**BOOK OF ABSTRACTS**

# XV REUNIÓN CIENTÍFICA SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES · SECyTA 2015

*XV SCIENTIFIC MEETING OF THE SPANISH SOCIETY OF CHROMATOGRAPHY AND RELATED TECHNIQUES · SECyTA2015*

# VII REUNIÓN NACIONAL SOCIEDAD ESPAÑOLA DE ESPECTROMETRÍA DE MASAS · SEEM 2015

*VII NATIONAL MEETING OF THE SPANISH SOCIETY OF MASS SPECTROMETRY · SEEM2015*

## CASTELLÓN
UNIVERSITAT JAUME I

**27-30 de octubre de 2015**
*October 27-30, 2015*
**www.secytaseem2015.com**

**Organiza**
*Organizer*

SECYTA SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES

S.E.E.M

**Colabora**
*Collaborate*

UNIVERSITAT JAUME I DES DE 1991 25 ANYS

# BIENVENIDA

El Instituto Universitario de Plaguicidas y Aguas (IUPA) de la Universitat Jaume I (UJI) de Castellón tiene el placer de daros la más calurosa bienvenida a la XV Reunión Científica de la Sociedad Española de Cromatografía y Técnicas Afines (SECyTA2015) así como a la VII Reunión Nacional de la Sociedad Española de Espectrometría de Masas (SEEM2015), que se celebran en un evento único del 27 al 30 de octubre de 2015 en Castellón de la Plana.

La organización de este Congreso combinado ha sido una apuesta difícil, en el período de crisis que sigue atravesando nuestro país, que asumimos en su momento con la ilusión de que con el esfuerzo y colaboración de todos, investigadores y patrocinadores, sería fácilmente superable.

En el programa de mano que leéis ahora figuran las contribuciones de investigadores y patrocinadores de SECyTASEEM2015, mostrando el éxito de la apuesta. Durante estos cuatro días de reunión, podremos disfrutar de Castellón mientras compartimos resultados y experiencias en las diferentes áreas temáticas que se abordan durante el congreso (medioambiente, alimentos, técnicas ómicas, análisis clínico, nuevos desarrollos instrumentales, etc.). La organización de este importante evento responde tanto al trabajo conjunto del comité organizador, que en este caso asumimos dos sociedades hermanas como son la SECyTA y la SEEM, a la labor del comité científico formado por colegas de reconocido prestigio internacional, como a la de otros miembros de las Juntas de gobierno, quienes nos han asesorado con sus años de experiencia, para elaborar un programa suficientemente atractivo para los asistentes.

La estructura del congreso consta de tres conferencias plenarias y cinco keynotes, 30 comunicaciones orales y casi 150 pósters, de los que una selección serán presentados mediante una comunicación flash. En esta ocasión, se siguen incluyendo sesiones plenarias para jóvenes investigadores que opten al Premio José Antonio García Domínguez, ya en su undécima edición, a la vez que se consolidan los Premios a las mejores comunicaciones en Espectrometría de Masas. Hay que resaltar que las comunicaciones presentadas en este Congreso pueden enviarse para ser publicadas en un Volumen Virtual Especial de Journal of Chromatography A, una de las revistas de mayor impacto en nuestro campo.

Por otra parte, nuestros patrocinadores llevarán a cabo diferentes lunch-seminars y dispondrán de una zona para exhibir sus productos e instrumentos. Reiterar nuestra gratitud por su participación y soporte financiero a la reunión.

Finalmente, manifestar nuestro agradecimiento por vuestra participación en este evento combinado de las dos sociedades. Esperamos que la estancia en estos días de Congreso responda a vuestras expectativas. Hemos preparado con ilusión este programa para asegurar que vuestra estancia sea científicamente provechosa y socialmente agradable.

Chairman
Dr. Juan Vicente Sancho Llopis

Co-Chairman
Dr. Joaquín Beltrán Arandes

Instituto Universitario de Plaguicidas y Aguas (IUPA)
Universitat Jaume I de Castelló (UJI)

# COMITÉS

## COMITÉ CIENTÍFICO

Esteban Abad Holgado, Institut de Diagnòstic Ambiental i Estudis de l'Aigua (CSIC)
Joaquín Beltrán Arandes, Universitat Jaume I de Castelló
Jordi Díaz Ferrero, Instituto Químico de Sarrià, Universitat Ramón Llull
Ana Mª García Campaña, Universidad de Granada
María José González Carlos, Instituto de Química Orgánica General (CSIC)
Belén Gómara Moreno, Instituto de Química Orgánica General (CSIC)
Joan Grimalt Obrador, Institut de Diagnòstic Ambiental i Estudis de l'Aigua (CSIC)
Félix Hernández Hernández, Universitat Jaume I de Castelló
Elena Ibáñez Ezequiel, Instituto de Investigación en Ciencias de la Alimentación (CSIC)
Begoña Jiménez Luque, Instituto de Química Orgánica General (CSIC)
Encarnación Moyano Morcillo, Universitat de Barcelona
Yolanda Picó Garcia, Universitat de València
Juan Vicente Sancho Llopis, Universitat Jaume I de Castelló
Fco. Javier Santos Vicente, Universitat de Barcelona
Mª Luz Sanz Murías, Instituto de Química Orgánica General (CSIC)
Rosa Ventura Alemany, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM)

## COMITÉ ORGANIZADOR

Juan Vicente Sancho Llopis, Universitat Jaume I de Castelló (Chair)
Joaquín Beltrán Arandes, Universitat Jaume I de Castelló (Co-Chair)
Esteban Abad Holgado, Institut de Diagnòstic Ambiental i Estudis de l'Aigua (CSIC)
Jordi Díaz Ferrero, Instituto Químico de Sarrià, Universitat Ramón Llull
María José González Carlos, Instituto de Química Orgánica General (CSIC)
Belén Gómara Moreno, Instituto de Química Orgánica General (CSIC)
Maria Ibáñez Martínez, Universitat Jaume I de Castelló
Francisco López Benet, Universitat Jaume I de Castelló
Encarnación Moyano Morcillo, Universitat de Barcelona
Elena Pitarch Arquimbau, Universitat Jaume I de Castelló
Tània Portolés Nicolau, Universitat Jaume I de Castelló
Antoni Francesc Roig i Navarro, Universitat Jaume I de Castelló
Roque Serrano Gallego, Universitat Jaume I de Castelló
Juan Solé Ribalta, HiTIC, S.A.
Rosa Ventura Alemany, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM)

# REUNIONES ANTERIORES

## SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TECNICAS AFINES (SECyTA)

| Presidente/s | Localización | Año | Edición |
|---|---|---|---|
| Jordi Mañes | Valencia | 2001 | I |
| Manuel V. Dabrio | Barcelona* | 2002 | II |
| Amadeo R. Fernández-Alba | Aguadulce (Almería) | 2003 | III |
| Coral Barbas | Boadilla del Monte (Madrid) | 2004 | IV |
| Juan Cacho | Barcelona* | 2005 | V |
| Ana Gago | Vigo (Pontevedra) | 2006 | VI |
| Ana María García Campaña | Granada | 2007 | VII |
| Joan O. Grimalt | Barcelona* | 2008 | VIII |
| Carmen Dorronsoro | San Sebastián | 2009 | IX |
| Yolanda Picó | Valencia | 2010 | X |
| Elena Domínguez | Barcelona* | 2011 | XI |
| Rosa María Marcé | Tarragona | 2012 | XII |
| Miguel Ángel Rodríguez y Alejandro Cifuentes | Puerto de la Cruz (Santa Cruz de Tenerife) | 2013 | XIII |
| Maria José González | Barcelona* | 2014 | XIV |
| Juan V Sancho y Joaquín Beltrán | Castellón de la Plana | 2015 | XV |

*Celebradas dentro de las correspondientes "Jornadas de Análisis Instrumental (JAIs)"

## SOCIEDAD ESPAÑOLA DE ESPECTROMETRÍA DE MASAS (SEEM)

| Presidente/s | Localización | Año | Edición |
|---|---|---|---|
| Alberto J Quejido | Madrid | 2001 | I |
| Mª Teresa Galcerán | Barcelona | 2003 | II |
| Alfredo Sanz-Medel | Oviedo | 2006 | III |
| Félix Hernández | Castellón de la Plana | 2009 | IV |
| José M. Vadillo | Málaga | 2011 | V |
| Antonio Molina | Úbeda (Jaén) | 2013 | VI |
| Juan V Sancho y Joaquín Beltrán | Castellón de la Plana | 2015 | VII |

# CONFERENCIANTES INVITADOS

**Dr. Facundo M Fernández**
Georgia Institute of Technology, Atlanta (USA)
*Ambient Ionization with Plasmas and Charged Droplets*

**Dr. Pablo Martínez-Lozano Sinues**
ETH Zurich (Switzerland)
*Breath Analysis: Transitioning from Bench to Bedside*

**Bruno José Fernandes Oliveira Manadas**
Coimbra University, Portugal
*Neuro-Mass Spectrometry: from Basic Research to Bed Side, and Back Again?*

**Prof. Dr. Jana Hajslova**
Institute of Chemical Technology, Prague (Czech Republic)
*Lipidomics Based on High Resolution Mass Spectrometry: A Novel Strategy Employed in Food and Nutrition Research*

**Dr. Eric Reiner**
University of Toronto, Toronto (Canada)
*Strategies and Techniques for Identifying Unknown Compounds in Environmental Samples*

**Prof. Dr. Paola Dugo**
University of Messina, Messina (Italy)
*Comprehensive Two-Dimensional Liquid Chromatography as a Powerful Tool for Food Applications*

**Dr. José Manuel Florêncio Nogueira**
University of Lisboa, Lisbon (Portugal)
*New Analytical Strategies for Sorption-Based Methods*

**Prof.Coral Barbas**
University of CEU San Pablo, Madrid (Spain)
*Analytical Developments and Biomedical Applications of Capillary Electrophoresis in Non-Targeted Metabolomics*

**Prof. Dr. Félix Hernández**
University Jaume I, Castellón (Spain)
*Investigation of Metabolites/Transformation Products of Emerging Contaminants in the Aquatic Environment by HRMS*

# SEEM2015
# SCIENTIFIC AND SOCIAL PROGRAM

## MONDAY OCTOBER 26

### Registration

**17:00 pm** Registration open
**20:00 pm** Registration close

## TUESDAY OCTOBER 27

### Opening Ceremony and Plenary Lectures

**08:00 am** **Symposium Secretary open**
**08:30 am** **SEEM2015 Opening Ceremony**

Vicent Climent Jordà - Rector
*Universitat Jaume I de Castelló*
Juan Vicente Sancho Llopis – Chairman
*Universitat Jaume I de Castelló*
Joaquín Beltrán Arandes – Co-Chairman
*Universitat Jaume I de Castelló*
Encarnación Moyano Morcillo – President of SEEM
*Universitat de Barcelona*

**Opening Plenary Lectures**
Session Chairs:
Jose Miguel Vadillo – Universidad de Málaga
Esteban Abad– IDAEA-CSIC, Barcelona

**09:00 am** ***PL-01 Ambient Ionization with Plasmas and Charged Droplets***
Facundo M Fernández
*Georgia Institute of Technology, Atlanta, USA*

**09:45 am** ***KN-01 Breath Analysis: Transitioning from Bench to Bedside***
Pablo Martínez-Lozano Sinués
*ETH Zurich, Switzerland*

**10:15 am** **Coffee break**
**Poster Session 1 & Exhibition**

## Morning Session 1

Session Chairs: Rosa Ventura – IMIM, Barcelona
Pablo Rodríguez – Universidad de Oviedo

**11:45 am** ***O-01 HRMS Tools for Direct Analysis of Recreative Drugs and Legal Highs***

Élida Alechaga, Encarnación Moyano, Maria Teresa Galceran
*Department of Analytical Chemistry, University of Barcelona*

**12:05 pm** ***O-02 Detection and Mass Spectrometric Characterization of Alternative Clostebol Long Term Metabolites in Human Urine***

Georgina Balcells[(1)], Oscar Pozo[(1)], Lorena Garrostas[(1)], Argitxu Esquivel[(1)], Xavier Matabosch[(1)], Aristotelis Kotronoulas[(1,2)], Jesús Joglar[(2)], Rosa Ventura[(1,3)]
*[(1)] Bioanalysis Research Group, IMIM, Hospital del Mar Medical Research Institute [(2)] Department of Biological Chemistry and Molecular Modeling, IQAC, CSIC [(3)] Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona*

**12:25 pm** ***O-03 Identification of Substances Migrating from Plastic Baby Bottles using High Resolution Mass Spectrometric Analyzers Coupled to Gas and Liquid Chromatography***

M. Onghena[(1)], E. Van Hoeck[(2)], J. Van Loco[(2)], M. Ibáñez[(3)], L. Cherta[(3)], T. Portolés[(3)], E. Pitarch[(3)], F. Hernandéz[(3)], F. Lemière[(4)], A. Covaci[(1)]
*[(1)]Toxicological Center, Faculty of Pharmaceutical Sciences, University of Antwerp, Belgium [(2)] Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium [(3)] Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain [(4)] Center for Proteome Analysis and Mass Spectrometry (CeProMa), University of Antwerp, Belgium*

**12:45 pm** ***O-04 The Use of Ion Mobility Separation and Collision Cross Section for the Comprehensive Analysis of Complex Samples***

Steve Preece, Daniel Weston, Emma Marsden-Edwards
*Waters Corporation, Wilmslow, UK*

**13:05 pm** ***O-05 When the Interpretation of MS Spectra Becomes Essential***

Josep Marcos[(1)], Maria Ibáñez[(2)], Juan V. Sancho[(2)], Oscar J. Pozo[(1)]
*[(1)] Bioanalysis Research Group. IMIM, Hospital del Mar, Barcelona, Spain [(2)] Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain*

**13:30 pm** **Lunch & Lunch-seminars**

**15:00 pm** **Poster Session 1 & Exhibition**

## Poster Flash Session 1

Session Chairs: Belén Gómara – IQOG-CSIC, Madrid
Sandra Pérez – IDAEA-CSIC, Barcelona

**16:00 pm** ***P-22 Effect of Glucocorticoid Administration on the Steroid Profile in Sports Drug Testing***

Sergi Coll[(1)], Xavier Matabosch[(1)], Lorena Garrostas[(1)], Clara Pérez-Mañá[(2,3)], Rosa Ventura[(1,4)]

*[(1)] Bioanalysis Research Group, IMIM, Barcelona, Spain [(2)] Human Pharmacology and Clinical Neurosciences Research Group, IMIM, Barcelona, Spain [(3)] Universitat Autònoma de Barcelona, Bellaterra, Spain [(4)] Universitat Pompeu Fabra, Barcelona*

**16:05 pm** ***P-04 Potential of GC-APCI-MS Based Strategies for Metabolic Studies***

Montse Raro[(1)], Tania Portoles[(1)], Elena Pitarch[(1)], Rosa Ventura[(2)], Juan Vicente Sancho[(1)], Oscar J. Pozo[(2)]

*[(1)] Research institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain [(2)] Bioanalysis Research Group. IMIM, Hospital del Mar, Barcelona, Spain*

**16:10 pm** ***P-32 High Precision Measurement of Dissolved Inorganic Carbon (DIC) by Isotope Dilution Mass Spectrometry for Climate Change Studies***

Laura Freije Carrelo[(1)], Laura Alonso Sobrado[(1)], Sergio Cueto Díaz[(2)], Jorge Ruiz Encinar[(1)], José Ignacio García Alonso[(1)]

*[(1)] Departamento de Química Física y Analítica, University of Oviedo, Spain*
*[(2)] Servicios Científico-Técnicos de la Universidad de Oviedo, Oviedo, Spain*

**16:15 pm** ***P-33 Atmospheric Pressure Ionization of Polyfluorinated Compounds***

Juan Francisco Ayala Cabrera, Francisco Javier Santos Vicente, Encarnación Moyano Morcillo

*Universitat de Barcelona, Barcelona, Spain*

**16:20 pm** ***P-35 Use of High Resolution Mass Spectrometry (Orbitrap) for Simulta neous Determination of Pesticides and Mycotoxins in Green Tea Nutraceutical Products***

Gerardo Martínez Domínguez, Roberto Romero González, Antonia Garrido Frenich, Ana Ruiz Delgado

*Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, University of Almería, Almería, Spain*

**16:25 pm** *P-43 Exploring Nanoflow Liquid Chromatography High Resolution Mass Spectrometry for Pesticide Testing in Food*

Juan F. García-Reyes, Patricia Pérez-Ortega, Antonio Molina-Díaz

*Analytical Chemistry Research Group, University of Jaén, 23071 Jaén, Spain*

**16:30 pm** **Coffee break & Exhibition**

## Keynote Lecture and Evening Session 1

Session Chairs: Montserrat Carrascal – LP CSIC-UAB, Barcelona
Encarnación Moyano – Universitat de Barcelona

**17:00 pm** *KN-02 Neuro-Mass Spectrometry: from Basic Research to Bed Side, and Back Again?*

Bruno Manadas[1,2]

[(1)] *CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal* [(2)] *Biocant - Biotechnology Innovation Center, Cantanhede, Portugal*

**17:30 pm** *O-06 Synthesis and Characterization of Minimally 13C-Labelled Peptide Standards for the Determination of Angiotensin II in Amniotic Fluid by Isotope Dilution Tandem Mass Spectrometry*

Adriana González-Gago[(1)], Diego O. Pastene Maldonado[(2)], Paula Núñez Martínez[(2)], Carmen Perillan[(2)], Juan Arguelles[(2)], Pablo Rodríguez-González[(1)], José Ignacio García Alonso[(1)]

[(1)] *Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo* [(2)] *Department of Functional Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Oviedo, Spain*

**17:50 pm** *O-07 UPLC-HRMS for the Detection and Identification of Pharmaceuticals Metabolites*

Jaume Aceña[(1)], Sandra Pérez[(1)], Damià Barceló[(1,2)]

[(1)] *Water and Soil Quality Research Group, IDAEA-CSIC, Barcelona (Spain)*
[(2)] *ICRA Catalan Institute for Water Research, Girona (Spain)*

**18:10 pm** *O-08 Gas Chromatography Orbitrap Mass Spectrometry: A Novel Analytical Tool to Help Emerge Iodinated Disinfection Byproducts in Chlorinated and Chloraminated Waters*

Cristina Postigo[(1)], Cristian I. Cojocariu[(2)], Susan D. Richardson[(3)], Damià Barceló[(1,4)]

[(1)] *Institute for Environmental Assessment and Water Research, (IDAEA-CSIC), Barcelona, Spain* [(2)] *Thermo Fisher Scientific, Runcorn, UK* [(3)] *Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, USA*
[(4)] *Catalan Institute for Water Research (ICRA), Girona, Spain.*

**18:30 pm**

*O-09 Submicrometric Characterization of Spatially-Structured Materials by Femtosecond Ionization and Time-Of-Flight Mass Spectro metry in On-Axis Configuration*

Samara Medina, Pablo Purohit, José M. Vadillo, J. Javier Laserna
*Universidad de Málaga, Departamento de Química Analítica, Málaga, Spain*

**18:50 pm** **SEEM2015 Closing Ceremony**

Juan Vicente Sancho Llopis – Chairman
*Universitat Jaume I de Castelló*
Joaquín Beltrán Arandes – Co-Chairman
*Universitat Jaume I de Castelló*
Encarnación Moyano Morcillo – President of SEEM
*Universitat de Barcelona*

**19:00 pm** **Plenary Meeting of SEEM**

**20:00 pm** **Social event**
**Villas de Benicàssim, Hotel Voramar**
**Welcome Cocktail**

## WEDNESDAY OCTOBER 28

### Opening Ceremony and Plenary Lectures

**08:00 am** **Symposium Secretary open**
**08:30 am** **SECyTA2015 Opening Ceremony**

Antonio Barba Juan – Vicerector of Research and Doctorate
*Universitat Jaume I de Castelló*
Juan Vicente Sancho Llopis – Chairman
*Universitat Jaume I de Castelló*
Joaquín Beltrán Arandes – Co-Chairman
*Universitat Jaume I de Castelló*
Mª José González Carlos – President of SECyTA
*IQOG-CSIC, Madrid*

### Opening Plenary Lectures

Session Chairs: Mª José González – IQOG-CSIC, Madrid
Yolanda Picó – Universitat de València

**09:00 am** ***PL-01 Lipidomics Based On High Resolution Mass Spectrometry: A Novel Strategy Employed In Food And Nutrition Research***
Jana Hajslova
*Institute of Chemical Technology, Prague, Czech Republic*

**09:45 am** ***KN-01 Strategies and Techniques for Identifying Unknown Compounds in Environmental Samples***
Eric J Reiner
*University of Toronto, Department of Chemistry, Toronto, ON Canada*

**10:15 am** **Coffee break**
**Poster Session 2 & Exhibition**

### Morning Session 2: Environmental Analysis & Sample Preparation

Session Chairs: Joan Grimalt – IDAEA-CSIC, Barcelona
Begoña Jiménez – IQOG-CSIC, Madrid

**11:45 am** ***O-01 Multi-compound GC-MS Analysis of Organic Tracers in Atmospheric Samples***
Barend L. van Drooge, Marta Fontal, Esther Marco, Joan O. Grimalt
*Department of Environmental Chemisty, Institute of Environmental Assessment and Water Research (IDÆA-CSIC), Barcelona, Spain*

**12:05 pm** ***O-02 Determination of Gasolina Range Organics in Water by Head Space Solid Phase Microextraction Gas Chromatography Mass Spectrometry***

Nuria Font-Cardona, José Luis Aranda-Mares, Salomé Ballester-Nebot
*IPROMA S.L. Camino de la Raya no 46, 12005 Castellón, Spain*

