targets, and the evaluation of treatment efficacy.

These examples illustrate how integrating advanced omics technologies supports discoveries in disease mechanisms, enhances our understanding of environmental and therapeutic influences, and paves the way for more effective, personalized medical care.

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P3-135

STREAMLINED APPROACH FOR RADIUM ISOTOPES QUANTIFICATION IN WATER SAMPLES BY α AND γ -SPECTROMETRY

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The determination of radium isotopes in environmental water samples is crucial for health physics and environmental protection [1, 2]. Among them, ^{226}Ra and ^{228}Ra are particularly significant due to their relatively long half-lives and widespread natural occurrence [3].

Several analytical methods have been developed for quantifying these isotopes in water samples, each with distinct advantages and limitations [3–8]. Given the limitations of existing methodologies, the main objective of this study is to develop a novel radiochemical method by integrating coprecipitation with $\text{Pb}(\text{Ba})\text{SO}_4$, microprecipitation and a sequential determination of ^{226}Ra and ^{228}Ra using alpha and gamma spectrometry. This combined approach mitigates the drawbacks of existing methods, such as lengthy sample preparation and the need for extended ingrowth periods, while ensuring compliance with the detection limits established by the Spanish Royal Decree 3/2023.

Optimal preconcentration and microprecipitation conditions were determined using a three-way ANOVA, which highlights the significant influence of lead and barium mass, as well as EDTA volume on recovery efficiency. The developed method was successfully validated with spiked and IAEA water samples, demonstrating high precision and accuracy. Minimum detectable activity (MDA) values for 5 L-samples ($0.11 \text{ mBq}\cdot\text{L}^{-1}$ for ^{226}Ra and $24 \text{ mBq}\cdot\text{L}^{-1}$ for ^{228}Ra) comply with Spanish Royal Decree 3/2023, confirming the method suitability for routine environmental monitoring. This efficient and cost-effective method is ideal for routine monitoring of drinking water and emergency scenarios that require rapid radium isotopes quantification.

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CHEMICAL CHARACTERIZATION OF CONCRETE: AN ANALYTICAL APPROACH

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Concrete is the second most consumed material by humans after water and constitutes approximately 70% of the built environment in the world¹. Despite its ubiquity, the perception of concrete remains largely confined to engineering and architectural domains. However, this material harbours a complex internal chemistry that is essential to its performance and longevity².

From a materials science perspective, the comprehensive characterization of concrete is crucial. While engineering approaches typically focus on macroscopic properties such as mechanical performance, workability, rheology, and durability³. Analytical chemistry provides the tools to investigate the material at the microstructural and molecular levels. These microscopic features are often the key determinants of macroscopic behaviour, making their study indispensable for a deeper understanding of concrete^{4,5}.

The chemical nature of concrete is rooted in the hydration reactions of cement, which lead to the formation of calcium silicate hydrate (C-S-H) gel—an amorphous, glassy phase that plays a central role in the mechanical strength of the material. Analytical characterization of this gel is therefore vital to unravel the underlying chemistry of concrete⁶.

This work presents the application of advanced analytical techniques to investigate the chemical processes occurring within cementitious matrices. Four analytical techniques of characterisation are employed: X-ray Diffraction (XRD), X-ray Fluorescence (XRF), Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM). XRD enables the identification of crystalline phases such as portlandite ($\text{Ca}(\text{OH})_2$) and ettringite ($\text{Ca}_6\text{Al}_2(\text{SO}_4)_3(\text{OH})_{12}\cdot 26\text{H}_2\text{O}$), which are key products of cement hydration. XRF provides elemental composition data, highlighting calcium and silicon as the predominant elements. FT-IR is used to monitor the formation of calcium silicate hydrate (C-S-H) gel through the analysis of Si–O vibrational bands. SEM offers microstructural insights, allowing visualization of the C-S-H gel, the interfacial transition zone (ITZ), and the surrounding matrix. The results obtained from these techniques have been correlated with mechanical performance data, establishing a direct relationship between microstructural features and macroscopic properties.

The integration of these techniques offers a comprehensive understanding of the chemical and structural evolution of concrete, bridging the gap between microstructural phenomena and macroscopic performance. This study underscores the pivotal role of analytical chemistry in the investigation of construction materials and advocates for a more interdisciplinary approach that unites chemistry and engineering in the study of concrete.