**12:25 pm** ***O-03 Analysis of Fullerenes in the Environment by LC-APPI-HRMS and Preliminary Metabolomic Studies***

Marinella Farré[(1)], Josep Sanchís[(1)], Mar Olmos[(1)], Cristina Bosch[(1)], Xavier Calafell[(2)] and, Damià Barceló[(1,3)]
*[(1)] Water and Soil Quality Research Group, IDAEA-CSIC, Barcelona, Spain [(2)] Sublimits, Avda. Platja d'Aro, 242, 17248 S'Agaró, Girona [(3)] Catalan Institute of Water Research (ICRA), Girona, Spain*

**12:45 pm** ***O-04 Evaluation of Retention Mechanisms of Benzotriazoles, Benzothiazoles and Benzenesulfonamides on Solid-Phase Extraction Sorbents with Strong Cationic or Anionic Mixed-Mode Properties***

Daniela Salas, Francesc Borrull, Rosa Maria Marcé, Núria Fontanals
*Department of Analytical Chemistry and Organic Chemistry, Faculty of Chemistry, Universitat Rovira i Virgili, Tarragona, Spain*

**13:05 pm** ***O-05 Application of HR-MS (Q-Exactive-MS) For The Evaluation of The Fate of Drug Glucuronides In Wastewater Treatment Plants***

Sandra Pérez[(1)], Bozo Zonja[(1)], Jaume Aceña[(1)], Damià Barceló[(1,2)]
*[(1)] Water and soil quality research group, IDAEA-CSIC, Barcelona, Spain [(2)] Catalan Institute of Water Research, ICRA, Girona, Spain*

**13:30 pm** **Lunch & Lunch-seminars**

**15:00 pm** **Poster Session 2 & Exhibition**

## Poster Flash Session 2

Session Chairs: Javier Moreno – IQOG-CSIC, Madrid
Joaquín Beltrán – Universitat Jaume I, Castellón

**16:00 pm** ***P-70 Screening and Quantification of Pharmaceuticals and Therapeutical Agents in Reauthorized Processed Animal By-Products***

Alberto Celma-Tirado[(1)], Jaime Nácher-Mestre[(1)], Roque Serrano[(1)], Marc H.G. Berntssen[(2)], Félix Hernández[(1)]
*[(1)] Research Institute for Pesticides and Water (IUPA), Univ Jaume I, Castelló, Spain [(2)] National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway*

**16:05 pm** ***P-73 Determination of Sulfonamide Residues in Chicken Muscle and Eggs by Liquid Chromatography with Photoinduced Fluorescence Detection Using Quechers As Sample Treatment***

José Fernando Huertas-Pérez[(1)], Natalia Arroyo-Manzanares[(1)], Lucie Havlíková[(2)], Laura Gámiz-Gracia[(1)], Petr Solich[(2)], Ana M. García-Campaña[(1)]

*[(1)] Department of Analytical Chemistry, University of Granada, Granada, Spain [(2)] Department of Analytical Chemistry, Charles University in Prague, Czech Republic*

**16:10 pm** ***P-56 Comprehensive Characterization of Goat Colostrum Oligosaccharides by Liquid Chromatography Coupled to Mass Spectrometry***

A. Martín-Ortiz[(1)], D. Barile[(2)], J. Salcedo[(2)], A. Bunyatratchata[(2)], F.J. Moreno[(3)], I. Martin-García[(4)], A. Clemente[(4)], M.L. Sanz[(1)], A.I.Ruiz-Matute[(1)]

*[(1)] Instituto de Química Orgánica General (CSIC), Madrid, Spain [(2)] Department of Food Science and Technology, University of California Davis, USA [(3)] Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), Madrid, Spain [(4)] Estación Experimental del Zaidín (CSIC), Granada, Spain*

**16:15 pm** ***P-52 Use of Gas Chromatography Coupled to Triple Quadrupole Mass Spectrometry for Identification and Quantification of Polycyclic Aromatic Hydrocarbons in Soy-Based Nutraceutical Products***

Ana Ruiz-Delgado, Gerardo Martínez-Domínguez, Roberto Romero-González, Antonia Garrido Frenich

*Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics (Analytical Chemistry Area), University of Almería, Almería, Spain*

**16:20 pm** ***P-01 Design and Construction of a Light-Emitting Diode Induced Fluorescence (LEDIF) Detector for Microchip Capillary Electrophoresis Devices***

Angel Puerta, Mercedes de Frutos, Jose Carlos Diez-Masa

*Institute of General Organic Chemistry (IQOG, CSIC), Madrid, Spain.*

**16:25 pm** ***P-04 3D Printed Columns for High Performance Liquid Chromatography***

Jose Javier Rubio-Sahuquillo, Enrique Carrasco-Correa, Guillermo Ramis-Ramos, José Manuel Herrero-Martínez, Ernesto Francisco Simó-Alfonso

*Department of Analytical Chemistry, University of Valencia, Valencia, Spain*

**16:30 pm** **Coffee break & Exhibition**

## Young Scientists Session 1

Session Chairs: Jordi Díaz-Ferrero – IQS, Barcelona
Rosa Mª Marcé – Universitat Rovira i Virgili, Tarragona

**17:00 pm**

***YS-01 Preliminary Biodegradation Studies of Different Commercial Homo-Polymers by MALDI-TOF MS Technique and Their Application to the Evaluation of River Metabolism***

Daniel Rivas[(1)], Arturo Elósegui[(2)], Antoni Ginebreda[(1)], Sandra Pérez[(1)], Jesús del Pozo[(2)], Carme Quero[(3)], Damià Barceló[(1,4)]

*[(1)] IDAEA-CSIC, Department of Environmental Chemistry, Barcelona, Spain [(2)] EHU University of the Basque, Lab. Ecology, Bilbao, Spain [(3)] IQAC-CSIC, Institute of Advanced Chemistry of Catalonia, Barcelona, Spain [(4)] ICRA, Girona, Spain*

**17:10 pm**

***YS-02 Ultrasensitive Emerging Illicit Drugs Profiling in Wastewater Using a Hybrid Quadrupole Time-Of-Flight Tandem Mass Spectrometry (Triple TOF 5600)***

María Jesús Andrés-Costa[(1)], Vicente Andreu[(2)], Yolanda Picó[(1)]

*[(1)] Environmental and Food Safety Research Group, University of Valencia, Valencia, Spain [(2)] Research Centre on Desertification-CIDE (CSIC-UV-GVA), Valencia, Spain*

**17:20 pm**

***YS-03 Prediction of Chromatographic Retention Time Using Artificial Neural Networks for the Identification of Emerging Contaminants in Environmental Waters***

Richard Bade[(1)], Lubertus Bijlsma[(1)], Leon Barron[(2)], Thomas Miller[(2)], Juan V. Sancho[(1)], Félix Hernández[(1)]

*[(1)] Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain [(2)] Analytical & Environmental Sciences Division, King's College London, UK*

**17:30 pm**

***YS-04 Classification and Characterization of Mycorrhizal Rosemary Plants by UHPLC-HRMS Compositional Profiles and Chemometrics***

Raquel Seró[(1)], Oscar Núñez[(1)], Javier Saurina[(1)], Cinta Calvet[(2)], Encarnación Moyano[(1)]

*[(1)] Department of Analytical Chemistry, University of Barcelona, Barcelona, Spain [(2)] IRTA, Protecció Vegetal Sostenible, Cabrils, Barcelona, Spain*

**17:40 pm** *YS-05 Fingerprinting Analysis of Extracts of Licorice by Comprehensive Two-Dimensional Liquid Chromatograhy*

Lidia Montero[(1)], Elena Ibáñez[(1)], Mariateresa Russo[(2)], Rosa di Sanzo[(2)], Luca Rastrelli(3), Anna Lisa Piccinelli[(3)], Rita Celano[(3)], Alejandro Cifuentes[(1)], Miguel Herrero[(1)]

*[(1)] Laboratory of Foodomics, Institute of Food Science Research (CIAL-CSIC), Madrid, Spain [(2)] Laboratory of Food Chemistry, Università Mediterranea di Reggio Calabria, Reggio Calabria, Italy [(3)] Dipartimento di Farmacia, Università di Salerno, Fisciano, Italy*

**17:50 pm** *YS-06 Capillary Electrophoresis Tandem Mass Spectrometry as a Simple Alternative for the Determination of Aminoglycosides in Honey using Molecularly Imprinted Polymers for Sample Treatment*

David Moreno-González, Francisco J. Lara, Laura Gámiz-Gracia and Ana M. García-Campaña

*Department of Analytical Chemistry, University of Granada, Granada, Spain*

**18:00 pm** **Plenary Meeting of SECyTA**

**20:30 pm** **Social event**
**Walking tour at Castellón City Center**

## THURSDAY OCTOBER 29

### Plenary Lectures

**08:30 am** **Symposium Secretary open**

### Opening Plenary Lectures

Session Chairs: Elena Ibáñez – CIAL, CSIC-UAM, Madrid
Lourdes Ramos – IQOG-CSIC, Madrid

**09:00 am** *PL-02 Comprehensive Two-Dimensional Liquid Chromatography as a Powerful Tool for Food Applications*

Paola Dugo[(1,2,3)]

*[(1)] University of Messina, Dipartimento SCIFAR, Messina, Italy [(2)] Centro Integrato di Ricerca, Università Campus Bio-Medico, Roma, Italy [(3)] Chromaleont s.r.l. A start-up of the University of Messina, Messina, Italy.*

**09:45 am**

***KN-02 New Analytical Strategies for Sorption-Based Methods***
J.M.F. Nogueira
*Centro de Química e Bioquímica, Universidade de Lisboa, Lisboa, Portugal*

**10:15 am**

**Coffee break**
**Poster Session 3 & Exhibition**

## Morning Parallel Sessions 3

**Session 3 A**
**Omics Techniques & Clinical Analysis**
Session Chairs: Fco. Javier Santos – Universitat de Barcelona
Mercedes de Frutos – IQOG-CSIC, Madrid

**11:45 am**

***O-06 Unveiling Post Mortem Changes in Mouse Brain by a Multiplat form Non-Targeted Metabolomic Study***
Carolina González-Riaño[(1)], Fernanda Rey-Stolle[(1)], Gonzalo León[(2)], Silvia Tapia[(2)], Laura Ravanetti[(1)], Antonia García[(1)], Javier De Felipe[(2)], Coral Barbas[(1)]
*[(1)] CEMBIO (Centre for Metabolomics and Bioanalysis), Universidad San Pablo CEU, Madrid, Spain [(2)] Centro de Tecnología Biomédica, UPM, Madrid, Spain*

**12:05 pm**

***O-07 UPLC-QTOF MS Metabolomics for Biomarker Discovery in Chemically Non-Diagnosed Diseases: Fetal Alcohol Spectrum Disorder***
Rubén Gil[(1)], Montse Raro[(1)], Xavier Joya[(2)], Oriol Vall[(2)], Judith Salat-Batlle[(2)], Gretsen Velezmoro-Jáuregui[(2)], Oscar García-Algar[(2)], Josep Marcos[(3)], Oscar J. Pozo[(3)], Juan Vicente Sancho[(1)]
*[(1)] Research institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain [(2)] Infant and Environment Research Group (URIE). IMIM, Hospital del Mar, Barcelona, Spain [(3)] Bioanalysis Research Group. IMIM, Hospital del Mar, Barcelona, Spain*

**12:25 pm**

***O-08 Untargeted Detection of Unaltered Glucuronoconjugated Metabolites of Metandienone in Sports Drug Testing by LC-MS/MS***
Argitxu Esquivel[(1)], Oscar J. Pozo[(1)], Lorena Garrostas[(1)], Georgina Balcells[(1)], Aristotelis Kotronoulas[(1,2)], Jesús Joglar[(1,2)], Rosa Ventura[(1,3)]
*[(1)] Bioanalysis Research Group, IMIM, Hospital del Mar Medical Research Institute, Barcelona, Spain [(2)] Department of Biological Chemistry and Molecular Modeling, IQAC, Barcelona, Spain [(3)] Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain*

**12:45 pm**

***O-09 Cyclobenzaprine Multi-targeted Mechanism Against Leishmania, Metabolomic and Funcional Study for a Drug Repurposing Candidate***

Marta Lopes Lima[1,2], Ángeles López-Gonzálvez[1], María Angeles Abengózar[3], Andre Gustavo Tempone[4], Luis Rivas[3], Coral Barbas[1]

*[1] Centro de Metabolomica y Bioanalisis (CEMBIO), Facultad de Farmacia, Universidad CEU San Pablo, Madrid, Spain [2] Tropical Medicine Institute, University of São Paulo (USP), Brazil [3] Centro de Investigaciones Biologicas (CSIC), Unidad Metabolomica, Interacciones y Bioanalisis (UMIB), Madrid, Spain [4] Adolfo Lutz Institute, Laboratory of Novel Drugs-Neglected Diseases, São Paulo, Brazil*

**13:05 pm**

***O-10 Common Ion Loss Scan for The Untargeted Detection of Bis-Sulfate Metabolites***

Malcom McLeod[1], Argitxu Esquivel[2], Georgina Balcells[2], Jordi Segura[2], Rosa Ventura[2], Oscar J. Pozo[2]

*[1] Research School of Chemistry Australian National University, Acton, Australia [2] Bioanalysis Research Group. IMIM, Hospital del Mar, Barcelona, Spain*

**Session 3B**
**Food Analysis & Fundamentals**

Session Chairs: Belen Gómara – IQOG-CSIC, Madrid
Juan Vicente Sancho – UJI, Castellón

**11:45 am**

***O-11 LC-MS/MS and LC-HRMS Targeted and Untargeted Approaches for the Characterization, Classification and Authentication of Natural Products***

Oscar Núñez[1,2], Riccardo Gagliardi[3], Lidia Parets[1], Raquel Seró[1], Élida Alechaga[1], Paolo Lucci[4], Javier Saurina[1], Deborah Pacetti[3], Natale G. Frega[3], Encarnación Moyano[1]

*[1] Department of Analytical Chemistry, University of Barcelona, Barcelona, Spain [2] Serra Húnter Fellow, Generalitat de Catalunya, Spain [3] Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy [4] Department of Food Science, University of Udine, Udine, Italy.*

**12:05 pm**

***O-12 Direct Analysis in Real Time - High Resolution Mass Spectrometry as a Valuable Tool for Polyphenols Profiling in Olive Oil***

Marinella Farre[1], Yolanda Picó[2], Damià Barceló[1,3]

*[1] Water and Soil Quality Research Group, IDAEA-CSIC Barcelona, Spain [2] Food and Environmental Research Group, Universitat de València, Valencia, Spain [3] Catalan Institute of Water Research (ICRA), Girona, Spain*

**12:25 pm**

***O-13 Valorization of Fruit and Olive Processing Byproducts***
María Concepción García López, Estefanía González-García, Romy Vásquez-Villanueva, María Luisa Marina Alegre
*Department of Analytical Chemistry, University of Alcalá, Alcalá de Henares, Madrid, Spain*

**12:45 pm**

***O-14 Determinación Ultrarrápida de Residuos de Plaguicidas mediante Cromatografia de Gases acoplada a Espectrometría de Masas Triple Cuadrupolo en Matrices Vegetales***
Javier López Flores, Diego Martín, Miguel Ángel Perez
*Bruker Española, SA, Rivas Vaciamadrid, Madrid, Spain*

**13:05 pm**

***O-15 Implementation of Methods in High Performance Liquid Chromatography using Serially-Coupled Columns***
María Celia García-Álvarez-Coque, José Ramón Torres-Lapasió, Tamara Álvarez-Segura
*Department of Analytical Chemistry, University of Valencia, Valencia, Spain*

**13:30 pm** **Lunch & Lunch-seminars**

**15:00 pm** **Poster Session 3 & Exhibition**

**Poster Flash Session 3**
Session Chairs: Rosa Ventura – IMIM, Barcelona
Joan Solé – HiTC, Barcelona

**16:00 pm**

***P-33 Determination of Human-Specific Biomarkers in Wastewater by Liquid Chromatography-Mass Spectrometry***
María Jesús Andrés-Costa[(1)], María Rico[(1)], Vicente Andreu[(2)], Yolanda Picó[(1)]
*[(1)] Environmental and Food Safety Research Group, University of Valencia, Valencia, Spain [(2)] Research Centre on Desertification-CIDE (CSIC-UV-GVA), Valencia, Spain*

**16:05 pm**

***P-44 UHPLC–API–MS/MS for the Determination of Polyfluorinated Compounds***
Juan Francisco Ayala Cabrera, Francisco Javier Santos Vicente, Encarnación Moyano Morcillo
*Department of Analytical Chemistry, University of Barcelona, Barcelona, Spain*

**16:10 pm** ***P-45 Multi-Class Determination of Polycyclic Aromatic Hydrocarbons, Pesticides and Polychlorinated Biphenyls in Aquaculture Samples By Gas Chromatography/ Tandem Mass Spectrometry with Atmospheric Pressure Chemical Ionization***

Borja Garlito[(1)], Tania Portolés[(1)], Jaime Nácher-Mestre[(1)], Marc H. G. Berntssen[(2)], Roque Serrano[(1)], Jaume Pérez-Sánchez[(3)], Félix Hernández[(1)]

*[(1)] Research institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain [(2)] National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway [(3)] Institute of Aquaculture Torre la Sal (IATS-CSIC), Castellón, Spain*

**16:15 pm** ***P-17 Dynamic Fabric Phase Sorptive Extraction for a Group Pharmaceuticals and Personal Care Products***

Sameer S. Lakade[(1)], Francesc Borrull[(1)], Kenneth G. Furton[(2)], Abuzar Kabir[(2)], Núria Fontanals[(1)], Rosa M. Marcé[(1)]

*[(1)] Department of Analytical Chemistry, Universitat Rovira i Virgili, Tarragona, Spain [(2)] International Forensic Research Institute, Department of Chemistry and Biochemistry, Florida International University, Miami, USA*

**16:20 pm** ***P-15 How to Avoid Agglomeration of Magnetic Beads for Immuno-Recognition and Analysis of Glycoproteins in Capillary Electrophoresis Systems***

Monica Gonzalez[(1)], Angel Puerta[(1)], Marta Fernandez-Garcia[(2)], Mercedes de Frutos[(1)], Jose Carlos Diez-Masa[(1)]

*[(1)] Institute of Organic Chemistry (IQOG-CSIC), Madrid, Spain [(2)] Institute of Polymer Science and Technology (ICTP-CSIC), Madrid, Spain*

**16:25 pm** ***P-30 Exploring Potential of Gas Chromatography with Atmospheric Pressure Chemical Ionization and Tandem Mass Spectrometry for Sensitive Determination of Ethyl Glucuronide in Hair***

Tania Portolés[(1)], Juliet Kinyua[(2)], Alexander van Nuijs[(2)], Delphine Cappelle[(2)], Juan V. Sancho[(1)], Félix Hernández[(1)]

*[(1)] Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain [(2)] Toxicologial Centre, University of Antwerp, Wilrijk, Belgium*

**16:30 pm** **Coffee break & Exhibition**

## Young Scientists Session 2

Session Chairs: Ana Mª García-Campaña – Universidad de Granada
Yolanda Picó – Universitat de València

**17:00 pm**

***YS-07 Photochemical Transformation of Cosmetic Preservatives into 2,8-Dichlorodibenzo-p-Dioxin and Other Unwanted Photoproducts in Topical Cream Applied onto Artificial Skin***

Gerardo Alvarez-Rivera, Maria Llompart, Carmen Garcia-Jares, Marta Lores

*Department of Analytical Chemistry, Nutrition and Food Science, University of Santiago de Compostela, Santiago de Compostela, Spain*

**17:10 pm**

***YS-08 Liquid Cromatography- and Capillary Electrophoresis-Mass Spectrometry Multiplatform for Broad Metabolomic Analysis of Dietary Polyphenols Effect on Colon Cancer Using in Vivo Models***

Tanize Acunha[(1,2)], Carolina Simó[(1)], Clara Ibáñez[(1)], Almudena Pérez[(3)], Vicente Micol[(3)], Alejandro Cifuentes[(1)]

*[(1)] Laboratorio de Alimentómica, CIAL, CSIC, Madrid, Spain [(2)] CAPES Foundation, Ministry of Education of Brazil, Brasília, Brazil [(3)] Institute of Molecular and Cellular Biology, University Miguel Hernández, Elche, Spain*

**17:20 pm**

***YS-09 Quality Classification of Olive Oils by Gas Chromatography with Atmospheric Pressure Chemical Ionization and Quadrupole Time Of Flight Mass Spectrometry using a Metabolomics-Based Statistical Approach***

Carlos Sales, María Inés Cervera, Rubén Gil, Tania Portolés, Elena Pitarch, Joaquín Beltrán *Research Institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain*

**17:30 pm**

***YS-10 Comprehensive Study of the Antiproliferative Activity of Rosemary Polyphenols on Colon Cancer Cells using Nano-Liquid Chromatography-Orbitrap MS/MS-Based Proteomics***

Alberto Valdés[(1)], Virginia García-Cañas[(1)], Konstantin Artemenko[(2)], Jonas Bergquist [(2)], Alejandro Cifuentes[(1)]

*[(1)] Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Madrid, Spain [(2)] Analytical Chemistry, Department of Chemistry-BMC, Uppsala University, Uppsala, Sweden*

**17:40 pm**

***YS-11 General Approach for Determination of Pharmaceuticals, Illicit Drugs and Personal Care Products in Water and Sediments***

Eric Carmona, Yolanda Picó

*Environmental and Food Safety Research Group, University of Valencia, Burjassot, Valencia, Spain*

**17:50 pm** *YS-12 Analytical Strategy Developed to Evaluate the Efficacy of Different Treatments for Removing Odours from Dishwashers*

Ignacio Ontañón, Vicente Ferreira, Ana Escudero
*Laboratorio de Análisis del Aroma y Enología, Departamento de Química Analítica, Universidad de Zaragoza, Zaragoza, Spain*

**20:30 pm** **Social dinner**
**Torrelamina Restaurant**

## FRIDAY OCTOBER 30

### Morning Sessions 4:

**New Developments in Instrumentation**

Session Chairs: Encarna Moyano – Universitat de Barcelona
José Carlos Diez-Masa – IQOG-CSIC, Madrid

**09:30 am** *O-16 Detection of Natural Additives in a Polylactic Based Polymer (Pla) using Pyrolysis Compound Specific Isotope Analysis (Py-CSIA)*

José A. González-Pérez[(1)], María Llana-Ruíz-Cabello[(2)], Silvia Pichardo[(2)], Nicasio T. Jiménez-Morillo[(1)], Gonzalo Almendros[(3)], Francisco J. González-Vila[(1)], Enrique Guillamón[(4)], José M. Bermúdez[(5)], Susana Aucejo[(5)], Ana M. Camean[(2)]
*[(1)] IRNAS-CSIC, Seville, Spain [(2)] Universidad de Sevilla, Seville, Spain [(3)] MNCN-CSIC, Madrid, Spain [(4)] DOMCA S.A., Alhendín, Granada, Spain [(5)] ITENE, Paterna, Valencia, Spain*

**09:50 am** *O-17 Identification of New Psychoactive Substances Based on the Complementary Use of Spectrometric Techniques*

Lubertus Bijlsma[(1)], Bram Miserez[(2)], María Ibáñez[(1)], Solomon Ma Ting Fung[(2)], John Ramsey[(2)], Trevor Shine[(2)], Eva Guillamón[(3)], Cristian Vicent[(4)], Félix Hernández[(1)]
*[(1)] Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain [(2)] TICTAC Communications Ltd., St George's University of London, UK [(3)] Department Química Física i Analítica, Universitat Jaume I, Castellón, Spain [(4)] Serveis Centrals d'Instrumentacio Científica, Universitat Jaume I, Castellón, Spain*

**10:10 am** *O-18 Potential of Gas Chromatography Coupled with Orbitrab-Based Mass Spectrometry for the Analysis of Halogenated Persistent Organic Pollutants in Food and Environmental Samples*

Esteban Abad[(1)], Manuela Abalos[(1)], Jordi Saulo[(1)], Sergio Guazzotti[(3)], Paul Silcock[(2)] and Cristian Ioan Cojocariu[(2)]
*[(1)] Spanish Council for Scientific Research (CSIC), Institute of Environmental Assessment and Water Research, Barcelona, Spain [(2)] Thermo Fisher Scientific, Runcorn, Cheshire, United Kingdom [(3)] Thermo Fisher Scientific, Austin, TX USA*

**10:30 pm**

***O-19 Autenticidad del Aceite de Oliva: Perfilado de Aceite de Oliva Extra Virgen usando GCQTOF MS. Desarrollo de un modelo de predicción geográfica para determinación de adulteraciones***

José Juan Rivero, Jaume Morales
*Agilent Technologies, Spain*

**10:50 am**

***O-20 Direct Characterization of Volatile Compounds by 2D Non-Chromatographic Separation by a Hybrid Planar Differential Mobility Analyzer and Mass Spectrometry***

José M. Vadillo[(1)], Samara Medina[(1)], Pablo Purohit[(1)], J. Javier Laserna[(1)] and Arturo Álvaro[(2)]
*[(1)] Universidad de Málaga, Departamento de Química Analítica, Málaga, Spain [(2)] SEADM, Fundación CARTIF, Valladolid, Spain*

**11:10 pm**

***O-21 Potential of Orbitrap GC-MS for Targeted and Unknown Compounds Analysis in Foods***

Sergio Guazzotti[(1)], Pau Castells[(2)], Javier Rodriguez[(2)]
*[(1)] Thermo Fisher Scientific, Austin, USA [(2)] Thermo Fisher Scientific, Spain*

**11:30 am** **Coffee break & Exhibition**

## Closing Plenary Lectures and Closing Ceremony

Session Chairs: Juan V Sancho – Universitat Jaume I, Castellón
Maria Teresa Galceran – Universitat de Barcelona

**12:00 pm**

***KN-03 Analytical Developments and Biomedical Applications of Capi llary Electrophoresis in Non-Targeted Metabolomics***

Coral Barbas
*CEMBIO (Centre for Metabolomics and Bioanalysis), Facultad de Farmacia, Campus Montepríncipe, Universidad San Pablo CEU, Madrid, Spain*

**12:30 pm**

***PL-03 Investigation of Metabolites/Transformation Products of Emerging Contaminants in the Aquatic Environment by HRMS***

Félix Hernández
*Institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain*

**13:15 pm** **SECyTA2015 Closing Ceremony**

Juan Vicente Sancho Llopis – Chairman
*Universitat Jaume I de Castello*
Joaquín Beltrán Arandes – Co-Chairman
*Universitat Jaume I de Castelló*
Encarnación Moyano Morcillo – President of SEEM
*Universitat de Barcelona*
Mª José González Carlos – President of SECyTA
*IQOG-CSIC, Madrid*

**Announcement of the Awards. Closing remarks**

**13:45 pm** **Farewell Cocktail**
**Paranimf Terrace**

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# VII NATIONAL MEETING OF THE SPANISH SOCIETY OF MASS SPECTROMETRY (SEEM2015)

## PL-01
## AMBIENT IONIZATION WITH PLASMAS AND CHARGED DROPLETS

Facundo M. Fernandez[1,2]*, Joel D. Keelor[1], Christina M. Jones[1], José J. Pérez[1(1)], Rachel Bennett [1], Jose Perez [1] , Matthew Bernier [1], Martin R. L. Paine [1], Maria Eugenia Monge [1], David A. Gaul[1,3], Jaeyeon Kim[4,9], Martin M. Matzuk[4,5,6,7,8,9], Long Q. Tran[10], Roman Mezencev[3], John F. McDonald[1], Nael A. McCarty[11], Arlene A. Stecenko[11], William T. Wallace[12], Daniel B. Gazda[12], Henrik I. Christensen[13].

*[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA.*
*[2]Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA 30332, USA.*
*[3]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA.*
*[3]School of Computing, Georgia Institute of Technology, Atlanta, GA 30332, USA.*
*[4]Departments of Pathology & Immunology, [5]Molecular and Cellular Biology, [6]Molecular and Human Genetics, and [7] Pharmacology, and Centers for [8]Drug Discovery and [9]Reproductive Medicine, Baylor College of Medicine, Houston, TX 77030, USA.*
*[10]College of Computing, Georgia Institute of Technology, Atlanta GA 30332, USA.*
*[11]Emory+Children's Center for Cystic Fibrosis Research and Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA 30322, USA.*
*[12] NASA Johnson Space Center, Houston, Texas 77058, United States.*
*[13]Institute for Robotics & Intelligent Machines, Georgia Institute of Technology, Atlanta, GA, 30332, USA.*

**fernandez@gatech.edu, Tel: +1-404-385-4432, Fax: +1-345-894-7452*

The introduction of desorption electrospray ionization mass spectrometry (DESI MS) by Cooks and co-workers in 2004 brought, for the first time, widespread attention to the concept of open air surface analysis under ambient conditions [1]. Contemporary with the disclosure of DESI, work carried in parallel by other research teams explored a similar philosophy in chemical analysis. Examples include the patent on the ion source named Direct Analysis in Real Time (DART) filed in December 2003 [2], Shiea's work on open air laser based ion sources [3], and work by the Van Berkel group at Oak Ridge National Laboratory on surface sampling probes (SSPs) for direct sampling of thin layer chromatography plates first published in 2002 [4], DESI, DART, and other ambient MS techniques enabled an exciting new perspective on ways to perform both qualitative and quantitative chemical investigations on samples not typically amenable to direct MS analysis [5].

As skeptical scientists, we should still ask ourselves what is truly new with respect to ambient MS approaches. Will the excitement about ambient MS withstand the challenge of time? Will we see ambient MS approaches being routinely used in laboratories worldwide 20 years from now?

To help answer these questions, in this presentation, I will review the various ambient ionization approaches based on the desorption and ionization principles involved, and highlight their unique advantages and potential weaknesses. Additionally, I will present what I believe to be unique capabilities of ambient ionization being currently exploited, including multimodal biological tissue imaging, rapid metabolome fingerprinting, rapid detection of counterfeit drugs, and three-dimensional robotic surface sampling.

[1] Z. Takats, J.M. Wiseman, B. Gologan, and R.G. Cooks, *Science*, **306** (2004) 471-473.
[2] R.B. Cody and J.A. Laramee, *Atmospheric Pressure Ion Source*. 2005, JEOL USA: USA.
[3] J. Shiea, M.Z. Huang, H.J. Hsu, C.Y. Lee, C.H. Yuan, I. Beech, and J. Sunner, *Rapid Communications in Mass Spectrometry*, **19** (2005) 3701-4.
[4] G.J. Van Berkel, A.D. Sanchez, and J.M.E. Quirke, *Analytical Chemistry*, **74** (2002) 6216-6223.
[5] M. Domin, R. Cody, and Royal Society of Chemistry (Great Britain), *Ambient ionization mass spectrometry*. New developments in mass spectrometry,. 2015, Cambridge, UK: Royal Society of Chemistry, 508 pages.

## KN-01
## BREATH ANALYSIS: TRANSITIONING FROM BENCH TO BEDSIDE

Pablo Martinez-Lozano Sinues[(1)*], Esther Schwarz[(2)], Robert Dallmann[(3)], Xue Li[(1,4)], Lukas Bregy[(1)], Steven Brown[(3)], Malcolm Kohler[(2)], Renato Zenobi[(1)]

[(1)]*ETH Zurich, Department of Chemistry and Applied Biosciences, Switzerland*
[(2)]*University of Zurich, Institute of Pharmacology and Toxicology, Switzerland*
[(3)]*University Hospital Zurich, Pulmonary Division, Switzerland*
[(4)]*Present affiliation: Jinan University, Guangzhou, China*

*pablo.mlsinues@org.chem.ethz.ch, *Tel: +41 44 633 4838, Fax: +41 44 632 1292*

Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject. However, in contrast to other biofluids (e.g. plasma), the analysis of breath remains far less explored. Here we present some recent examples of how real-time breath analysis may contribute to the fields of disease diagnosis and drug monitoring.

We modified the entrance of a commercial quadrupole time-of-flight mass spectrometer to allow for the real-time analysis of breath via secondary electrospray ionization-mass spectrometry [1]. We have studied i) exhaled metabolic changes produced by obstructive sleep apnea (OSA) in a randomized controlled trial and ii) breath levels of ketamine and its main metabolites in mice injected with ketamine.

In the study aiming to OSA-specific metabolic changes, we found a panel of breath metabolites that were significantly enhanced in breath during the disease development. The figure (a) shows one such example (pentanal). Further identification of the compounds enabled gaining insights into OSA.

In the study aiming to monitor ketamine metabolism [2], we could trace pharmacokinetic curves of ketamine in real-time. The figure (b) shows time-dependent ketamine signal for 4 different doses: 15, 30, 45, and 60 mg/kg. Each dose was injected in a different mice (n=4).

We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant clinical and pharmacological problems, which are currently investigated through the analysis of body fluids other than breath [3].

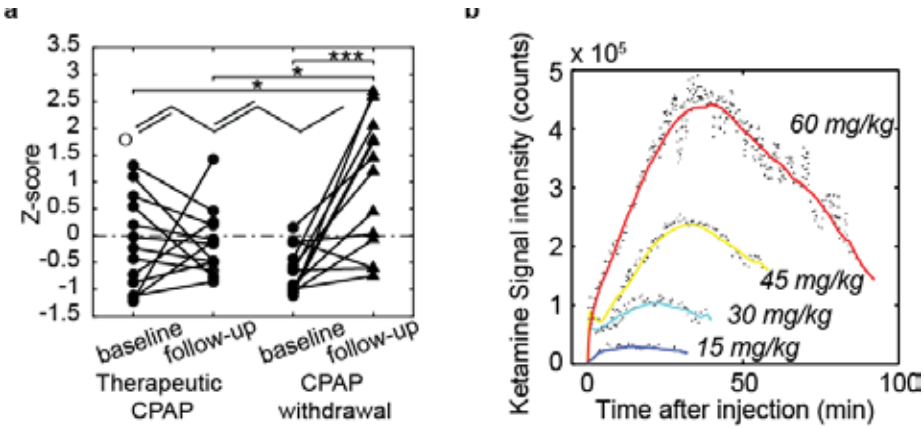


Figure (a) treatment withdrawal (i.e. CPAP) leads to a significant increase in breath pentanal levels (* represents $p \leq 0.05$; $p \leq$ *** 0.001); (b) dose-response curves of ketamine at 4 different injected doses in 4 different mice.

[1] C. Wu, W. F. Siems, H. H. Hill, *Anal. Chem.* **72** (2000) 396-403.
[2] X. Li, P. Martinez-Lozano Sinues, R. Dallmann, L. Bregy, M. Hollmén, S. Proulx, S. A. Brown, M. Detmar, M. Kohler, R. Zenobi, *Angew. Chem. Int. Ed.* (2015), in press.
[3] P. Martinez-Lozano Sinues, R. Zenobi, M. Kohler, *Chest* **144** (2013), , 746-749.

## KN-02
## NEURO-MASS SPECTROMETRY: FROM BASIC RESEARCH TO BED SIDE, AND BACK AGAIN?

Bruno Manadas[1,4], Sandra Anjo[1,2],Liliana Loureiro[1,3], Lúcia Sabála[1,3], Matilde Melo[1], Vera M. Mendes[1], Tiago Faria[1], Pedro Castanheira[4], Rui Brito[1], Mário Grãos[1,4]

[1] *CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal;*
[2] *Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal;*
[3] *University of Aveiro; Aveiro, Portugal*
[4] *Biocant - Biotechnology Innovation Center, Cantanhede, Portugal*

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Parkinson´s disease (PD), the second most common progressive neurodegenerative disorder, is a multifactorial disease caused by both genetic and environmental factors. Among the genes associated with PD, DJ-1 is a multifunctional protein involved in oxidative stress response and neuroprotection. DJ-1 mutations, such as L166P, M26I and E163K lead to loss of protein function causing early onset autosomal recessive PD. Moreover, the residue C106 is considered crucial in DJ-1 function as a sensor of oxidative stress. In this study, two missense mutations (M26I and E163K) and two engineered mutations in the residue C106 (C106A and C106DD) were produced and characterized in order to evaluate the neuroprotective effect of each mutation and also characterize their dynamic interactome.

The dynamic interactome[1] of endogenous DJ-1 revealed over 1119 proteins binding to DJ-1, with a quantitative dynamic determination of 811 proteins. This dynamic interactome was further evaluated in recombinant proteins under oxidative stress conditions: DJ-1 WT and the mutant forms C106DD and C106A. A wide number of binding partners were identified and quantified (AP-SWATH)[1] in the different conditions. These interactors have a broad range of functions but the majority are associated with cellular response to oxidative stress.

Although interactomics studies are becoming common, there is a lack of quality control on the recombinant proteins commonly used in pull down assays, with a potential impact on their structure and therefore on their interactome. In this study, besides thermal shift, size exclusion chromatography and circular dichroism, also comprehensive mass spectrometry approaches were used including bottom-up proteomics (to clearly identify the point mutations), intact protein characterization (LC-MS and CESI-MS) and top-down (LC-MS/MS and CESI-MS/MS).

A combination of secretome analysis[2] using short-GeLC-SWATH[3], comprehensive 2D-LC-MS/MS[4,5] along with targeted approaches for protein characterization and quantification[1,6] are key aspects to turn basic research into useful tools for bed-side science.

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## O-01
## HRMS TOOLS FOR DIRECT ANALYSIS OF RECREATIVE DRUGS AND LEGAL HIGHS

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Drug consumption has been regarded as one of the most important concerns of our society. It has been estimated that nowadays between 16 and 39 million people worldwide are regular drug users or have drug dependence issues [1]. The adulteration of the illegal drugs with other substances, which sometimes are not deemed as safe for human consumption, is known but not considered in most reports regarding drug use. Some of these adulterating agents may represent a higher health risk due to its own toxicity or synergic effects with the main component [2]. Moreover, nowadays new psychoactive substances (Legal Highs) which often contain synthetic drugs such as synthetic cathinones [3] and β-cannabinoids [4] are increasingly consumed. In this context the continuous and quick development of new analogues rapidly outdates most of the available analytical methods for their control. So, there is a need for wide-range screening methods able to identify illicit drugs, adulterants and new psychoactive substances and also able to be easily adapted to the constant introduction of new substances.

In this work the capabilities of a powerful hybrid mass analyzer such as the quadrupole-orbitrap for the direct screening analysis of psychoactive substances have been evaluated. Samples were simply dissolved in a 1:1 mixture of acetonitrile:methanol and injected into the HRMS system by FIA, using ESI as ionization source. MS acquisition in both, positive and negative modes was performed in a Q-Exactive mass spectrometer combining full-scan and data dependent tandem mass spectrometry acquisition (FullMSddMS/HRMS) to obtain high quality information for the identification of the sample components. For target compounds, data processing was performed with ExactFinder v.2.0 software, a custom-made database containing more than 440 compounds was built and the accurate mass and the isotopic pattern of the ions observed in full scan mode and also the main product ions obtained in the product ion scan were used as identification criteria. For unknowns identification, online spectral libraries and/or in-silico fragmenter softwares in combination with on-line chemical databases was used, and confirmation was performed analyzing the respective standards. Examples of the analysis of real samples will be used to illustrate the strategies applied for the identification of their components.

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## O-02
## DETECTION AND MASS SPECTROMETRIC CHARACTERIZATION OF ALTERNATIVE CLOSTEBOL LONGTERM METABOLITES IN HUMAN URINE

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Clostebol, the 4-chloro derivative of the hormone testosterone, is a synthetic anabolic androgenic steroid (AAS) used by sportsmen to increase muscle growth. Clostebol misuse is commonly detected by monitoring different metabolites excreted conjugated with glucuronic acid using gas chromatography mass spectrometry (GC-MS) after hydrolysis with β-glucuronidase and derivatization [1]. Sulfation is an important phase II metabolic pathway and sulfate metabolites of some AAS have been recently described as long-term metabolites [2,3]. The aim of this work was to evaluate the sulfate fraction of clostebol metabolism by liquid chromatography tandem mass spectrometry (LC-MS/MS).

In order to investigate sulfate metabolites of clostebol, samples obtained after administration of this compound were extracted with ethyl acetate and extracts were analysed by LC MS/MS using electrospray ionization in negative mode. Different mass spectrometric methods were applied to detect sulfate metabolites of clostebol: neutral loss scan method of HCl (36 Da and 38 Da, due to isotopic pattern of chlorine); precursor ion scan method of m/z 97 (characteristic product ion of steroid sulfates); and selected reaction monitoring method (SRM) including characteristic ion transitions of potential clostebol metabolites resulting from oxidations, reductions and hydroxylations ($[M-H]^-$→ $[M-H-HCl]^-$, $[M-H]^-$→97, $[M-H-HCl]^-$ →97, $[M+2-H]^-$→ $[M+2-H-HCl]^-$, $[M+2-H]^-$→97). Sixteen sulfate metabolites were detected in post-administration samples.

Potential structures for these metabolites were suggested after solvolysis, mass spectrometric experiments and comparison with GC-MS results obtained from the glucuronide fraction. One of the metabolites, S1, was detected up to 31 days after the administration of a single oral dose of clostebol (10 mg). S1 structure was suggested as 4ξ-chloro-5ξ-androst-3β-ol-17-one 3β-sulfate. The retrospectivity of this sulfate metabolite in the detection of clostebol misuse was compared with that obtained with previously described markers. Metabolite S1 improves the period in which the misuse can be reported with respect to the currently monitored metabolites. Inclusion of its measurement in screening methods will be useful for doping control purposes.

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## O-03
## IDENTIFICATION OF SUBSTANCES MIGRATING FROM PLASTIC BABY BOTTLES USING HIGH RESOLUTION MASS SPECTROMETRIC ANALYZERS COUPLED TO GAS AND LIQUID CHROMATOGRAPHY

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The European Union recently prohibited the production of polycarbonate (PC) baby bottles due to the toxic effects of the PC monomer Bisphenol-A (EU 10/2011). Therefore, baby bottles made of alternative materials, such as polypropylene, polyethersulphone, polyamide, Tritan® or silicone, have appeared on the market. The principal aim of our study was the identification of major organic compounds migrating from these baby bottles.

The use of simulants is prescribed in the EU Regulation 10/2011 to mimic the testing of real foods, leading to the selection of simulant D1 (water:EtOH (50:50)) as a simulant for milk. After sterilization, three consecutive migrations for 2h at 70°C were performed. Afterwards, a non-targeted liquid-liquid extraction with ethyl acetate:n-hexane (1:1) was performed on the simulant samples.

The extracts were first analyzed by GC-MS followed by an untargeted database search using Wiley® and NIST® libraries. Various compounds, such as alkanes, phthalates, amides, fatty acids, antioxidants, etc. were detected. Unidentified peaks were further investigated by GC-(EI)TOF-MS and APGC-QTOF-MS to specifically elucidate the structure of these unknown compounds.

Additionally, extracts were analysed also by LC-QTOF-MS under $MS^E$ mode. Data were processed using a home-made database (around 1200 contaminants) based on EU Regulation 10/2011 and expected migrants, such as anti-oxidants, plasticizers, etc. Several compounds not identified by GC-MS were detected and identified by LC-MS.

## O-04
## THE USE OF ION MOBILITY SEPARATION AND COLLISION CROSS SECTION FOR THE COMPREHENSIVE ANALYSIS OF COMPLEX SAMPLES

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The detection and characterization of low level compounds in complex matrices poses a significant analytical challenge as components can be hidden due to elevated background levels or may be mis-assigned due to co-elution of interferences with similar mass to charge ratio. Ion mobility coupled to liquid chromatography and time of flight mass spectrometry (LC-IM-MS) provides additional resolving power and selectivity to that which can be achieved by improving chromatographic or mass resolution alone. A novel geometry travelling-wave IMS-QTof has been used for the characterization of compounds in complex samples, even in the presence of co-eluting species that were either isomeric (*i.e*. same elemental formula) or isobaric (*i.e.* similar accurate-mass values).