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P3-137

DEVELOPMENT OF A NOVEL PYRIDINIUM-BASED DERIVATIZATION PLATFORM FOR THE ANALYSIS OF AMINO COMPOUNDS BY LC-MS

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We report the synthesis and initial evaluation of a new pair of derivatizing agents designed to enhance the detection of amino-containing compounds by liquid chromatography–mass spectrometry (LC-MS). The reagents were synthesized via alkylation of 3-aminopyridyl-N-hydroxysuccinimidyl carbamate (APDS)[1] with methyl iodide and its isotopically labeled analogue, iodomethane-d₃. The reaction was performed under reflux conditions using a 1:4 molar ratio (APDS:alkyl iodide) in acetonitrile as solvent, and the products were isolated after solvent removal under reduced pressure, yielding crystalline materials with an overall yield of 47%. These novel pyridinium-tagged reagents are part of an emerging derivatization platform[2, 3, 4] aimed at improving ionization efficiency in electrospray ionization (ESI) and enabling reliable quantification through stable isotope coding. The current work establishes the synthetic feasibility of this platform and lays the groundwork for its application in the quantitative analysis of amino acids and related metabolites in biological samples. The method was applied to the quantification of a panel of amino acids including Asp, Asn, Ala, Ser, Gly, Thr, Val, Ile, Leu, Tyr, Trp, Phe, Glu, and Gln. Calibration curves were constructed over the range of 0.02 to 2.5 nmol/mL, yielding coefficients of determination (R²) greater than 0.99 for all compounds.

These results highlight the excellent linearity and sensitivity of the approach, which is compatible with a variety of biological matrices. LC-MS analysis was performed on a Shimadzu LCMS-8030 triple quadrupole system operating in multiple reaction monitoring (MRM) mode and positive ionization mode. All derivatized amino acids generated a common fragment ion at m/z 135.1 for the light form and m/z 138.1 for the deuterated analogue. This uniform fragmentation pattern not only enables simplified method development and quantification through internal standards, but also opens the possibility of using precursor ion scan modes for untargeted profiling of amino compounds. Moreover, the consistent fragmentation facilitates isotopic distribution analysis of labeled amino acids when stable isotope tracers (e.g., labeled glucose) are used in metabolic experiments. Chromatographic separation was achieved in a total runtime of 12 minutes using a biphenyl column (150 × 2.1 mm, 2.7 μm particle size). The mobile phase A consisted of 25 mM ammonium formate buffer adjusted to pH 6.0, and mobile phase B was composed of methanol:acetonitrile (80:20, v/v). The gradient conditions allowed for effective retention and resolution of all target analytes, including structurally similar amino acids. The method has been successfully applied to both monolayer (2D) cultures and cellular spheroids (3D) derived from various mammalian cell lines, enabling the quantification of the selected amino acids under different growth conditions. This versatility demonstrates the robustness of the approach for diverse biological systems. Method validation included recovery studies in metabolite extracts from MEF cells, showing recovery rates between 80% and 105% for all amino acids at both low and high concentrations relative to the calibration range. These values confirm the method's accuracy and reproducibility when applied to real biological matrices. Further evaluation will include matrix effect studies, precision, and broader applicability to other classes of nitrogenous metabolites. This platform is expected to support both targeted and untargeted metabolomic workflows, improving the accuracy and robustness of amino compound quantification in complex samples.

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P3-138**SIMULTANEOUS ELECTROCHEMICAL DETECTION OF ETHYL PARABEN, DICLOFENAC, AND EPINEPHRINE USING Zn-2MI@V-COF-1@MWCNTs SENSOR****Zac R, Antonia Trisha¹; Tsai, Yao-An²; Yeh, Chih-Ling²; Lee, Hui-Ling².***¹Fu Jen Catholic University, Department of Chemistry, New Taipei City, India; ²Fu Jen Catholic University, Department of Chemistry, New Taipei City, Taiwan.*

The development of practical and cost-effective electrochemical sensors capable of simultaneously detecting ethyl paraben (EPB), diclofenac (DIC), and epinephrine (EPN) remains a critical analytical challenge. In this study, we report a novel and economical sensor based on screen-printed carbon electrodes (SPCE) modified with a hybrid nanocomposite of Zn-2MI@V-COF-1@MWCNTs. The hybrid material was synthesized via a simple room-temperature method by combining an octahedral imidazole-based metal-organic framework (Zn-2MI) with a two-dimensional vinylene-linked covalent organic framework (V-COF-1), forming Zn-MOF@V-COF-1. To enhance conductivity and sensitivity, multi-walled carbon nanotubes (MWCNTs) were incorporated into the hybrid structure. Structural and morphological analyses using PXRD, SEM, and TEM confirmed the high crystallinity, porosity, and expanded electroactive surface area of the hybrid material. These properties, along with abundant adsorption sites, contributed to the sensor's high sensitivity toward the target analytes. The modified SPCE sensor achieved low detection limits of 0.05 μM for both DIC and EPN, and 0.5 μM for EPB. The sensor's practical utility was demonstrated through differential pulse voltammetry (DPV) analysis in real samples, including chili powder, liquid sweetener, and energy drinks. Recovery rates ranged from 83.62% to 131.65%, confirming its accuracy and reliability. These results highlight the Zn-2MI@V-COF-1@MWCNTs/SPCE sensor as a promising platform for rapid, sensitive, and economical detection of multiple pharmaceutical and personal care products in complex matrices.