Samples were analyzed using an IMS-QTof operating with electrospray ionization and a non-targeted HDMS$^E$ mode of acquisition where alternating high energy and low energy TOF spectra were generated with ion mobility enabled. When compared to non-mobility enabled acquisition, the resulting files contained cleaner data through spectral clean-up, using mobility-resolved precursor and fragment-ion spectra. This approach allowed the detection and characterisation of low level drug metabolites in rat hepatocytes, even in the presence of chemical noise at orders of magnitude higher.

The use of a travelling-wave ion mobility separation device also allowed the generation of collision cross section (CCS) data based on the relative ion mobility (size and shape) of components in the samples. Precise (≤ 2%) CCS values were automatically generated for all detected components. Unlike chromatographic retention times, CCS values are independent of matrix or chromatographic conditions and provide unique identifiers that reduce the possibility of incorrect assignments [1]. Using these CCS data in combination with m/z and fragment ion information resulted in an increase in the number of relevant components detected and characterized correctly for both drug metabolites in rat plasma and for pesticides screened in a variety of food matrices.

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## O-05
## WHEN THE INTERPRETATION OF MS SPECTRA BECOMES ESSENTIAL

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Interpretation of both full scan and CID spectra of an analyte is often not required for target analysis. However, in some applications such as the identification of metabolites in metabolic studies and the markers identification in metabolomic approaches, a proper interpretation starts to play an essential role. In several cases, a previous knowledge of the expected metabolism (like the one of similar substances) or the occurrence of the marker as such in an available data base are enough for the final elucidation. However, the situation is extremely more difficult when these two aspects are lacking and the interpretation of the MS spectra is the only tool available. In fact, the analytical chemist facing up to the spectra of a completely unknown substance can have a kind of blank page syndrome.

In this presentation, we illustrate how an accurate interpretation of the MS data was critical in the elucidation of some unexpected metabolites and previously unreported markers. Several aspects like the use of accurate mass measurements, the careful interpretation of the data in both ionization modes, the identification of unlikely fragmentation pathways and the use of adduct formations will be discussed.

## O-06
## SYNTHESIS AND CHARACTERIZATION OF MINIMALLY 13C-LABELLED PEPTIDE STANDARDS FOR THE DETERMINATION OF ANGIOTENSIN II IN AMNIOTIC FLUID BY ISOTOPE DILUTION TANDEM MASS SPECTROMETRY

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The development of new reference measurement procedures for the absolute quantification of proteins and peptides is required to ensure the accuracy and comparability of the results under different conditions or biological states. Isotope Dilution Mass Spectrometry (IDMS) provides highly accurate and precise results which are directly traceable to the International System of units (SI). Most of the current IDMS methodologies applied for the absolute quantification of proteins resort to multiply labelled peptides to avoid spectral overlap between natural and labelled analogues. Under these conditions, the ratio of intensities for the natural and labelled peptides is assumed to be equal to the molar ratio. However, this assumption needs to be demonstrated in practice as different isotopic enrichments of the labelled material, potential isotopic effects or spectral interferences can be significant sources of error.

We propose a new IDMS strategy based on the use of minimally labelled peptides and Selected Reaction monitoring (SRM) [1]. In contrast to common approaches resolution of the first quadrupole is reduced to transmit the whole parent ion cluster to the collision cell for monitoring accurate isotopic distributions of the molecular fragments. In this way, molar fractions of labelled and natural abundance peptides are directly obtained from the experimental mass spectra of the in-cell fragment ions and the concentration of the endogenous peptide can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs. This strategy is applied to the development of an accurate and precise analytical method for the SI traceable determination of angiotensin II (ang II), a peptide hormone involved in blood pressure regulation, obtained from amniotic fluid from Wistar rats using an in-house synthesized minimally labelled $^{13}C_1$-ang II, previously characterized by amino acid analysis.

[1] Spanish Patent ES2472724. Priority date 21/03/2014.

## O-07
## UPLC-HRMS for the detection and identification of pharmaceuticals metabolites

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The presence of pharmaceuticals residues in the aquatic environment has caused concerns about potential adverse effects on exposed wildlife. Many of the pharmaceuticals are considered as being biological active compounds capable to act as endocrine disruptors. Several studies have been reported the occurrence of pharmaceuticals in different organisms, such as fish, crustaceans or plants, but still very little is known about their metabolism. Furthermore, aquatic organisms are capable of metabolizing pharmaceuticals taken up from polluted waters.

Nowadays, due to recent improvements in the instrumental analysis, the high sensitivity of accurate mass measurements by ultra-performance liquid chromatography coupled to high resolution mass spectrometry (UPLC-HRMS) allow the identification of pharmaceutical metabolites and the development of screening methods allowing the detection of large number of pollutants without prior selection of compounds.

In this context, preliminary studies were focused on the identification of metabolites of two of the most commonly detected drugs, carbamazepine and ibuprofen, on different organisms at laboratory conditions by UPLC-HRMS. With this approach several phase I metabolites, corresponding to different hydroxylation reactions, and phase II metabolites such as glucuronide, hexoside and taurine conjugates were tentatively identified. Different metabolic pathways were observed for the different organisms. Identified metabolites were used to predict different metabolites of other pharmaceuticals. For suspect screening of pharmaceuticals and their predicted metabolites in environmental samples, elemental compositions and structures were used to create a database of exact ion masses for the expected protonated or deprotonated molecules.

Finally, a sampling campaign was designed to collect different fish from the Llobregat river, a high polluted river basin from Spain. Bile and muscle samples were analyzed using different analytical methodologies. The HRMS analysis of pharmaceuticals and their metabolites was carried out on the Q Exactive (Orbitrap)-MS system and on the Q-TOF system, bout with MS/MS capability. The analysis of fish bile has allowed the detection of predicted phase I and phase II metabolites. The suspect analysis of fish muscle allowed the detection of more than ten different pharmaceuticals. Their identities were proposed by matching their accurate MS and MS/MS data against different libraries and, if available, reference standards were used to obtain the first level of confirmation of the compound identity. Thus this approach notes that UPLC–HRMS is a powerful tool for simultaneous quantitative and qualitative analysis, allowing the search for suspected metabolites, their identification and the quantitation of target compounds.

## O-08
## GAS CHROMATOGRAPHY ORBITRAP MASS SPECTROMETRY: A NOVEL ANALYTICAL TOOL TO HELP EMERGE IODINATED DISINFECTION BYPRODUCTS IN CHLORINATED AND CHLORAMINATED WATERS

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Iodinated disinfection byproducts (DBPs) formed during disinfection of waters containing iodide or iodine sources may pose a health risk for the population exposed to them. *In vitro* studies have shown for some chemical classes, e.g., haloacids and haloacetamides, that iodinated compounds are more genotoxic and cytotoxic than the corresponding brominated and chlorinated analogues [1,2]. However, iodinated DBPs identified to date do not account for the total iodinated organic fraction formed during disinfection treatments. Thus, complete characterization of this DBP class is crucial to further investigate its occurrence in disinfected waters and potential toxicity effects. In this context, the main objective of this study was to detect and characterize the iodinated DBPs formed during chlorination and chloramination of source waters with different iodide and bromide levels.

The identification of emerging iodinated DBPs in water is a challenging task due to the complexity of this matrix and their often low concentrations. Moreover, analytical techniques with high resolving power, high mass accuracy, and sensitivity like those based in Orbitrap mass spectrometry technology are also required. This technology has now been coupled to gas chromatography (GC) in the Q Exactive™ GC hybrid quadrupole Orbitrap mass spectrometer system, and therefore, this novel configuration opens up new possibilities for GC-amenable compounds such as volatile and semivolatile DBPs. For iodinated DBP discovery, DBP mixture extracts were generated from lab-scale chlorination and chloramination reactions and analysed in the full scan mode using gas chromatography (GC) electron ionization high-resolution accurate mass Orbitrap mass spectrometry with a Q Exactive™ GC system. The workflow applied allowed compound identity confirmation based on accurate mass measurements and fragment rationalization, and proved that this novel technology in combination with the powerful Mass Frontier™ software is a very useful tool to help emerge iodinated DBPs in disinfected waters.

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## O-09
## SUBMICROMETRIC CHARACTERIZATION OF SPATIALLY-STRUCTURED MATERIALS BY FEMTOSECOND IONIZATION AND TIME-OF-FLIGHT MASS SPECTROMETRY IN ON-AXIS CONFIGURATION

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Advanced technologial areas are demanding manufacturing techniques capable of reducing the spatial dimensión of the devices in lateral and depth scales. Such reality pushes the analytical techniques to keep up with such challenge to provide the industry with the required answers. In this sense, depth-profile analysis represents a required tool to define precisely the layer-by-layer composition of specific materials for quality control, R&D or critical point analysis.

Some of the requirements demanded to theanalytical techniques include the non-destructiveness, speed, robustness or accuracy. Laser ionization mass spectrometry (LIMS) is one of the promising techniques to perform direct analysis on solids with little or none destruction on the sample. Coupled to time-of-flight analyzers, LIMS allows the simultaneous monitoring of atomic and molecular species. When ultrashort pulses are used, the minimum heat-affected zone in the laser-matter interaction process allows a much better resolution of the interfaces in comparison to that commonly obtained with nanosecond pulses.

The present communication will show applications in the characterizatin of layered materials by using a combination of 35-fs width laser pulses at 800 nm with an all-reflective pierced Cassegrain focusing optics that acts as focusing element and electrostatic lens. In such geometry, the excitation and collection axis are collineal, improving the obtention of full mass-range spectra from craters as small as 3 microns.

# EU 252/2012 Y EU 278/2012

# ANÁLISIS REGULADO DE DIOXINAS EN ALIMENTOS Y PIENSOS

The Measure of Confidence

## Sistema de Cuadrupolo Triple para GC/MS

Las últimas modificaciones de la **Regulación de la UE** para el análisis de dioxinas en Alimentos y Piensos, establece el uso de cromatografía de gases/espectrometría de masas en tandem (GC-MS/MS) como un método apropiado y regulado de comprobación de los niveles máximos permitidos de estos compuestos.

El sistema Agilent 7010 de Cuadrupolo Triple para GC/MS ofrece una detección lineal, reproducible y sensible para el análisis de dioxinas (PCDDs), furanos (PCDFs) y PCBs, cumpliendo los requisitos de la normativa de la Unión Europea para alimentos y piensos para animales, siendo la opción más fiable para este análisis.

*"It is the time to revise the current strategy by using only confirmatory tools to monitor the food-feed web for PCDD/Fs and selected PCBs"*

*Jean-François Focant*
*University of Liège*


**El analizador de Agilent para la detección de dioxinas en alimentos y piensos incorpora las últimas innovaciones en materia de GC y MS/MS y es el fiel reflejo de la importancia que otorgamos a la calidad y las prestaciones. Este analizador incluye todo lo que se indica a continuación:**

**Proceso de fabricación**

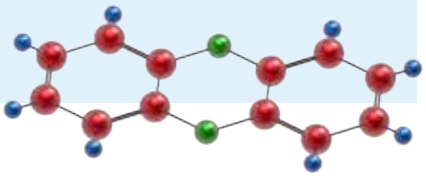
- Configuración y pruebas de fugas del sistema.
- Comprobación del instrumento.
- Instalación de una columna GC DB-5ms UI.
- Método de comprobación en fábrica en el que se utiliza una mezcla patrón específica para el analizador de dioxinas.

**Equipo suministrado**

- Manual del usuario con instrucciones para llevar a cabo el método.
- Software complementario para automatizar la elaboración de informes.
- CD-ROM con archivos de parámetros del método y datos de comprobación para poder comenzar a trabajar con el analizador nada más recibirlo.
- Incluye consumibles para la aplicación en cuestión (no requiere pedirlos por separado).
- Información que facilita la realización de pedidos de reposición de consumibles.

**Instalación**

- Duplicación de la comprobación en fábrica con una muestra de comprobación (in situ, con la ayuda de un ingeniero de soporte formado en fábrica).
- Opción de contratar servicios de consultoría de aplicaciones.




Octubre 2015

Agilent Technologies

## P-01
## A NOVEL NONCOVALENT POLYMERIC COATING FOR FAST AND EFFICIENT ANALYSIS OF PEPTIDES, BASIC PROTEINS AND ANIONIC METABOLITES PROFILING BY CAPILLARY ELECTROPHORESIS AND CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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In this work, the suitability of a new group of polymers as coatings in capillary electrophoresis (CE) has been investigated for the analysis of peptides, basic proteins, and anionic metabolites profiling by CE-UV and CE-MS. This polymer family has been designed to modify the EOF and to minimize or completely prevent peptide/protein-capillary wall interactions. Four different polymers have been prepared and evaluated: a homopolymer that comprises only cationizable repetitive units of TEDETAMA, and three copolymers that randomly incorporate TEDETAMA together with neutral hydrosoluble units of N-(2-hydroxypropyl) methacrylamide (HPMA) at different molar percentages (25:75, 50:50 and 75:25). It is demonstrated that the composition of the copolymers influenced the EOF and, therefore, the CE separation. Among the novel polymers studied, poly-(TEDETAMA-co-HPMA) 50:50 copolymer was successfully applied as coating material of the inner capillary surface in CE-UV and CE-MS. It exhibited durability and good chemical stability without generating ESI source contamination. Fast and efficient baseline separation of peptides and basic proteins could be achieved with this co-polymeric coating, as well as the profiling of anionic metabolites with very good reproducibility. The feasibility of the CE-MS method was corroborated by the analysis of lysozyme (a highly basic protein) in cheese, and by obtaining the profiling of anionic metabolites of orange juice and wine samples.

## P-02
## ANALYSIS OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) AND ITS BROMINATED ANALOGUES IN CHLORINE-TREATED WATER BY GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY (GC-QQQ-MS/MS)

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A simple, selective and sensitive method for the analysis of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and its brominated analogues (BMXs) in chlorine-treated water has been developed. The method is based on gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS), previous liquid-liquid extraction (LLE) of a smaller sample volume compared to other methods [1-3] and on-line derivatization with a silylation reactive [4,5]. GC-QqQ-MS/MS has been raised as an alternative easier to perform than gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) [6,7] for the analysis of MX and BMXs, and it allows to achieve low LODs (0.3 ng/L for MX and 0.4-0.9 ng/L for BMXs). This technique had not been previously described for the analysis of MX and BMXs.

Quality parameters were calculated and real samples related to 3 drinking water treatment plants (DWTPs), tap water and both untreated and chlorinated groundwater were analyzed. Concentrations of 0.3-6.6 ng/L for MX and 1.0-7.3 ng/L for BMXs were detected. Results were discussed according to five of the main factors affecting MX and BMXs formation in chlorine-treated water (organic precursors, influence of bromide ions, evolution of MX and BMXs in the drinking water distribution system, groundwater chlorination and infiltration of water coming from chlorination processes in groundwater).

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## P-03
## IMPLEMENTATION OF DIELECTRIC BARRIER DISCHARGE IONIZATION (DBDI) SOURCE WITH LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY INSTRUMENTATION FROM DIFFERENT MANUFACTURERS

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Liquid chromatography-mass spectrometry (LC-MS) with electrospray ionization (ESI) is generally used for the analysis of relatively polar compounds. However, the analysis of nonpolar species is commonly undertaken by GC-MS, as they are not effectively ionized in the ESI source. With the aim of extending the applicability of LC-MS coupling towards a wider range of compounds with different physicochemical properties, alternative ionization sources have been proposed. The main alternative to ESI is so far atmospheric pressure chemical ionization (APCI) source. Another alternative that has been proposed is atmospheric pressure photoionization (APPI). Besides, several mass spectrometry vendors have proposed hybrid ionization techniques (*eg.* ESI/APCI). Recently, an ion source for LC-MS based on dielectric barrier discharge principle was reported by Hayen *et al*. [1]. Due to the different species generated in the plasma jet, the DBDI source offers the ability to generate not only positive but also negative ions, as various mechanisms including electron capture and proton transfer apply at the same time. The eventual combination of this ionization source coupled to a mass spectrometer featuring fast polarity switching (i.e. > 5-10 Hz) may provide a universal method covering a vast range of compounds with different physicochemical properties [2]. In this communication, we have coupled this dielectric barrier discharge plasma jet with different LC-MS instruments from three manufacturers featuring atmospheric pressure interfaces with completely different geometry and conditions: AB Sciex (TurboIonSpray™, QTRAP 4000) Agilent Technologies (orthogonal APCI source, Agilent TOF 6220) and Thermo Fisher Scientific (IonMax™, Exactive Orbitrap). Selected examples on the application of the HPLC-DBDI-MS coupling are shown including the determination of multiclass lipids, trace analysis of contaminants of emerging concern such as pharmaceuticals in wastewater and pesticide determination in foodstuffs.

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## P-04
## POTENTIAL OF GC-APCI-MS BASED STRATEGIES FOR METABOLIC STUDIES

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Metabolic studies are crucial in several application fields like environmental analyis, drug development or doping control analysis. One of the key aspects for the structural elucidation of the metabolites is the establishment of their molecular weight. For this reason, soft LC-ESI-MS strategies are preferred against common GC-EI-MS approaches which exhibit high fragmentation making the determination of the molecular weight difficult. Recently, an APCI interface which promotes soft ionization has been marketed. This interface generates mainly $[M+H]^+$ or $M^{+\cdot}$ ions as the base peak of the spectrum.

In this study, the potential of GC-APCI-MS based strategies for the untargeted detection of metabolites has been evaluated. The steroid 4-chloromethandienone (4-ClMTD) has been selected as a model compound. The presence of a chlorine atom in the 4-ClMTD structure was an additional help in the assignation of metabolites due to its characteristic isotopic pattern. Urine samples collected before and 8 hours after 4-ClMTD administration were hydrolyzed with β-glucuronidase. After a liquid-liquid extraction, the extract was derivatized in order to obtain the trimethylsilyl derivatives. The chromatograms obtained for both urines were compared in order to detect the potential metabolites.

Three different strategies have been evaluated: (i) a precursor ion scan of the ions at m/z 77, 91 and 105 coming from the steroid skeleton using a triple quadrupole analyzer, (ii) a full scan approach using a QTOF analyzer and (iii) a common fragment strategy using the $MS^E$ chromatogram in a QTOF analyzer.

The suitability of each strategy for metabolic studies has been evaluated by several aspects such as the capability to detect reported 4-ClMTD metabolites, the number of metabolites detected for each approach or the number of compounds detected which cannot be associated with 4-ClMTD metabolites.

## P-05
## DETECTION AND CONFIRMATION OF VERY LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN GILTHEAD SEA BREAM (Sparus aurata L.) RETINA EXTRACTS BY GAS CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION

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Long-chain and very long-chain polyunsaturated fatty acids (LC-PUFAs, $C_{12-22}$; VLC-PUFAs, $C_{24-38}$), play an important role in retinal, neural and reproductive functions [1,2]. Some studies have stablished a relationship between the dietary intake of LC-PUFA and the prevalence of retinal diseases in humans [3], being this closely related to the amount of retinal (V)LC-PUFA. However, despite being fish the main source of LC-PUFA in human diets, epidemiology studies in this matrix have been scarcely carried out. Thus, the study of the effects of a dietary shortage of LC-PUFA on their subsequent concentration in fish has been recently of great interest in the aquaculture field from a sustainability and profitability point of view.

Gas chromatography coupled to mass spectrometry (GC-MS) with election ionization (EI) has been widely applied to the study of (V)LC-PUFA (previous derivatization). The extensive fragmentation of these compounds in EI source involves the partial or total loss of molecular ions ($M^{\bullet+}$) in the EI spectrum. This, together with the fact that fatty acids (FA) with the same unsaturation degree and position show common fragment ions and very similar (or same) EI spectra hinder the identification process. For this reason, atmospheric pressure chemical ionization (APCI) recently implemented in GC-MS instruments and characterized by its soft ionization, has resulted a useful alternative to EI.

In the present work, an APCI source coupled to a quadrupole time-of-flight analyzer (QTOF) has been tested to study the ionization and fragmentation behavior of ten LC-FA methyl esters (LC-FAMEs). The protonated molecule ($[M+H]^+$) became the base peak of the spectrum for all the studied compounds promoted, furthermore, by the addition of a 1% formic acid solution as a modifier in the source. In addition, specific fragment ions related with the FAMEs unsaturation degree and position have been identified. The application of GC-APCI-(Q)TOF MS for the screening of gilthead sea bream retina extracts made possible the tentative identification of several VLC-PUFA. The reliable identification of these compounds was supported by accurate mass measurements of $[M+H]^+$ together with the isotopic pattern of $^{13}C$ and the presence of two representative *m/z* fragment ions in the spectrum.

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## P-06
## COMPARISON OF CCS (N2) MEASUREMENTS OBTAINED FROM TWO DIFFERENT T-WAVE ION MOBILITY SYSTEMS WITH DIRECT MEASUREMENTS USING A DRIFT TUBE ION MOBILITY SYSTEM

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With recent developments in ion mobility (IM) separation instrumentation, interest has increased in the determination of collisional cross-sections (CCS) of various classes of compounds. These CCS measurements can be used to augment screening of complex samples both by reducing interfering effects of matrix ions and as an additional identification criterion [1]. Additionally they assist in structural confirmation of isoforms of protein complexes [2] and structural isomers of small molecules. The widely used T-Wave IM system relies on calibration to provide CCS values as there is no direct analytical solution for the complex motion of ions through the device. Here the efficacy of T-wave CCS calibration is investigated by comparison of CCS values obtained from a linear-field drift tube IM system, theoretical calculations and available literature values.

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## P-07
## NEW METHOD FOR THE DETERMINATION OF PARABENS AND BISPHENOL A IN HUMAN MILK SAMPLES USING ULTRASOUND-ASSISTED EXTRACTION AND CLEAN-UP WITH DISPERSIVE SORBENTS PRIOR TO UHPLC–MS/MS ANALYSIS

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Early life is a highly vulnerable period of development and disturbances in the developmental process. Abnormalities in this life stage can result in persisting structural and functional changes that can manifest later in life in the form of impairment of cognitive function, chronic diseases, pubertal development or adult obesity [1,2]. Assessment of children exposure to the many environmental contaminants is of special interest. Over the last decades, industry has grown steadily to meet the needs of today's society. This has resulted in an increase of the number of chemical compounds used to improve the standards of living. Is particularly important the case of endocrine disrupting chemicals (EDCs). The group of compounds covers a wide range of chemical substances, able to alter the normal hormone function of wildlife and humans, consequently causing adverse health effects. Bisphenol A (BPA) and parabens (PBs), belong to this group of compounds [3, 4]

A sensitive and accurate analytical method for the determination of methyl-, ethyl-, propyl- and butylparaben and bisphenol A in human milk samples has been developed and validated. The combination of ultrasound-assisted extraction (UAE) with acetonitrile and a simplified and rapid clean-up step with a C18 sorbent has been successfully applied for the preparation of samples prior to ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) analysis. Negative electrospray ionization (ESI) in the selected reaction monitoring (SRM) mode was used for MS detection. Deuterium-labelled ethylparaben-$d_5$ (EPB-$d_5$) and deuterium-labelled bisphenol A-$d_{16}$ (BPA-$d_{16}$) were used as surrogates. The limits of quantification ranged from 0.4 to 0.7 ng $mL^{-1}$, while inter- and intra-day variability was under 11.1% in all cases. In the absence of certified reference materials, recovery assays with spiked samples using matrix-matched calibration were used to validate the method. Recovery rates ranged from 93.8% to 112.2%. The proposed method was satisfactorily applied for the determination of five EDCs in human milk samples obtained from nursing mothers living in the province of Granada (Spain).

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## P-08
## DETERMINATION OF CANNABINOIDS IN HEMP SEEDS, COW LIVER AND COW MILK BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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The whole hemp plant would be, due to its high fibre content, a suitable feed material for ruminants, and daily amounts of 0.5 to 1.5 kg whole hemp plant dry matter could likely be incorporated in the daily ration of dairy cows. Hemp seeds have a low content of tetrahydrocannabinol (THC), mainly found on the outside of the seeds, as result from physical contamination by the plant leaves. Based on a very limited number of studies performed in farm animals, it may be assumed that both the parent compound and its metabolites are distributed in the different tissues and organs, and excreted by milk [1]. The aim of the present study is to develop a simple, fast and reliable method for the determination of THC and its main metabolites 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and 11-Hydroxy-delta-9-tetrahydrocannabinol (THC-OH) in three different matrices (seeds of hemp plant, three brands of cow milk and cow liver).

Several parameters of the method to determinate THC, THC-COOH and THC-OH in seeds of hemp plant, three brands of cow milk and cow liver, were optimized including sample size. Regarding the solid-phase extraction (SPE), 7 cartridges were tested (Thermo scientific C18 200mg/3mL, Thermo scientific C8 200mg/3mL, Supelco HLB SPE 60mg/3mL, Supelco SPE, 500mg/3mL, Phenomenex Strata-X 500mg/6mL, Phenomenex Strata-X 200mg/6mL and Oasis HLB VAC RC 60mg/20mL) and 6 different elution volumes (2, 4, 6, 8 and 10mL) were tested. Extracts were reconstituted to 1 mL of methanol. THC, THC-COOH and THC-OH were extracted by SPE using Oasis HLB VAC RC 60mg/20mL (Waters, UK). Samples were trapped through the cartridges under vacuum at a flow rate of 10 mL $min^{-1}$. Analytes were eluted with 2 mL of methanol. Chromatographic separation was performed using an Agilent 1260 UHPLC. The separation was carried out with a Phenomenex Kinetex C18 at constant flow rate of 0.2 mL $min^{-1}$. The mobile phase was eluent A (formic acid 0.1% in water) and eluent B (formic acid 0.1% in methanol) in gradient. The UHPLC was coupled to an Agilent 6410 triple quadrupole mass spectrometer with an electrospray ionization source working in positive mode (ES+).

In the present study, the developed methodology was applied to determinate THC, THC-COOH and OH-THC in seeds of hemp plant and cow liver and milk. The results show the presence of THC in hemp seeds and THC-COOH was detected in the three brands of milk and in the cow liver at low concentrations.

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## P-09
## QuEChERS-BASED METHOD FOR DETERMINATION OF CARBAMATES IN AROMATIC HERBS BY UHPLC-MS/MS

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Aromatic herbs are frequently used to impart flavor and aroma to foods with, sometimes the addition of color. Their high consumption requires intensive supply from agriculture which, to protect the crops and increase the yield, uses pesticides, including carbamates (CRBs).In this work a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) were used to determine 28 CRBs in different edible fresh aromatic herbs namely: parsley, basil, spearmint, thyme, cilantro, celery and salvia from Cameroon and Spain.The separation was achieved in less than 6 min, using a Zorbax Eclipse plus RRHD $C_{18}$ column (50 mm × 2.1 mm, 1.8 µm), with a mobile phase of water and methanol, both of them with 0.01% formic acid. The analytes were detected in ESI+ with multiple reactions monitoring mode; fragmentation conditions were optimized in order to obtain the highest sensitivity. QuEChERS methodology was chosen as sample treatment. The best results in terms of recovery and matrix effect were obtained using $C_{18}$ as sorbent for dispersive solid phase extraction. Full validation was performed with parsley samples free of CRBs, while recovery studies were performed in other samples. Limits of quantification ranged from 0.04–3.39 µg $kg^{-1}$, below maximum residue limits (MRL) established for these samples. With the exception of asulam in basil, and propamocarb in thyme, the method allowed recoveries between 60 and 125%, with relative standard deviations lower than 15%. Some CRB residues were found in the analyzed samples, such as carbemedazim-benomyl in celery (23.15 ± 2.11 µg $kg^{-1}$) and parsley (12.84 ± 0.26 µg $kg^{-1}$) from Cameroon; pirimicarb and pirimicarb desmethyl in spearmint (53.35 ± 0.58 µg $kg^{-1}$ and 12.09 ± 0.31 µg $kg^{-1}$, respectively), wild mint (16.92 ± 0.74 µg $kg^{-1}$ and 3.42 ± 0.09 µg $kg^{-1}$, respectively) and cilantro (836.43 ± 14.46 µg $kg^{-1}$ and 328.96 ± 4.55 µg $kg^{-1}$, respectively), all from Spain. However, all of them shown concentrations of CRBs below the MRLs. The proposed method combines the advantages of QuEChERS methodology (such as simplicity and effectiveness for cleaning-up complex samples) and the high sensitivity, selectivity, short analysis time and identification capability of UHPLC-MS/MS, showing its usefulness for the simultaneous monitoring of these residues in a wide range of edible fresh herbal products, which are scarcely explored matrices.

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## P-10
## VALIDATION OF A METHOD BASED ON SALTING OUT ASSISTED LIQUID-LIQUID EXTRACTION AND UHPLC-MS/MS FOR THE DETERMINATION OF BETA-LACTAM ANTIBIOTICS IN INFANT DAIRY PRODUCTS

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β-lactam (BL) antibiotics are one of the most widely applied groups of antimicrobial drugs in current veterinary. Their residues in foods derived from animals can cause some adverse human health effects, such as allergic reactions and bacterial resistance. The European Union (EU) has established maximum residue limits (MRLs) for the presence of BLs in foodstuff of animal origin such as milk and meat but no specific regulation is established for baby and infant foods.

In this work a new multiresidue method has been developed for the determination of 15 BLs in infant milk and yogurt by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The separation was achieved in 6 min, using a Kinetex Biphenyl Core-Shell column (50 mm x 2.1 mm 1.7 µm), with a mobile phase of water with 0.05% acetic acid and methanol. This new column allows better resolution, sharper peaks, and greater sensitivity than traditional C18 columns, in addition to its 100 % aqueous stability and the enhanced polar basic selectivity. The analytes were detected in ESI+ with multiple reaction monitoring mode and fragmentation conditions were optimized to obtain the highest sensitivity. Moreover, ion-pair salting-out assisted liquid/liquid extraction (IP-SALLE) has been optimized for the satisfactory extraction of these compounds. In this methodology, 2 ml of $K_2HPO_4$ buffer (pH 8) and 1066 µl of ion pair agent hexadecyltrimethylammonium bromide (CTAB) was added to 1 g of sample in order to form the ion-pair. After that, 4 ml of ACO/ACN (75:25) was added and stirred in vortex during 2 min. Then, the mixture solution was centrifuged during 5 min. Ammonium sulphate (1.315 g) was added, stirred in vortex during 2 min and centrifuge at the same conditions. Finally, the upper phase was evaporated under nitrogen stream current, reconstituted in 1 ml of water and the extract was filtered and injected into the UHPLC system. Under optimum conditions, recoveries for fortified samples ranged from 79 to 93 %, (RSD(%)< 7.5). The limits of quantification were lower than 9.0 µg/kg, showing the high sensitivity and applicability of this fast and simple method. The results in terms of analysis time, sensitivity and accuracy showed the suitability of this procedure for the monitoring of BL residues in different infant foods.

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## P-11
## IN VIVO ANTITUMORAL ACTIVITY OF A ROSEMARY EXTRACT-RICH DIET IN MICE: PROTEOMICS EVALUATION USING DIMETHYL-LABELING AND NANO-LIQUID CHROMATOGRAPHY-ORBITRAP MS/MS ANALYSIS

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*In vitro* studies have already demonstrated the anti-proliferative effect of rosemary extracts against HT-29 human colon cancer cells [1]. In the present work, we present a step forward to corroborate this antiproliferative bioactivity using an *in vivo* model. To do this, a proteomics approach employing dimethyl-labeling and a nanoLC-orbitrap MS/MS has been carried out to evaluate the molecular mechanisms underlying the antitumoral activity of a rosemary extract-rich diet in xenograft mice. To attain this goal, xenograft mice were daily fed with a diet containing 200 mg/kg of a rosemary extract for two weeks before the inoculation of the human adenocarcinoma HT-29 cells, and the treatment was extended for 5 weeks more. After that period, tumors obtained from mice were weighted and proteins were extracted as previously reported [2]. Equal amounts of proteins from treated and control groups were digested in-solution with trypsin, and then labeled using different isotope dimethyl reagents. Five samples were prepared by mixing two labeled samples (treated and control) with a pool of all the samples as a normalizer. These mixtures were cleaned up using C18 SPE columns and redissolved in 0.1% formic acid prior to nanoLC−Orbitrap MS/MS analysis. The raw data generated by this technique were processed by MaxQuant and Perseus [3], two bioinformatic open-access tools, and the list of differentially expressed proteins were used for a posterior pathway analysis. The results obtained indicated that the treatment with rosemary extract reduced the volume of the tumor significantly (30%), without affecting the body weight of mice. The application of the proposed proteomic approach provides the relative quantification of 417 proteins in tumor samples, and the detection of a set of 30 statistically significant altered proteins in the tumors from mice fed with rosemary extract with respect to controls. Moreover, the pathway analysis of the differentially expressed proteins suggested that translation pathway was significantly altered in tumors from mice fed with rosemary extract rich-diet, which might be related with the reduction of the tumor volume.

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## P-12
## TASTE MADE OBJECTIVE: DETERMINING VOLATILE COMPOUNDS WITH A SIGNIFICANT EFFECT ON SENSORIAL TRAITS OF POTATOES BY TD-GC-MS

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Potato is the third most important food crop in the world after rice and wheat in terms of human consumption [1]. The flavor of a potato comes from a combination of taste, aroma and texture. Many of the taste and aroma compounds come from Maillard reactions and breakdown processes (of lipids and sugars) when being prepared [2]. HZPC's Pommonde Project has the goal to create a model capable of predicting (cooked) potato characteristics based on the chemicals present and produced by the potato. For this model, chemical data needs to be related to characteristics rated by a trained sensorial panel. The chemical data mostly comes from the analysis of volatiles, released from boiled potatoes, by a GC-MS. Research exist on identifying these aroma compounds in potatoes and relating them to certain characteristics [1-4]. But there is, as far as is known to HZPC, no model created with this data to predict potato (taste) characteristics.

For this project, a dataset of 104 varieties (in duplicate) to were characterized by a trained sensorial panel based on 47 different traits and by GC-MS in an untargeted way, simultaneously. The chromatographic data were pre-processed in order to get the majority of the peaks present in the samples. Afterwards, Multivariate ANOVA was applied to remove outliers and, finally, GC-MS and sensorial data were analyzed by Partial Least Square (PLS) Regression.

The results showed that all of the studied traits can be predicted by a PLS model using less than 200 variables (m/z fragments) per trait while maintaining $R^2$ higher than 0.7 (70% fit). For all traits this comes to a total of 2418 fragments. Form the significant fragments, up to 280 compounds, which influence sensorial traits, were elucidated. This results fulfill the expectation and indicates that the approach taken is robust (based on the PLS cross-validation data). Furthermore, marker compounds were compared with the literature to confirm their validity.

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## P-13
## EVALUATION OF THE ANTIMALARIC BEHAVIOUR OF INHIBITORS OF C40-CBP PATHWAY IN INFECTED ERYTROCYTES BY UHPLC-QTOF MS METABOLOMICS

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Nowadays the power of analytical instrumentation together with new data processing treatments have rendered into new research approaches such as metabolomics. The great versatility of this methodology linked to the high universality, sensitivity and high m/z range of HRMS have directly risen up MS-based metabolomics in many application. In health area, metabolomics have been used to highlight biomarkers from diseased organisms faced to control ones but also to determine the metabolic pathways altered by drug administration.
Malaria is a major global threat responsible for million cases worldwide caused by the parasite *Plasmodium*. Malaria symptoms and complications are primarily associated with a systemic infection of erythrocytes. Therefore, this stage is the main focus for most of antimalarial therapies. Heme polymerization has been by far the most explored parasitic pathway for the research of antiplasmodial drugs. However, parasite resistance towards these drugs is increasing claiming to the research of novel Plasmodium-specific metabolic routes. In this scenario, inhibitors selectively targeting the plant-like apicoplast organelle of the parasite have received special attention. Several essential pathways strongly depend on this organelle being some of them probably related with the carotenoid biosynthtesis (C40-CBP pathway). Although this route is poorly understood in *Plasmodium*, several inhibitors of C40-CBP could be explored as alternative and parasite-specific antimalarials. Metabolomic approaches can reveal the metabolites recognized by such drugs as well as carotenoid downstream functions in *Plasmodium.*
In this study, *Plasmodium* cultures were washed several times and cultured in the presence of C40-CBP inhibitors for 24 h. C40-CBP1 and C40-CBP2 inhibitors were assayed separately and in combination with a control culture without infection. Samples were lyophilized and after reconstitution divided in two aliquots. One aliquot was evaporated and reconstituted in acetonitrile:water for its analysis by HILIC chromatographic separation. The other one was directly injected in a reverse phase column. All samples were analyzed by UHPLC-QTOF MS using both positive and negative ionization modes.
Data generated was extracted with XCMS (R free package), aligned, normalized and multivariate analysis was carried out. The most variable features from infected samples (with fold changes up to 10 relative to the control) were selected in order to obtain the most significant biomarkers. These changes (more than 700 different ions) were compared with samples treated with C40-CBP inhibitors, to identify the metabolites affected by these treatments. Elucidation of these markers will be carried out to understand the effects caused by antimalaric drugs to *Plasmodium.*

## P-14
## DETERMINATION OF CYSTATIN C IN HUMAN URINE BY ISOTOPE DILUTION MASS SPECTROMETRY USING MINIMALLY LABELLED PAPETIDES AND LC-MS/MS

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Cystatin C is a cationic non-glycosylated low molecular weight protein of 13.3 kDa that can be used for determination of the Glomerural Filtration Rate (GFR) which is employed for the diagnosis of renal diseases. Traditionally serum creatinine has been employed to calculate GFR however Cystatin C offers several advantages over creatinine such as its lower variability in serum and its independence on age, sex and muscle mass. An additional advantage is that urinary Cystatin C concentration increases by a factor of 200 when the patients have the first signs of kidney failure whereas only a 2 fold increase is expected in serum. This is particularly interesting because urinary Cystatin C can be employed for the early diagnosis of renal diseases. The main disadvantage is its very low secretion in urine as it is almost entirely absorbed by the proximal tubule. For this reason Cystain C level in urine is in the $\mu g\ L^{-1}$ range and thus, the development of rapid and sensitive methods for Cystatin C determination in human urine is challenging.

The absolute quantification of proteins by Isotope Dilution Mass Spectrometry usually resorts to multiply labelled peptides assuming that the ratio of intensities for the natural and labelled peptides is equal to the molar ratio. However, this assumption needs to be demonstrated in practice as different isotopic enrichments of the labelled material, isotopic effects or spectral interferences can lead to significant errors. We propose here an approach based on the use of Mass Overlapping Peptides (MOPs) minimally labelled in $^{13}C$ and the application of a Selected reaction monitoring (SRM) mode in which the resolution of the first quadrupole is reduced to the extent that the whole parent ion cluster is transmitted to the collision cell [1,2]. In this way, accurate isotopic distributions of the molecular fragments can be monitored and the concentration of the natural abundance protein can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs. This strategy is applied here to develop a method capable of quantifying Cystatin C in human urine in patients with normal and impaired renal function.

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## P-15
## A PROTEOMICS CHARACTERIZATION OF THE HUMAN T LYMPHOCYTE ACETYLOME

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Lysine acetylation is a reversible posttranslational modification [PTM] of proteins which is known to play a key role in regulating gene expression [1]. The emergence of specific antibodies against this PTM and recent breakthroughs in the identification and quantification of lysine acetylation by mass spectrometry have increased the knowledge of this modification. Recent studies are revealing that acetylation is involved in many other biological processes, through the regulation of protein interactions, enzyme activity, and protein localization [2]. In consequence, mapping the human acetylome has become a priority task as it has the potential for discovering novel properties and regulatory functions of this modification.

In this study we report on the characterization of the human T lymphocyte acetylome. Although acetylation is now believed to play an important role on signal transduction, the degree of protein acetylation and its dynamics during lymphocyte activation remain largely unknown.

Like other PTMs, acetylation often occurs at a low stoichiometry. Thus, for its detection, the enrichment in acetylated peptides by immunopurification using acetyllysine-specific antibodies prior to their analysis is crucial [2] [3] [4]. Even using these specific concentration methods, these studies require high amounts of starting material making the analysis of the T-cell acetylome a difficult challenge.

To reduce these problems, we have developed a FASP-based method that allows the analysis up to 1.2 mg of protein extract. The method avoids common steps of protein precipitation or sample cleaning, providing of a high protein recovery. Using this method, we

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## P-16
## MULTIELEMENTAL ANALYSIS OF TOXIC METALS IN WHOLE BLOOD SAMPLES USING ICP-MS

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**Introduction:** Toxic metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) has been recognized as toxins for centuries. Diagnosis of metal toxicity requires demonstration of the source of metal exposure; the patient must show signs and symptoms typical of the metal; and abnormal metal concentration in the appropriate sample must be found. The clinical laboratory plays a key role in this process, since accurate analysis can make a major difference in correct diagnosis.

**Objective:** To develop and validate a method for measurement of toxic metals (As, Cd, Hg, and Pb) in whole blood samples (EDTA K2) by inductively coupled plasma mass spectrometry (ICP-MS).

**Methods:** The analysis was performed on an ICP-MS 7700x (Agilent). Standards, quality controls and whole blood samples (200 µL) are diluted 1:20, before analysis, with an ammoniacal solution containing 20 µg/L of internal standards ($^{72}$Ge, $^{103}$Rh, $^{193}$Ir and $^{209}$Bi). Integration times are 2, 2, 3 and 1.5 s for $^{75}$As, $^{111}$Cd, $^{201}$Hg and $^{206+207+208}$Pb, respectively, and 0.3 s for each internal standard. Measurements are performed in triplicate and total time of analysis is less than 3 min per sample. A multielemental calibration standard is prepared from monoelemental solutions containing 1000 mg/L on each metal (High-Purity™ Standards). Method validation (linearity, precision, limit of detection (LoD) and quantification (LoQ) and carryover) was performed according to CLSI guidelines. The results were analyzed with MedCalc statistical software, version 15.2.2.

**Results:** Linearity was established from 2.5 to 40 µg/L for As, 1.25 to 20 µg/L for Cd and Hg, and 5 to 80 µg/dL for Pb (correlation coefficients higher than 0.995). The LoD for As, Cd and Hg was below 0.25 µg/L and the LoD for Pb was 0.2 µg/dL. Precision was calculated by analysis in triplicate (for five days) of three levels of internal quality controls (Clinchek®, Recipe) and three levels of external quality controls (OELM program). The intra-assay and within-laboratory coefficients of variation (CVs) were within 5.2% for As, 6.7% for Cd, 10% for Hg and 5.1% for Pb at all levels tested. The LoQ was set as the lowest calibration standard, with within-laboratory CVs of 6.9%, 9.7%, 5.7% and 2.9% for As, Cd, Hg and Pb, respectively. No significant carryover was observed for this assay.

**Conclusion:** This ICP-MS method is a reliable and sensitive assay for quantitation of As, Cd, Hg and Pb in whole blood samples, allowing the diagnosis of metal toxicity.

## P-17
## ANALYSIS OF PLASMA FREE METANEPHRINES BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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**Introduction:** Plasma free metanephrines (metanephrine (MN) and normetanephrine (NMN)) are measured in the clinical lab as screening test for presumptive diagnosis of pheochromocytomas or paragangliomas, catecholamine-producing tumours arising from adrenal and extra-adrenal chromaffin tissue. Measurement of these two metabolites of catecholamines provides a highly sensitive test for diagnosis of these tumours in patients with sustained hypertension or an incidentally discovered adrenal mass.

**Objective:** To develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for measuring free metanephrines (MN and NMN) in plasma.