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P3-139**DETERMINATION OF OCHRATOXIN A IN FOODS BY FLUORESCENCE SPECTROSCOPY AND MULTIVARIATE CALIBRATION: COMPARISON OF DATA ORDERS****Bravo, Manuel; Gonzalez, Albani.***Pontificia Universidad Catolica de Valparaiso, Valparaiso, Chile.*

Ochratoxin A (OTA) is one of the most toxic secondary metabolites produced by fungi, contaminating a wide range of food products. It is well recognized for its nephrotoxic and immunosuppressive effects and is classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen (Group 2B). Consequently, there is a critical need for highly sensitive and selective analytical methods to quantify OTA in commonly consumed matrices such as coffee, tea, and wine. However, conventional methodologies based on extraction, cleanup, and chromatographic determination are often laborious, solvent-intensive, and generate significant chemical waste, which conflicts with the principles of green analytical chemistry.

In this study, the analytical performance of two greener methods was evaluated for OTA determination in selected food samples, relying on fluorescence spectroscopy coupled with multivariate calibration (second- and third-order). Second-order data were acquired through excitation-emission fluorescence matrices (EEFM), while third-order data were obtained by registering EEfMs at varying irradiation times following the photodecomposition of OTA. All data sets were successfully modeled using the multivariate curve resolution–alternating least squares (MCR-ALS) algorithm, achieving explained variance up to 90%, even in the presence of kinetic-mode deviations (breaking quadrilinearity).

Comparatively, the third-order approach demonstrated superior analytical figures of merit, with a lower limit of detection (0.11 µg/L) and a prediction error of less than 4%, despite potential interferences. When applied to real samples, including tea infusions and red wine, the third-order methodology offered distinct advantages such as minimal sample pretreatment, negligible matrix effects, and satisfactory recoveries ranging from 84% to 105%. These results clearly highlight the third-order advantage for robust OTA quantification in complex matrices using advanced fluorescence-based multivariate calibration.

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P3-141**BIOBASED CeO_{2-x} NANOPARTICLES DERIVED FROM ULVA LACTUCA FOR FUNCTIONAL PAPER-BASED SENSORS****Ozyurt, Dilek.***Istanbul University, Istanbul, Turkey.*

Marine algae possess unique biochemical compositions rich in polyphenols, flavonoids, polysaccharides, and carotenoids, which endow them with strong antioxidant properties. These compounds protect algae from oxidative stress caused by harsh environmental conditions. Unlike most terrestrial plants, marine algae do not require fertile soils and can be harvested multiple times per year. Moreover, their natural growth rate, even without chemical additives, is approximately twice that of land-based plants. These advantages have led to increased interest in using algae for nanoparticle (NP) synthesis (Dahoumane et al., 2017; Mukherjee et al., 2021). Nanoparticle-enhanced paper-based biosensors have emerged as promising tools for the qualitative and quantitative detection of various analytes in food safety, environmental monitoring, and biomedical diagnostics (Karimi et al., 2016).

In this study explores the green synthesis of cerium oxide nanoparticles with oxygen vacancies (CeO_{2-x}NPs) using *Ulva lactuca*, a type of macroalgae, through a microwave-assisted synthesis approach. Macroalgal extracts acted as both reducing and stabilizing agents in the synthesis process. The extraction of bioactive compounds from *Ulva lactuca* was carried out using ultrasound-assisted extraction (UAE) in an aqueous medium, which allowed for efficient, rapid, and environmentally friendly isolation of secondary metabolites without the use of organic solvents. The CeO_{2-x}NPs synthesized via this green method were characterized using fourier-transform infrared spectroscopy (FT-IR), x-ray photoelectron spectroscopy (XPS), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX), and zeta potential analysis. These techniques confirmed the successful formation and surface properties of the nanoparticles. This integrated approach highlights the dual benefit of macroalgae: as a raw material for both nanoparticle production and cellulose extraction. The residual macroalgal pulp, after extraction, was utilized to derive cellulose to serve as a support matrix in the development of paper-based biosensors. This strategy aligns with zero-waste and green chemistry principles, ensuring both environmental and economic sustainability. We present a facile and cost-effective method to fabricate a robust, portable enzyme-based biosensor using CeO_{2-x}NPs. These redox-active nanoparticles serve two critical functions: (i) providing a high surface area for enzyme immobilization and (ii) enabling colorimetric detection of hydrogen peroxide (H₂O₂), a by-product of oxidase enzyme activity on target substrates. This study demonstrates the potential of macroalgae as a valuable resource for green nanoparticle synthesis and biosensor development. Therefore, they can serve as a sustainable and economically viable input for high-value technological applications.

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