**Methods:** Instrumentation included an AB Sciex QTRAP 5500 triple quadrupole mass spectrometer coupled to an Ekspert ultra liquid chromatography 100 system from Eksigent. Metanephrines were extracted from 500 µL of EDTA-plasma using weak cation exchange solid-phase extraction cartridges and analyzed by LC-MS/MS with a 7 min gradient (0.2% formic acid in ammonium formate, pH=3.2 (Buffer A); and 0.2% formic acid in methanol (Buffer B)). Method validation (analyte recovery, linearity, precision and carryover) was performed according to CLSI guidelines. A comparison study was accomplished on 22 samples simultaneously assayed with an ELISA used in a reference laboratory from Barcelona.

**Results:** Absolute recovery of analytes, assessed by comparison of two pre- and postextraction spiked pools at two levels of methanephrines (267 and 1333 pg/mL) and analyzed for five days (20 replicates), was from 56 to 83% for MN (70% on average) and from 31 to 89% for NMN (52% on average). Linearity was established from 10 to 2000 pg/mL for MN and NMN (correlation coefficients higher than 0.995). Precision was calculated by analysis in triplicate (for five days) of four levels of metanephrines in aqueous solution (10, 20, 100 and 500 pg/mL) and one plasma quality control from Chromsystems (60 and 100 pg/mL of MN and NMN, respectively). The intra-assay and within-laboratory coefficients of variation (CVs) were within 7.5% for MN and 7.8% for NMN. The limit of quantification of 10 pg/mL showed a within-laboratory CV = 11% for MN and 10.9% for NMN. No significant carryover was observed for this assay. Method comparison with ELISA revealed a correlation coefficient of 0.68 for MN and 0.98 for NMN. The mean difference was -28% for MN (statistically significant) and 16% for NMN (not statistically significant).

**Conclusion:** This LC-MS/MS method is a reliable and sensitive assay for quantitation of plasma metanephrines and will be implemented in our routine laboratory for screening of patients with suspected pheochromocytoma/paraganglioma.

## P-18
## ANALYSIS OF BIS-GLUCURONIDE METABOLITES OF ANABOLIC ANDROGENIC STEROIDS IN SPORTS DRUG TESTING

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Anabolic androgenic steroids (AAS), included in the list of prohibited substances by the World Anti-Doping Agency, are the substances most frequently detected in doping control analyses. Most AAS are extensively metabolized and excreted in urine mainly as phase II metabolites, however most of the metabolic profile remains unknown. Traditionally, studies on steroid metabolism allow only for the detection of metabolites conjugated with glucuronic acid hydrolysable using β-glucuronidase enzymes and unconjugated metabolites. LC-MS allows for the direct analysis of steroid conjugates and it has been recently used to identify new AAS phase II metabolites, such as glucuronoconjugates resistant to β-glucuronidase hydrolysis, sulfates or conjugates with cysteine. Other phase II metabolites not systematically studied for AAS are bis-glucuronides. Bis-glucuronoconjugated metabolites, in which two separated functional groups are conjugated with glucuronic acid, have been described for compounds such as bilirubin and morphine.

The objective of this study was to detect bis-glucuronide metabolites of AAS. For this purpose, the first step is to study ionization and CID behaviour of AAS bis-glucuronides. Because these metabolites are not commercially available as standards, we synthesized bisglucuronides from steroids with two free hydroxyl groups. Synthesis conditions (the amount of the reagents $Ag_2CO_3$ and acetobromo-α-D-glucuronic acid methyl ester, and the reaction time) were optimized for each analyte. Several compounds were synthesized that can be divided in different groups according to their structure: androstandiols, estrandiols, 5-androstendiols, 4-androstendiols and 17-methyl-androstandiols. Briefly, bis-glucuronides ionized as $[M+NH_4]^+$, in positive mode, and they formed the ions $[M-H]^-$ and $[M-2H]^{2-}$ resulting from the deprotonation of one or two acidic group, respectively. In positive mode, the most common fragments of steroid bis-glucuronides were result from the loss of 211, 229, 387 and 405 Da, and the ions at m/z 141, 159 and 177 coming from the glucuronide moiety. The CID-spectra of the $[M-H]^-$ ion show only the neutral loss of 176 Da (corresponding at the loss of one glucuronide group) and the ions at m/z 75, 85 and 113 coming from the glucuronide moiety. Fragmentation of $[M-2H]^{2-}$ show ion losses of 175 and 75 (corresponding to loss of glucuronide and $HOCH_2CO^{2-}$, respectively). The common ionization and fragmentation behavior observed for steroid bis-glucuronides will be used as a basis for the development of open scan methods (PI and NL methods) and SRM methods to detect this type of metabolites in excretion study samples collected after administration of AAS.

## P-19
## DETERMINATION OF 5-NITROIMIZALE DRUGS IN URINE SAMPLES BY CAPILLARY ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY

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5-nitroimidazoles (5-NDZs) are antimicrobials employed in clinical medicine for treating infections mainly due to protozoan such as *Trichomonas vaginalis*, *Entamoeba hisolytica* or *Giardia lamblia*, although they have proved to be also effective against to bacterial pathogens *Helicobacter pylori*, *Clostridium difficile*, *Gardnerella vaginalis* and *Bacteroides fragilis.* Their importance as human antibiotics has been reflected in the World Health Organization (WHO) Model List of Essential Medicines, where metronidazole (MNZ) is listed as one of the two essential antiamoebic and antigiardiasis medicines [1]. Although 5-NDZ determination has been traditionally carried out by liquid chromatography [2], capillary electrophoresis (CE) has been proved to be a powerful technique in clinical analysis.

In this work, we propose for the first time the coupling of CE with tandem mass spectrometry (CE-MS/MS) as a novel method for the determination of eight 5-NDZs and three of their main metabolites in urine samples. As sample treatment, a molecular imprinted polymer solid phase extraction (MISPE) procedure was applied to 2 mL urine samples. MISPE elutes were dried under nitrogen current at 40°C and they were reconstituted in 200 µL of deionized water. A hydrodynamic injection at 50 mbar for 40 s was established. 5-NDZ separation was performed in a fused silica capillary (110 cm x 50 µm) using formic acid 1.0 M (pH 1.8) as background electrolyte (BGE). A separation voltage of 28 kV and a temperature of 25°C were considered. Moreover, a pressure of 50 mbar was applied to the inlet vial during separation in order to improve migration time reproducibility. A sheath flow interface was employed for the CE-MS coupling, considering a sheath liquid composition of isopropanol/water/acetic acid (60/38.8/0.2, (v/v/v)) with a flow rate of 0.2 mL/min. Electrospray ionization (ESI) in positive mode was used under a nebulization pressure of 7.4 psi, a dry gas flow of 6 L/min and a dry gas temperature of 160°C. Matrix-matched calibration curves showed satisfactory linearity ($R^2 \geq 0.994$) for the proposed method. Detection limits from 0.02 to 0.31 µg/mL were obtained. Precision studies resulted in relative standard deviations (RSDs) lower than 16.1%. Recoveries over 79.2% were obtained for all studied compounds. The method shows an useful alternative for the therapeutical drug monitoring of 5-NDZs in urine samples.

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## P-20
## DETERMINATION OF TESTOSTERONE IN URINE BY ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY USING ISOTOPE PATTERN DECONVOLUTION CALCULATION

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In this work we present a new quantification method of total testosterone (T) in human urine samples by ultra high pressure liquid chromatography and tandem mass spectrometry (UHPLC-ESI-MS/MS). The proposed methodology, Isotope Pattern Deconvolution (IPD) is able to reduce the total analysis time since no methodological calibration curves are needed. For comparison purposes, two quantification methods were developed and validated: isotope dilution mass spectrometry (IDMS) using IPD and calibration with internal standard. Double $^{13}C$-labeled testosterone ($^{13}C_2$-T) was used as internal standard in both cases.

Briefly, the liberation and extraction of testosterone was accomplished by applying a procedure based on an already established method [1]. Hydrolysis of the glucuronide conjugates was performed by means of enzymatic reaction with β–glucuronidase *E. coli*, followed by liquid-liquid extraction with methyl tert-butyl ether.

Fifteen urine samples were collected from healthy volunteers of both genres and different ages. After quantification, six synthetic samples were prepared by mixing different pairs of samples with similar concentration levels. True concentration values of the synthetic samples were determined by standard additions and values ranging from 2.11 ng/mL to 81 ng/mL were obtained, with RSD values lower than 10% in all cases. One sample replicate for each one of the six mixed urines were analyzed in five consecutive weeks. Both accuracy and precision of IPD and calibration with internal standard were compared against the reference standard additions method. Calibration with internal standard provided inter-day relative standard deviations (RSD) lower than 16% in all samples, while RSDs lower than 4% were obtained by IPD even at the lowest concentration level. Trueness of the inter-day averages was assessed as the percentage difference with the reference method. Results obtained for both developed methods ranged between 80% and 102%.

The main advantage of the proposed IPD methodology is that it provides concentration results of a sample in a single injection without the need to perform any methodological calibration curve. Besides, it has also been demonstrated that IPD quantification provided a lower inter-day variability than calibration with internal standard quantification method, demonstrating its applicability to steroid determination in urine samples.

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## P-21
## REFERENCE MATERIALS FOR ANTIDOPING CONTROL: PREPARATION OF LYOPHILIZED URINE SAMPLES.

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Anti-doping laboratories accredited by ISO 17025 and WADA have to participate in interlaboratory comparisons to assure quality of analytical results. One method used for ensuring stability during distribution, transport and storage of reference materials used in interlaboratory comparisons is lyophilisation which is a process based in the elimination of the water by sublimation and desorption. The objective of this project was to study the lyophilisation conditions to prepare reference materials of 11- nor- Δ9- tetrahydrocannabinol- 9- carboxylic acid (THC-COOH), Benzoylecgonine (BE), Ethyl glucuronide/Ethyl sulphate (EtG/EtS) and ethanol (EtOH).

Lyophilisation conditions were optimized and preliminary tests to choose the adequate containers and volume of sample were carried out. Urine samples containing THC-COOH (300 ng/mL), BE (1500 ng/mL), EtG/EtS (10 µg/mL) and EtOH (4 g/L) were prepared from blank urine, previously filtered and stabilized. Different storage conditions were tested. Liquid urine samples were stored at 4ºC and -20ºC (used as reference aliquots), the rest of the aliquots were lyophilized and stored at 4ºC. Homogeneity was evaluated in lyophilized aliquots. Samples were quantified using specifics quantitative procedures.

Results of all samples demonstrate homogeneity. The percentages of change obtained between lyophilized aliquots and reference aliquots were minimum in samples of THC-COOH (-4,6%) and BE (-1,6%). Therefore, it could be attributed to the variability of the method and not to a loss of the compound during the lyophilisation process. However, higher differences were observed for the sample containing EtG (-8,6%) and EtS (-15,6%). If these differences were maintained constant in subsequence quantifications, they could be attributed to the evaporation of the analytes during the lyophilisation process. Results obtained for EtOH (-100%) show a completely loss of the analyte due to its high volatility.

## P-22
## EFFECT OF GLUCOCORTICOID ADMINISTRATION ON THE STEROID PROFILE IN SPORTS DRUG TESTING

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The steroid profile is a powerful tool to detect the misuse of endogenous anabolic androgenic steroids in sports. Due to wide interindividual variability in the markers of the steroid profile, the Athletic Biological Passport (ABP) is used to individually detect alterations on the steroid profile that could indicate the use of drugs. Glucocorticoids (GCs) may modify the steroid profile because their effect on the hypothalamic-pituitary-adrenal (HPA) axis. The aim of the work was to verify if GCs administered by conventional systemic routes (oral or intramuscular) or by local routes (topical) could affect the urinary steroid profile.

Urine samples collected after GCs administration were hydrolysed with β-glucuronidase enzymes, subjected to liquid-liquid extraction with tert-butyl-methyl ether and analysed by gas chromatography coupled to mass spectrometry after derivatization (trimethylsilyl ether). The method was applied to urines collected in clinical studies where different GCs were administered to volunteers by different routes: a single oral dose of prednisone (5 mg, n=2 volunteers), prednisolone (10 mg, n=2 volunteers) or methylprednisolone (40 mg, n=4 volunteers), a single intramuscular (IM) dose of betamethasone (12 mg, n=2) or triamcinolone acetonide (40 mg, n=1; 80 mg= 1) and topical doses of prednisolone (10 mg) for five consecutive days followed by a single oral dose of prednisolone (5 mg) to 6 volunteers.

Concentrations of testosterone, epitestosterone, androsterone, etiocholanolone, 5α-androstandiol and 5β-androstandiol and ratios between them were evaluated. As expected, large interindividual differences in urinary concentrations of steroids were observed and no clear trend was observed for most of the administration routes. After IM and oral administration of GCs, daily excretion of all evaluated endogenous steroids decreased significantly. No effects were observed after topical administration. Even though a significantly decrease in the androgen excretion was observed, all studied ratios between analytes of interest of the steroid profile were not significantly altered. Therefore, it seems that administered GCs cannot lead to suspicious samples on the steroid profile.

## P-23
## IMPROVING EFFECTIVENESS OF METHOD DEVELOPMENT USING A SYSTEMATIC PROTOCOL

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Method development involves screening a range of chromatographic parameters to generate conditions for optimum separation. These often include investigating different column chemistries, organic solvents, pH, gradient slopes, flow rates, approaches to method development such as one-factor-at-a-time (OAFT), systematic protocols and quality-by-design (QbD), the final goal is the same, to develop a robust method that separates all components and generates an accurate result.

In this work, we present the development of a UPLC method for metoclopramide HCl and related substances using a pre-defined systematic protocol that incorporates scouting, screening, and optimization steps [1]. At each step, we evaluated the impact of different chromatographic parameters on the separation and selectivity of sample components. Chromatographic results from each step of the method development process were analyzed using automated reporting features to remove bias in decision making process. In addition, an integrated compact mass detector was used for identification and tracking of sample components.

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## P-24
## ADVANCED DECONVOLUTION SOFTWARE IN THE SUSPECT AND NON-TARGET SCREENING OF WASTE-WATER SAMPLES

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In recent years, the study of emerging contaminants has become prevalent in analytical environmental chemistry circles. For their detection and/or identification, there are commonly three approaches: target, suspect/post-target and non-target. The latter is of increasing interest but notoriously difficult to undertake, as, strictly speaking, no a priori information is available. Even with the help of automated peak-picking software, thousands of peaks can be identified in an individual sample. Subsequent steps must therefore be made to reduce the number of peaks to a more manageable number, including molecular formula derivation, isotopic pattern, mass defect analysis and retention time prediction. Further confidence in the "potential positives" remaining can be gained through the use of fragmentation in a subsequent MS/MS injection and comparison with in silico fragmentation and/or mass spectral libraries.

This work presents the use of two software (MsXelerator and Sieve 2.1) in the analysis of influent and effluent wastewater samples following injection in LC-LTQ-Orbitrap MS. An initial suspect/post-target approach was undertaken, using an in-house database of more than 250 pharmaceuticals and illicit drugs, with numerous compounds being detected across the software, including the illicit drug cocaine and its metabolite benzoylecgonine and the pharmaceuticals carbamazepine, gemfibrozil and losartan, among others. To get a further understanding of potential compounds present, a non-target approach, using the ChemSpider lookup feature of Sieve was made. The compounds found using both approaches were combined, and isotopic pattern and retention time prediction were used to filter out false positives. The remaining potential positives were reanalysed by MS/MS with their fragment ions compared with literature and/or mass spectral libraries.

The inclusion of ChemSpider led to the confirmation of several compounds previously found by suspect screening and the tentative identification of several additional compounds and metabolites, including the pharmaceuticals phenazone and phenacetin and the metabolite carboxylosartan. Furthermore, three isomeric compounds detected by the software were able to be distinguished by their fragment ions.

## P-25
## IDENTIFICATION OF MYCOTOXINS BY UHPLC-QTOF MS IN AIRBORNE FUNGI AND FUNGI ISOLATED FROM INDUSTRIAL PAPER AND ANTIQUE DOCUMENTS FROM THE ARCHIVE OF BOGOTÁ

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Mold deterioration of historical documents in archives and libraries is a frequent and complex phenomenon that may have important economic and cultural consequences. In addition, exposure to toxic fungal metabolites can produce health problems. Powerful analytical methodology is required to investigate the large number of mycotoxins that can be found in different environments and sample matrices. Their detection and reliable identification is an analytical challenge due to the high number of possible compounds, the similarity in their chemical structures, the lack of mass spectra in common analytical libraries, and the limited availability and unaffordable cost of many reference standards.

In this work, samples of broths of fungal species isolated from the documentary material and from indoor environmental samples of the Archive of Bogotá have been analyzed. HRMS made possible to investigate a large number of mycotoxins, even without reference standards available at the laboratory. For this purpose, a screening strategy based on UHPLC-QTOF MS under $MS^E$ mode was applied. A customized home-made database containing elemental composition for around 600 mycotoxins was compiled. The presence of the (de)protonated molecule measured at its accurate mass was searched in the samples. When a peak was detected, collision induced dissociation fragments and characteristic isotopic ions were evaluated and used for tentative identification, based on structure compatibility and on comparison with literature data (if existing).

44 mycotoxins were tentatively identified by UHPLC-QTOF MS. 34 of the candidates were confirmed by subsequent analysis using a targeted LC-MS/MS method in another lab. The compounds identified included Fumigaclavines A, B and C; Isofumigaclavine A; Fumagillin; Kojic acid; and Chanoclavine, among others. The remaining 11 compounds (e.g. Pyranonigrin, Aspergillic acid or Nigragillin) were tentatively identified based on information provided by QTOF MS, which was supported by the fragment ions reported in the literature; however, they could not been confirmed as these compounds were not included in the LC-MS/MS method. The discovering of these mycotoxins in the samples may help to reinforce safety measures for researchers and staff who work on reception, restoration and conservation of archival material, not only at the archive of Bogotá but worldwide.

## P-26
## GUAZATINE ANALYSIS IN SURFACE AND WASTE WATER BY HIGH PERFOMARANCE LIQUID CHROMATOGRAPHY (HPLC) COUPLED WITH A TANDEM MASS SPECTROMETER (MS/MS)

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Guazatine is a non-systemic contact fungicide that disrupts the cell membrane of fungi. It is composed of a mixture of reaction products from polyamines, in which the diamine derivatives account for 40% of the constituents of guazatine, triamines for 46%, tetramines for 11% and other amine derivatives for 3%. The World Health Organization (WHO) has classified it as moderately dangerous. It is widely used in agriculture to prevent a broad spectrum of plagues in cereals. In citrus fruits, it is used to prevent the rottenness caused by fungi. The guazatine mixture is applied submerging the fruit in it or spraying in the packing line.

Not many methods for the determination of guazatine have been developed and the few that have been developed analyze guazatine residues in vegetables. This is due to the complexity that involves the analysis of a mixture of several compounds under a unique name (Guazatine). Since it is a mix with fairly constant technical products used in proportions, the analysis of major components of the mixture will take place to determine as well the total concentration of guazatine. In the standards and technical mixtures, the guazatine comes formulated as guazatine acetate. The major components are assigned the total of guazatine acetate concentration, for in this way, calibrating with standards of them we can calculate the total concentration of acetates of guazatine in the sample. A HPLC-MS/MS method was developed to meet the current maximum pesticide levels in waste water of 50 µg/L for total pesticides (RD 849/86 and its modification RD 606/2003).

The method is based on a direct sample injection in the HPLC-MS/MS system. The waste water sample is centrifuged, filtered and 10 µL is directly injected. The results and calculations are made expressing the guazatine as guazatine acetate. Weighted least squares linear adjustments (1/x) are employed. The calibration is linear in the concentration range (16-601 µg/L). In these conditions the analysis of guazatine can be done with an accuracy of 85%, a precision of 7% and a limit of quantification of 25 µg/l (as guazatine acetate) with an established uncertainty of 25%.

The developed method was employed in a wastewater monitoring program. As the guazatine acetate was found in the inspection samples, the industries that produced the wastewater introduced wastewater treatment protocols that drastically reduced the guazatine residues in the waste water.

## P-27
## DEVELOPMENT OF A METHOD BASED ON ISOTOPE DILUTION MASS SPECTROMETRY FOR THE DETERMINATION OF TRIBUTYLTIN IN WATER SAMPLES USING GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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The use of organotin compounds, particularly Tributyltin (TBT), as a toxic additive in antifouling paints has been responsible of their anthropogenic widespread introduction into the aquatic environmental and in the food chain. TBT persist in water killing not only the sea life attached to the hulls of ships but also other non-target organisms. Due to the extremely high toxicity and the endocrine disrupting power of this tin compound injurious effect in many marine species such as imposex in gastropods like dog whelks (occurring at TBT concentration levels as low as 0.5 ng Sn L-1) or deformities in oysters have been reported worldwide.

The European Water Framework Directive (WFD) 2013 / 39/ UE, requires for Tributyltin (TBT) a maximum allowable concentration of 0.0002 µg L-1 in surface water samples. With the aim of meeting these requirements, a procedure based on Isotope Dilution Mass Spectrometry (IDMS) in combination with Isotope Pattern Deconvolution (IPD) for the determination of TBT in water samples using GC-MS/MS instrumentation, has been developed. The fragmentation pattern of the TBT in the electron ionization source, the determination of possible losses of TBT during the sample preparation as well as the influence of different volumes of water samples on the recovery of TBT were also important issues studied in this work.

The capabilities of the method developed in this work for routine analysis were evaluated. For this purpose, the implementation of the proposed methodology was carried out in the analysis laboratory IPROMA S. L. (Castellon, Spain) and it was subsequently accredited by the Spanish National Accreditation Body (ENAC, Entidad Nacional de Acreditación) according to the requirements of UNE-EN-ISO/IEC 17025.

The developed MRM method along with IDMS allows the determination of TBT at the concentration level required by the WFD, no significant TBT losses occur during the preconcentration step and satisfactory recoveries were obtained for 100 and 250 ml of water samples. The accredited method validation was performed in drinking, continental and sea water with an average precision and accuracy of 8% and 91% respectively.

## P-28
## CONCENTRATION, PROFILE PATTERNS AND SPATIAL DISTRIBUTION OF PERFLUOROALKYL SUBSTANCES IN WATER, SEDIMENT AND BIOTA OF THE EBRO AND GUADALQUIVIR RIVERS (SPAIN)

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The occurrence and sources of 21 perfluoroalkyl substances (PFASs) were determined in water, sediment and biota of the Ebro and Guadalquivir River basins (Spain). In **water** samples, of 21 analytes screened, 11 were found in Ebro and 9 in Guadalquivir. In both basins, the most frequents were PFBA, PFPeA and PFOA. Maximum concentration was detected for PFBA, up to 251.3 ng $L^{-1}$ in Ebro and 742.9 ng $L^{-1}$ in Guadalquivir. Regarding the **sediments**, 8 PFASs were detected in the samples from Ebro and 9 in those from Guadalquivir. The PFASs most frequently detected were PFBA, PFPeA, PFOA and PFOS. Maximum concentration in Ebro samples was, in dry weight, for PFOA (32.3 ng $g^{-1}$) and in Guadalquivir samples for PFBA (63.8 ng $g^{-1}$). In water and sediments, short-chain perfluoroalkyl acids were the most frequents [1]. For **biota**, 12 PFASs were detected in fish from the Ebro River and only one (PFOS) in that from Guadalquivir River. In the Ebro, the most frequents were PFBA, PFHxA, PFOA, PFBS, PFOS and PFOSA. Maximum concentration in Ebro samples was, in wet weight, for PFHxA with 1280.2 ng $g^{-1}$, and in Guadalquivir samples for PFOS with 79.8 ng $g^{-1}$. PFASs can be detected in the Guadalquivir and Ebro rivers in water, sediments and biota showing different patterns and were detected in the whole course of the rivers including the upper parts. In some points contamination can be related to anthropogenic impact (e.g. ski resorts, military activities or urban areas). Furthermore, the low PFASs levels found in waters from abrupt areas where there are not point sources of these compounds, suggests a widespread diffuse contamination from atmospheric deposition, runoff or infiltration/exfiltration processes [2]. PFASs concentrations in the Guadalquivir and the Ebro rivers do not suppose a potential risk to biota, except in the case of PFTeDA.

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## P-29
## THE FATE OF PESTICIDES IN THE EBRO RIVER

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The Ebro River, located at the north eastern of Spain, drains an area of approximately 85.000 $Km^2$, has 929 Km in length and receives water from several tributaries. It is influenced by industrial and agricultural activities (vineyards and rice) at lower-medium course. Therefore, a widespread range of organic pollutants have been detected along the river as happen in other Mediterranean areas [1, 2].

In this work, 50 currently used pesticides were monitored from the end of September to the middle of October 2010 and 2011 in water, sediments and biota along the River.

Pesticides were extracted from water by solid-phase extraction (SPE) and sediment and fish by QuEchERS method. The resulted extract was then analyzed by LC-ESI-MS/MS in positive mode. Separation was carried out on a Luna C18 column (150 × 2.0 mm, 3 µm) using a gradient elution profile with mobile phase consisting of water-methanol both, 10 mM ammonium formate.

The results show that in water samples the most frequent pesticides were chlorfenvinphos, chlorpyrifos, pyriproxyphen, buprofezin and hexythiazox which appeared in more than 90 % of the samples during 2010 campaing; and the highest concentrations was for imazalil (409.8 ng/L). Nevertheless, in 2011 carbendazim was the most frequent (70%) and the highest concentration detected was for imazalil (121 ng/L). Regarding the spatial distribution, the concentrations were variable along of the rivers, however the highest concentrations in both years was found in the station SEG which belongs at Segre tributary near to LLeida.

In sediments, chlorpyrifos was the most frequent in 2010 and 2011 (45% and 82%, respectively), and the highest concentrations (9.6 and 36.2 ng/L d.w, respectively) had in both campaigns. Finally, the only pesticide detected in biota samples was Chlorpyrifos. It is important monitoring the quality water to preserve its health and the ecosystem.

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## P-30
## OPTIMIZATION AND VALIDATION OF THE DETERMINATION OF PESTICIDES IN BEESWAX BY LC-MS/MS

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Beeswax is a very complex mixture of lipophilic compounds synthetized by bees. They use it mainly for building the honeycombs, and recycle it widely when establishing a new hive. Consequently, pesticides applied in Varroa mite control and other environmental pollutants can enter the hive directly or indirectly and accumulate in it causing an emerging problem for apiculture [1].

The aim of this work was to develop a sensitive multiresidue method for monitoring a large number of pesticides in beeswax by liquid chromatography-tandem mass spectrometry. The study selected 60 pesticides including neonicotinoids acetamiprid, imidacloprid and thiamethoxam because of their high toxicity for honeybees. Acaricides usually applied in hives as amitraz (together with its metabolites dimethylamine, dimethylformamide and 2,4-dimethylphenyl formamide), acrinathrin, coumaphos, fluvalinate and flumethrin were also included as well as many other compounds from different chemical families such as carbamates, organophosphates, pyrethroids, triazines, chloroacetanilides, benzimidazoles, azoles and some fungicides and acaricides of difficult chemical classification.

Prior to the chromatographic analysis, beeswax samples were extracted with acetonitrile, melt in a water bath at 80 ºC and then, the tube was left to cool to room temperature and put into the freezer (−18 °C, for at least 2 h) for precipitation of the wax. Finally, 25 mg PSA and 25 mg C18 were added for clean-up and pH was adjusted to 5.

The resulted extract was then analyzed by LC-ESI-MS/MS in positive mode. Separation was carried out on a Luna C18 column (150 × 2.0 mm, 3 µm) using a gradient elution profile with mobile phase consisting of water-methanol both, 10 mM ammonium formate. The two most intense precursor ion → product ion transitions were monitored to obtain unambiguous confirmation of the compound identity.

Multiresidue screening method showed recovery percentages from 70 to 96% and precision values were under 20% for all analytes except for atrazine-desethyl, carbofuran, fenthion-sulfoxide and omethoate. The detection limits ranged from 0,0005 to 0,25 ng/g whereas quantification limits oscillated from 0,0015 to 0,15 ng/g. The method developed meets the stated objective and it is suitable for the monitoring of the selected pesticides in beeswax matrix to control environmental quality.

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## P-31
## POTENTIAL OF ATMOSPHERIC PRESSURE CHEMICAL IONIZATION SOURCE FOR THE QUANTIFICATION OF A WIDE RANGE OF HALOGENATED PERSISTENT POLLUTANTS IN COMPLEX SAMPLES

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Gas chromatographs coupled to mass spectrometers (GC-MS) have been extensively applied for the determination of volatile and semi-volatile, non-polar, compounds in fields like food safety, environmental or toxicological studies. Most methods reported in the literature use electron ionization (EI) [1], which may result in extensive fragmentation of analytes compromising both, selectivity and sensitivity. This makes also difficult the application of tandem MS due to lack of specific/abundant precursor ions [2].
The analysis of persistent organic pollutants, including brominated flame retardants (BFRs), polychlorinated dibenzodioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls (dl-PCBs), relies on the use of methods based on GC-MS operating in EI or negative chemical ionization (NCI) modes using quadrupole, triple quadrupole, ion trap or magnetic sector analyzers [3]. These halogenated contaminants are examples of compounds for which a softer, reproducible and robust ionization technique might be favorable since they show high fragmentation in EI and low specificity in NCI [4]. In this work the potential of atmospheric pressure chemical ionization (APCI) combined with GC and triple quadrupole mass analyzer has been investigated, using BFRs, PCDD/PCDF and dl-PCBs as model compounds for their determination in different complex samples, including marine samples, milk, feed and animal fat and interlaboratory studies samples.
Ionization and fragmentation behavior of 14 PBDEs and 3 novel BFRs as well as 10 PCDD/PCDF and 7 dl-PCB congeners by APCI has been studied. The formation of highly abundant (quasi) molecular ions has been the main goal because of the expected improvement in specificity obtained when using it as precursor ion in tandem MS. The convenience of using modifiers for the ionization step has been studied and discussed for each family of compounds with the aim of obtaining the maximum sensitivity in combination of acceptable reproducibility and optimum specificity/selectivity. The improved detectability (LODs between 1 and 20 fg) achieved when using APCI compared to EI has been demonstrated. The application to different complex samples has shown the feasibility of the methods at trace levels for unambiguous identification and determination of the compounds investigated. These results highlight the potential of APCI as the reference for quantification of halogenated pollutants in the next years.

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## P-32
## HIGH PRECISION MEASUREMENT OF DISSOLVED INORGANIC CARBON (DIC) BY ISOTOPE DILUTION MASS SPECTROMETRY FOR CLIMATE CHANGE STUDIES

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The influence of $CO_2$ release due to anthropogenic activities is well known nowadays. The oceanic absorption of anthropogenic $CO_2$ has led to an acidification of seawater, what causes an important impact in biological and biochemical processes in oceans and coastal waters. Dissolved inorganic carbon (DIC) is one of the most important parameters for calculating partial pressure of $CO_2$ in seawater in order to study ocean acidification and Climate Change. DIC concentration varies from 1250 to 2400 µmol/kg in coastal waters and from 1950 to 2200 µmol/kg in open sea. Considering that DIC concentration increases around 1 µmol/kg per year, the quantitative assessment of such process would require analytical methodologies that provide overall precisions around 0.05% RSD. Nowadays the standard approach is based on coulombimetric measurements but it is a very complex procedure that demands for a highly experienced operator as well. Other approaches mostly based on Infrared and spectrophotometric measurements do not reach the precision level required.

Herein, a methodology for high precision measurement of DIC based on LC-IRMS and post-column isotope dilution has been evaluated. Bicarbonate ion of the sample is mixed on line with an isotopically enriched $H^{13}CO_3^-$ flow. Then, the mixture reacts with phosphoric acid and the equilibrium shifts to the formation of $CO_2$. Once isotopic equilibrium between natural $^{12}CO_2$ from the sample and $^{13}CO_2$ from the tracer is reached, formed $CO_2$ is separated from the liquid phase by a membrane separation unit. The $CO_2$ permeates the membrane and is transported by a dry helium gas stream through two Nafion™ gas dryer units. The dried gas is introduced via an open-split into the IRMS ion source. A magnetic sector field analyzer separates mass-to-charge ratios of the isotopologues of $CO_2$ (44, 45) and the separated ion beams are collected in two Faraday cups. Instrumental parameters have been optimized.

Precision achievable was evaluated in 2 different ways. In the first approach, the sought ratio was computed as area ratio of fast and gaussian flow injection peaks. In the second approach, it was continuously measured in the plateau of intentionally made flat-topped peaks. The developed methodology has been applied to DIC measurement of bicarbonate standards and a DIC certified reference material.

## P-33
## ATMOSPHERIC PRESSURE IONIZATION OF POLYFLUORINATED COMPOUNDS

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Fluorotelomers are polyfluorinated compounds partially saturated by fluoride ions which present hydrophilic groups (alcohol, sulfonamide and sulfonamido ethanol). They have been synthetized by telomerization and it has been shown they are subjected to degradation to the environmentally persistent and toxic perfluorinated carboxylic acids (PFOA) and sulfonates (PFOS). The use of fluorotelomers has been widespread due to the multiple uses in consumer products [1], and their presence in the environment has caused an environmental and health risk concern.

Nowadays, fluorotelomers (volatile and neutral compounds) are generally analyzed by gas chromatography coupled to mass spectrometry (GC–MS), but it has been observed some ionization and sensitivity problems [2]. PFOS and PFOA are ionic compounds that are usually determined by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) using electrospray (ESI) as atmospheric pressure ionization source. However, for the simultaneous determination of the whole family, PFOS and PFOA have to be derivatized to be analyzed by GC–MS and fluorotelomers show a strong ion suppression when using the additives (acids and bases) needed for the LC separation of the ionic compounds. For this reason, a more thoroughly study of the ionization of fluorotelomers with alternative ionization sources is required to develop alternative methods that improve the determination of this family of compounds by LC–MS/MS and compatible with PFOS and PFOA.

In this work, the ionization of fluorotelomers have been studied using different atmospheric pressure ionization sources (ESI, APCI and APPI). The influence of the mobile phase constituents on the ionization has been evaluated. ESI shows the worst performance being inefficient to deprotonate fluorotelomers, but favoring the formation of adducts ($Cl^-$, $HCOO^-$ or $CH_3COO^-$). Post–column addition of $NH_3$ could be a solution to obtain the $[M–H]^-$ although the high pH values required could be a problem for the mass spectrometry instrumentation maintenance and performance. APCI and APPI show the best response of the $[M–H]^-$ being 9 and 15 times better than ESI, respectively. Nevertheless, for APCI best performance MeOH/$H_2O$ as mobile phase is required, while acetonitrile is the organic modifier to be recommended when working with APPI using toluene as dopant. Additionally, tandem mass spectrometry has been performed to characterize the product ions observed using the complementary information obtained by both ion trap and triple quadrupole. These fragmentation studies allowed us to propose fragmentation pathways for the different families of fluorotelomers.

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## P-34
## DESI-HRMS FOR THE IDENTIFICATION OF UKNOWNS IN AN ADULTERATED PHYTOSANITARY PRODUCT

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For the analysis of unknowns in complex environmental matrices laborious sample treatments and chromatographic separations are currently used. However, these methods can be discriminatory and some unknown compounds of interest can be lost. In this context, ambient ionization techniques such as desorption electrospray ionization (DESI) and direct analysis in real time (DART) that allow the direct analysis of the samples in their native environment or with minimal sample manipulation, can be an alternative. However, these techniques provide complex mass spectra containing ions from all the components of the sample. So, the use of high resolution mass spectrometry (HRMS) is mandatory. HRMS provides accurate mass measurements for the correct assignment of elemental compositions, high quality isotope patterns free of interferences and moreover, structural information can be obtained by tandem high resolution mass spectrometry (MS/HRMS). This information can be combined for the reliable characterization of unknowns.

The aim of this work is to evaluate the applicability of DESI-HRMS (Q-Orbitrap) for the analysis of a phytosanitary product suspected of being adulterated. A simple sample manipulation consisting on impregnating a filter paper with the sample was used. The mass spectrum obtained by the direct analysis of the filter paper by DESI-HRMS revealed the presence of metal ions with pattern distributions characteristics of organotins with two and three Sn atoms. The accurate mass measurements, isotope pattern fits and structural information obtained by DESI-MS/HRMS allowed identifying the presence of bis(triphenyltin) oxide, a biocide extensively used for agricultural purposes and regulated by the European Communities, under the Directive 2009/425/EC, for causing persistent and widespread pollution. The analysis of the standard by DESI-HRMS and DESI-MS/HRMS confirmed the presence of bis(triphenyltin) oxide in the sample and moreover, it indicated that other organotins were not present in the sample since all of the organometallic isotope clusters observed in the sample were also found in the standard. This is the first time that an organotin compound is analyzed by DESI-HRMS.

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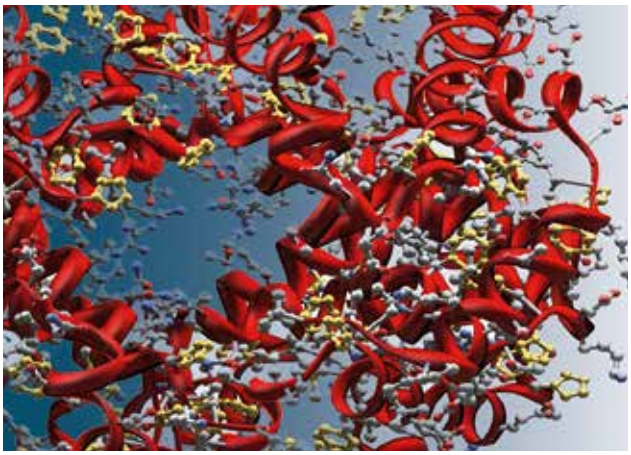

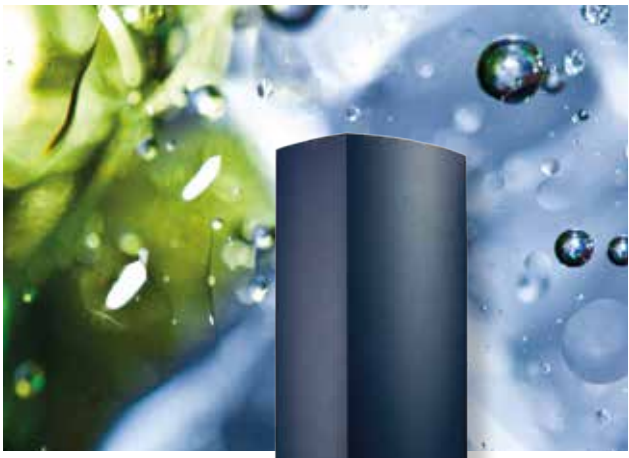

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## P-35
## USE OF HIGH RESOLUTION MASS SPECTROMETRY (ORBITRAP) FOR SIMULTANEOUS DETERMINATION OF PESTICIDES AND MYCOTOXINS IN GREEN TEA NUTRACEUTICAL PRODUCTS

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Nutraceutical products are an alternative to pharmaceuticals and they can be classified in specific nutrients, dietary supplements, herbal extracts and even transgenic food. A nutraceutical product is usually a concentrated form of a food or plant, and it is possible to find toxic substances that can be presented in the raw material, as pesticides and mycotoxins. There are legislation in the European Union that covers maximum residues limits (MRLs) for these toxic substances, being the Regulation EC 396/2005 [1] for pesticides and Regulation EC 1881/2006 [2] for mycotoxins. However, these regulations do not cover nutraceuticals, only the raw material.

In this work, pesticides and mycotoxins (272 substances) were simultaneously determined in green tea nutraceuticals using high resolution mass spectrometry (Orbitrap-MS). The extraction method was a generic *"dilute and shoot"* procedure with acidified acetonitrile (1% formic acid v/v) followed by a clean-up step using $C_{18}$. For the separation and quantification, ultra high pressure liquid chromatography coupled to high resolution mass spectrometry (UHPLC-Orbitrap-MS) was used. The method was validated, determining linearity, trueness, precision, limits of detection (LODs) and quantification (LOQs). Recoveries were observed in the range of 70 and 120% with precision below 25% (RSD values) for all the compounds studied. Both LODs and LOQs were below 10 μg $kg^{-1}$.

The validated method was tested in eleven commercial green tea nutraceuticals. Nine pesticides were detected in some of them, being paclobutrazol (47.9 μg $kg^{-1}$), acetamiprid (42.0-43.3 μg $kg^{-1}$) and imidacloprid (36.7 μg $kg^{-1}$) the compounds detected at higher concentration. In the case of mycotoxins, aflatoxin B1 (5.4 μg $kg^{-1}$) was found in one sample.

Acknowledgments

The authors gratefully acknowledge the Spanish Ministry of Economy and Competitiveness (MINECO) and FEDER (project ref. CTQ2012-34304) for financial support.

[1] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC
[2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

## P-36
## MONITORING OF THE OCCURRENCE OF DIOXINS AND FURANS IN WASTEWATER EFFLUENTS FROM ANDALUSIA

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Dioxins and furans are toxic chemicals that persist in the environment and accumulate in the food chain. They can cause adverse effects on the nervous, immune and endocrine systems, so these contaminants represent an important public health issue worldwide.

The determination of dioxins and furans in environmental samples such as water and sediments is standardized by methods as the United States Environmental Protection Agency (EPA) method 1613 Revision B [1], which is the reference method for this kind of analysis. This method describes the extraction of dioxins and furans from water by means of liquid-liquid extraction (LLE), although in the case of wastewater a previous filtration is recommended followed by the analysis of both phases [1], if necessary.

Although the last trend regarding the determination of these contaminants seems to be the development of analytical methods based on the use of low resolution mass spectrometry (LRMS) for routine analysis, from an analytical point of view, gas chromatography (GC) coupled to high resolution magnetic sector mass spectrometry (HRMS) provides both better selectivity and unequivocal confirmation in comparison with LRMS, as well as it is considered to be one of the most sensitive techniques for the measurement of halogenated toxicants at ultra-trace levels [2].

Based on the EPA Method 1613 Revision B for the determination of dioxins and furans in surface waters, the purpose of this work is the implementation of an analytical method based on the use of GC-HRMS with isotope dilution for the analysis of dioxins and furans in wastewater effluents and superficial waters. In consequence, the monitoring of the contamination with dioxins and furans of wastewater effluents from two provinces of Andalusia (Almeria and Seville) has been carried out, obtaining toxic equivalents up to 26 pg/L.

Acknowledgments

The authors gratefully acknowledge Andalusian Regional Government (Regional Ministry of Innovation, Science, and Enterprise) and FEDER (Project Ref. P12-FQM-1838) for financial support.

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## P-37
## ACRYLAMIDE DETERMINATION IN STARCHY FOOD (POTATO AND ASPARAGUS) BY LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY

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The presence of acrylamide in several heat-treated carbohydrate-rich food is a concern because, according to International Agency for Research on Cancer, acrylamide is a potential human carcinogen. In 2002, the presence of relevant amounts of acrylamide in high starch-based products was reported when they were heated at high temperatures during frying, grilling or baking. Among those products, potato is the most investigated because it is highly consumed. However other products, such as asparagus, also contain high concentration of acrylamide. Currently, there are not regulatory maximum limits for acrylamide in food. However the European Commission (EC) has introduced 'indicative values' for those food groups, which are considered to significantly contribute to consumer dietary exposure to acrylamide and they are based on the European Food Safety Authority (EFSA) monitoring data from 2007-2012 [1].

The aim of this study has been the evaluation of an analytical procedure for the determination of acrylamide in different starchy food as potato chips and roasted asparagus. For that purpose, high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (HPLC-QqQ-MS/MS) was used for separation and quantification. A clean up step based on solid phase extraction (SPE) or dispersive solid phase extraction (d-SPE) was needed. Thus, for potato extracts, the SPE procedure was applied using polymeric cartridges (OASIS HLB). By contrast, for asparagus extracts, aluminum oxide ($Al_2O_3$) was sued as sorbent in d-SPE.

The methodologies were validated taking into account linearity, trueness, precision, limits of detection (LOD) and quantification (LOQ). LODs and LOQs were calculated as 4 and 12 µg/kg for potato chips and 2 and 5 µg/kg for roasted asparagus, respectively. Recoveries were obtained in the range of 90% to 117% for potato chips and 90% to 116% in case of roasted asparagus. Precision was below 20%. These methodologies were tested in real samples detecting concentrations in potato chips around 100-800 µg/kg, and in roasted asparagus around 250-1000 µg/kg.

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## P-38
## PASSIVE SAMPLING AND THERMAL DESORPTION–GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF TRICHLOROANISOLE AND TRICHLOROPHENOL IN WINERIES' AMBIENT AIR

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Chloroanisoles (CAs), particularly 2,4,6-trichloroanisole (TCA), are well-known wine contaminants, responsible for a variety of unpleasant musty aromas and taste. TCA represents a serious economic problem for wineries because wine spoilage can affect a few bottles or even the entire production of wine. TCA has been extensively studied due to the economic impact on the wine market. The sources of contamination are well-known [1] and recent investigations have shown that corks are just one of many possible sources of contamination. These include some kinds of wood preservatives and flame retardants used on wineries. The regular monitoring of these sources is highly recommended [1]. TCAs are produced by fungal biodegradation of halophenols (HPs). This degradation is not only caused by the formation of haloanisoles (HAs) from their respective HPs, but also low halogenated HAs can be formed by the removal of chlorine or bromine atoms to produce less chlorinated CAs from tetra– and penta–halophenols [2]. Since previous scientific papers have shown these compounds to be responsible for wine taint, 2,4,6–tricholoranisole and 2,4,6–trichlorophenol (TCP), were selected for the present study [3].

The present work describes the calibration of selected passive samplers used in the quantitation of trichlorophenol and trichloroanisole in wineries' ambient air, by calculating the corresponding sampling rates. The method is based on passive sampling with sorbent tubes and involves thermal desorption-gas chromatography-triple quadrupole mass spectrometry analysis. Three commercially available sorbents were tested using sampling cartridges with a radial design instead of axial ones. The best results were found for Tenax TA™. Sampling rates (R-values) for the selected sorbents were determined. Passive sampling was also used for accurately determining the amount of compounds present in the air. Adequate correlation coefficients between the mass of the target analytes and exposure time were obtained. The proposed validated method is a useful tool for the early detection of trichloroanisole and its precursor trichlorophenol in wineries' ambient air while avoiding contamination of wine or winery facilities.

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## P-39
## CHEMICAL CHARACTERIZATION OF PARTICULAR PHLOROTANNINS OF *Sargassum muticum* BY LC x LC-DAD-MS/MS

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Phlorotannins are phenolic compounds exclusively found on brown algae. The interest of phlorotannins is growing related to their potential beneficial health effects. Several bioactivities associated with phlorotannins have been reported, like antioxidant, anti-inflammatory, antibacterial, antidiabetic, antiadipogenic or antiproliferative activities.
The chemical structure of phlorotannins consists of polymers of phloroglucinol (PG) units with different degree of polymerization (DP). Depending on the kind of bond that links the PG units, phlorotannins can be classified in several groups: fuhalols and phlorethols (ether linkage), fucols (phenyl linkage), fucophlorethols (ether and phenyl linkage) and eckols (benzodioxin linkage). Consequently, they present a huge structural complexity, and therefore, the complete chemical analysis and characterization of this complex mixture becomes a great analytical challenge, unattainable using conventional monodimensional liquid chromatography. Thus, powerful analytical techniques are required to achieve an appropriate separation of phlorotannins. In this regard, comprehensive two-dimensional liquid chromatography coupled to mass spectrometry (LC × LC-MS) has proven to be a useful tool to carry out the complete analysis, separation and identification of samples with great level of complexity.

In this work, phlorotannins from the brown alga *Sargassum muticum* collected at four very different locations from Portugal and Norway were extracted by pressurized liquid extraction (PLE). The separation and chemical characterization of phlorotannins from the 4 locations was carried out by a LC x LC-MS method. The LC × LC method developed was based on the use of a HILIC separation in the first dimension and a reversed phase separation in the second dimension. An IT was the mass analyzer employed working in MS/MS mode allowing the fragmentation of each separated compound and their structural elucidation.
By using this approach, the separation of 56 compounds on the Portugal samples and 73 compounds on the Norwegian ones was reached. Most of these compounds were characterized as phlorotannins, particularly fuhalols, phlorethols and a new structure of phlorotannins, namely hydroxyfuhalols, which were tentatively identified in this seaweed for the first time. These new phlorotannins consisted on fuhalols with one or more additional hydroxyl groups. Phlorotannins with a DP from 3 to 11 PG units and up to 4 additional hydroxyl groups were detected.
These results demonstrate the great potential of the LC × LC-MS method developed for the separation and the characterization of samples with large complexity, as it is the case of phlorotannins of *Sargassum muticum*.

## P-40
## LC-HRMS/MS (ORBITRAP) METABOLOMIC FINGERPRINTING FOR THE CHARACTERIZATION AND AUTHENTICATION OF CRANBERRY-BASED NATURAL PRODUCTS AND PHARMACEUTICALS

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American red cranberries (*Vaccinium macrocarpon*) have gained great popularity among consumers because their beneficial health properties against urinary tract infections by controlling urine acidity and inhibiting *E. coli* proliferation which is the principal responsible for this kind of infections. Their antibacterial activity is attributed to its high content in different types of polyphenols such as flavonoles, anthocyanins, and phenolic acids and, more specifically, to the presence of A-type proanthocyanidins (PACs), which are oligomeric and polymeric forms of flavan-3-ols and they vary in cranberry according to the nature of the interflavan linkage. A-type PACs are those in which monomeric units are linked through one bond between the C4 position of the upper unit and the C6 or C8 positions of the lower unit (C4-C6 or C4-C8) and one bond between the C2 position of the upper unit and the hydroxyl group at C5 or C7 of the lower one (C2-O-C5 or C2-O-C7). Conversely, B-type PACs, which are not capable of inhibiting the adhesion of bacteria to urinary tract tissues, are those were only the first bond is present. Today, there are some concerns that some of the products sold in the market claiming to be derived from red cranberry come from other fruits like grapes or blueberries, which do not contain the adequate polyphenols to fight these infections. This fact shows the importance of developing analytical methodologies for the characterization and authentication of natural products and pharmaceuticals.

In this work, grape- and cranberry-based processed products and cranberry-based pharmaceutical preparations were analyzed by UHPLC-HRMS/MS (Orbitrap, Thermo Fisher Scientific). Separation was performed by C18 reversed-phase chromatography (Ascentix Express 150x2.1 mm, 2.7 µm) using 0.1% formic acid water and acetonitrile solutions as mobile phases. Full scan MS (*m/z* 100-1,500) at a resolution of 70,000 FWHM (full-width half-maximum) and data dependent MS/MS product ion spectra at a resolution of 17,500 FWHM were used to obtain metabolomic fingerprint profiles. On an untargeted approach, full scan MS raw data was employed as fingerprints to be treated by principal component analysis (PCA). After correcting and improving MS signal quality by using blank and standard mixture data, full scan MS metabolomic fingerprints allowed the classification of the analyzed products regarding the fruit of origin. On a targeted approach, polyphenolic profiles (including PACs) were also employed for characterization. For that purpose, MS data was processed by ExactFinder v2.0 software by applying a customized target database list of polyphenols. The most remarkable polyphenols were identified and selected to achieve characterization of natural products and pharmaceuticals.

## P-41
## CAPABILITIES OF HIGH RESULUTION MASS SPECTROMETRY TO ASSESS SMALL ODOR-ACTIVE THIOLS IN FOOD AT SUB-ng/kg LEVELS

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Volatile thiols are powerful odor compounds that contribute significantly to the sensory properties of several food and beverages such as wine, beer, and coffee, and other foods undergoing Maillard reaction [1]. Due to their extremely low odor thresholds, they exhibit significant sensory impact even at very low concentrations. The analytical assay of volatile thiols in food is mainly hindered by their low concentration and by the high reactivity of the free thiol (-SH) group. A selective derivatization strategy is the key for the stabilization of free thiol group, and for volatile thiol labeling.

With the aim to dispose of a simple, reliable, selective and sensitive method for the assessment of volatile thiols at trace levels in foods and beverages, with minimum sample manipulation, a single-step derivatization/extraction method followed by HPLC-ESI-HRMS was developed, using ebselen as derivatization reagent [2]. The analytical conditions were optimized in model system and real samples of olive oil, wine and beer, and coffee.

HRMS provided incomparable confirmatory performances with excellent quantitative capabilities. The method was evaluated in terms of sensitivity, precision, accuracy and selectivity, and then applied to real samples. Moreover, the advantage of acquiring in full scan mode when working with HRMS, permitted a non-target approach, based on the fragmentation of thiol derivatives yielding a main product ion at m/z 275.9922 $[C_{13}H_{10}ONSe]^+$ and which corresponded to the ebselen moiety of the derivatives.

Sub-ppt experimental LOQs were below the sensory threshold available in the literature, and a significant influence of thiols on virgin olive oil, coffee and beer aroma was revealed by combining derivatization-ESI-LC-HRMS and sensory analysis. In addition, the non-target screening allowed identifying in these foods and beverages, new volatile thiols whose sensory influence deserves to be further investigated.

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## P-42
## DIRECT CHARACTERIZATION OF FOODSTUFFS USING LOW-TEMPERATURE PLASMA (LTP) AMBIENT IONIZATION MASS SPECTROMETRY

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The increasing concern about the potential health risks posed by the presence of toxic residues in the human diet has focused sight on food quality and safety. This results in a need for authentication of food samples by means of straightforward approaches to determine their provenance, quality, and possible adulteration. Ideal analytical methods require minimal or no sample preparation, such as for instance those based on ambient mass spectrometry, which combines the classic outstanding performance of mass spectrometry in terms of sensitivity and selectivity along with convenient features related with the lack of sample workup required. Amongst ambient mass spectrometry methods, Low Temperature Plasma (LTP) probe is a plasma-based ambient direct ionization technique constituted as follows: a glass tube, an internally grounded metal electrode centered axially, and an electrode surrounding the outside of the tube [1]. An AC voltage and helium are used to generate the plasma, and also to transport the analyte ions towards the inlet of the mass spectrometer (MS). The sampling plasma torch operates at low temperature (30 ºC) interacting directly with the sample, leading to desorption and ionization of the surface molecules in the ambient environment.

In the present communication, the usefulness of LTP-MS as a quick method for rapid food analysis is shown with two examples of high-value commodities: extra virgin olive oil and wine. Two different approaches were examined: (i) direct analyses of wine or olive oil with no prior treatment, and; (ii) analyses of sample extracts obtained after a simple dilution in the case of wines or a quick liquid-liquid extraction to shift the measurement towards a specific part of the composition of the edible oil (i.e. polyphenol rich fraction or lipid/fatty acid profile). Examples of relevant compounds identified in a wide range of concentration levels, from post-harvest fungicides pesticides to polyphenols such as elenolic acid or oleocanthal are included to demonstrate the performance of this type of ionization methods.

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## P-43
## EXPLORING NANOFLOW LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY FOR PESTICIDE TESTING IN FOOD

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Pesticide testing in food is based on the use of multiresidue methods based on a generic extraction procedure, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [1], followed by analysis using GC-MS/MS and UHPLC-MS/MS. Usually, in LC-MS/MS, LC flow rates exceed 400 µL/min and combined with small-particle size columns provide excellent peak shape and results although at the expense of relatively high solvent consumption. The reduction of flow rates in electrospray detection yields to an increase in sensitivity, which can be used for increasing the ruggedness of methods by means of, for instance the dilution of the sample extracts, thus minimizing matrix effects. Recently, the use of microflow-liquid chromatography tandem mass spectrometry has been proven to be an interesting alternative to standard analytical size approaches [2], provided the significant benefits in terms of sensitivity and matrix effect reduction.

In this sense, the use of nanoflow liquid chromatography coupled to nanospray mass spectrometry detection has been restricted so far to selected bioanalytical applications (*eg.* proteomics), bearing in mind the difficulties associated to adapt such specialized approaches to routine applications. The relatively recent introduction of more robust and reproducible ultra-high pressure nanoflow LC instrumentation along with new column technology integrating nanoLC column and nano-ESI spray emitter in an easy-to-use plug-and-play fashion has made accessible such sophisticated approach to routine work, avoiding typical nanoLC issues such as leaks and minimizing other problems related to dead volumes/junctions. In this communication, the performance of nano-flow UHPLC combined with high-resolution mass spectrometry (using an orbital ion trap analyzer) has been evaluated for pesticide determination in food. Selected aspects such as retention time and peak area reproducibility, method sensitivity, column tolerance and endurance to different matrices and matrix effects have been evaluated for a suite of over 60 multiclass pesticides.

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## P-44
## SCREENING FOR HUNDREDS OF PESTICIDE RESIDUES USING A GC/Q-TOF WITH AN EXACT MASS PESTICIDE DATABASE IN VARIOUS FOOD MATRICES

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With increased international trade in food and food ingredients, there is even more emphasis on food safety. State-of-the-art pesticide screening requires the consideration of more than 1,000 pesticides and their metabolites. Of these, as many as 600 to 700 compounds can be included in routine monitoring programs. Testing approaches must be able to handle many compounds at a time whilst being able to avoid matrix interferences coming from many different food matrices. The increasing global emphasis on pesticide screening is reflected in the implementation of European Union (EU) guideline SANCO/12571/2013. The most recent revision specifies criteria for qualitative screening without the use of expensive standards for each pesticide in each batch of samples. An accurate-mass approach in pesticide screening using quadrupole time-of-flight mass spectrometry (Q-TOF) ensures reliable pesticide identification under this approach, and allows for a virtually unlimited number of compounds to be screened simultaneously. For many of the most important compounds gas chromatography (GC) coupled to a Q-TOF mass spectrometer is the ideal analytical tool for screening, confirmation, and quantification of both target and unexpected compounds at trace levels, even in complex matrices.

This workflow automates the screening for more than 700 pesticides that are contained in a Personal Compound Database and Library (PCDL). This Library is easy to customize, e.g adding new compounds.

For any unexpected compounds the user can quickly verify the identities of such compounds with high resolution accurate mass data, and if subsequent quantitative screening is considered important for future work then they can easily export the critical ion information into a quantitative method, if necessary hundreds of pesticides can be quantified in a single analysis.

The 7200 Series GC/Q-TOF combined with the MassHunter Qual and the GC/Q-TOF Pesticide PCDL, can be used effectively to screen for pesticide residues in a variety of matrices. The advantages of the GC/Q-TOF include the increased confidence in compound confirmation provided by accurate mass-high resolution data, the ability to perform retrospective analysis (particularly for unexpected peaks) and the ability to seamlessly go from qualitative to quantitative analysis.

This is encouraging because as new compounds appear on our laboratory's radar, we cannot only re-interrogate data we collected in the past but we will also have a quick way to create and expand optimized quantitative methods for the future.

## P-45
## ADVANCED MULTI-TARGET COMPARATIVE SCREENING USING HIGH RESOLUTION AND ACCURATE MASS LC-MS/MS

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A fast and straightforward method to screen for various classes of residual chemicals, including pesticides, mycotoxins, plant toxins, and veterinary drugs, in food and feed matrices was developed. Samples were extracted using a simple extraction with acidified water and acetonitrile followed by dilution. Sample extracts were analyzed using liquid chromatography (LC) and high resolution accurate mass tandem mass spectrometry (MS/MS) using the AB SCIEX TripleTOF™ 5600 system.
TOF-MS information was used to screen for targeted food contaminants. Quantitative information was achieved by performing single concentration standard addition at the level of the Maximum Residue Level (MRL). Identification was based on retention time, accurate mass quasimolecular ion, isotopic pattern and MS/MS fragmentation pattern.
Data were processed using a newly developed smart data review tool allowing quantitative comparison of samples and automatic compound identification based on retention time, accurate mass, isotopic pattern, and MS/MS library searching.

## P-46
## IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN EXHALED BREATH USING SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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The vast majority of analytical methods for detecting and monitoring diseases are based on blood or urine analysis. Diagnosis based on breath analysis is less developed and not yet regularly used in clinical practice but it offers some advantages over conventional medical tests like their non- invasive nature, low cost and safety, [1,2]. Human exhaled breath contains more than 3,000 different Volatile Organic Compounds (VOCs), which could be potential biomarkers for the early detection of malignant diseases [3].

This work presents the identification of several VOCs in exhaled breath using a methodology that combines solid-phase microextraction (SPME) with a fiber of 65µm polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 1L Tedlar bags. The extracted volatile compounds were analyzed by GC-MS with an electron ionization source. The method allowed the identification of more than 40 different compounds using a 60m long chromatographic column. Also, the measurement of $\delta\ ^{13}C$-values using GC-C-IRMS (gas chromatography combustion isotope ratio mass spectrometry) was carried out to check if these compounds came from exhaled breath or from other contamination sources like Tedlar bags laboratory atmosphere or SPME fiber.

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## P-47
## INVESTIGATION OF NEW PSYCHOACTIVE SUBSTANCES IN WASTEWATERS FROM SWITZERLAND

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New psychoactive substances (NPS) are analogues of conventional illicit drugs like cocaine, cannabis and amphetamines, which are designed to produce effects similar to the controlled substances. Introducing only minor modifications to chemical structures they may evade law enforcement [1]. NPS, often purchased through Internet, have become a major issue in recent years in numerous countries around the world. Their prevalence among the general population is a recurring question raised by stakeholders in Switzerland. According to information provided by the police and analyses carried out in forensic laboratories, the number of seizures containing these substances is limited. The majority of seizures are made by Swiss customs inspecting suspect parcels entering the territory. These are then analyzed and characterized by a centralized laboratory that feeds a database containing 262 different compounds. According to the authorities, 12 out of these were recurrent (seized more than once) in the past six months. Still, little is known about the consumption prevalence and the current belief is that these substances represent only a niche of the actual drug market. However, there is no data to support this hypothesis.

In an attempt to answer these questions, the present work investigated the possibility of detecting the consumption of these substances by analysing wastewater samples with high-resolution mass spectrometry. Samples collected in Lausanne, Neuchâtel and during two music festivals in Switzerland were analysed for their content in NPS. A wide-scope screening was applied based on the use of quadrupole time-of-flight (QTOF) analyzer, which provides sensitive, full-spectrum acquisition MS data with high mass resolution and accuracy (<5 ppm) [2]. Samples were analysed under $MS^E$ mode and results were confronted to a database containing around 300 NPS. The potential presence of a compound was based on the accurate mass of the (de)protonated molecule (low energy $MS^E$ function). Subsequently, fragmentation patterns for the candidates (high energy $MS^E$ function) were included in data analysis and confronted to the molecular structure and to the literature (when available). Using this approach, it was possible to focus on five NPS potentially present in the samples without the use of reference standards. These were finally purchased and used to confirm (or confute) the identity of the retained compounds.

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## P-48
## EXPLORING RELATIONSHIPS IN COMPLEX MASS SPECTROMETRY DATA USING AGILENT MASS PROFILER PROFESSIONAL CHEMOMETRIC DATA ANALYSIS AND VISUALIZATION SOFTWARE

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Mass Profiler Professional (MPP) is an statistical & data visualization tool designed to address challenges associated with Chemometrics data which allows researchers to easily import, analyze and visualize GC/MS, LC/MS, CE/MS and ICP-MS data from large and complex data sets.

MPP provides rapid and powerful mass data analysis of metabolomics, proteomics, food safety, environmental, forensics and toxicology datasets in the context of biological pathways.

It features selected normalization methods, an extensive array of plotting functions and a comprehensive hypothesis testing scheme including also tools as ANOVA, PCA, t-tests, volcano plots, clustering algorithms and class prediction algorithms

MPP supports multiple organism pathway ontologies and seamlessly integrates data from different sources.

Compound identification is supported by an integrated identification tool (ID Browser) to search against LC/MS Databases (METLIN, pesticides, forensics), GC/MS libraries (NIST and Agilent Fiehn Metabolomics), and LC-MS/MS libraries

The Graphical User Interface (GUI) is designed to be very intuitive and user friendly.

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# XV SCIENTIFIC MEETING OF THE SPANISH SOCIETY OF CHROMATOGRAPHY AND RELATED TECHNIQUES (SECyTA2015)

## PL-01
## LIPIDOMICS BASED ON HIGH RESOLUTION MASS SPECTROMETRY: A NOVEL STRATEGY EMPLOYED IN FOOD AND NUTRITION RESEARCH

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In this study, we focused on a comprehensive analysis of lipids that represent a large and diverse group of naturally-occurring compounds in living organisms. For an in-depth structural and quantitative characterization of various lipid classes and their distribution within biotic matrices, instrumental platforms based on advanced chromatographic methods coupled with high resolution mass spectrometric (HRMS) detection represent currently the most challenging option. In our experiments, we focused on an application of these novel strategies for monitoring of lipids changes under heat processing / storage. i.e.. under conditions when they may undergo various degradative processes such as oxidation. Worth to notice that compounds generated through oxidation reactions are related to undesirable sensory and biological effects. In addition to cytotoxic and genotoxic compounds, free radicals, products of lipids peroxidation also co-oxidize some vitamins, and thereby impair the nutritional quality of the foods. Although a high number of methodologies enabling determination of both primary and secondary oxidation products has been developed and implemented, some of these ´classic´ approaches such as peroxide value illustrating early stages of lipids oxidation, are highly empirical and their accuracy is rather questionable Not surprising that introduction of modern instrumental techniques that enable rapid obtaining of more comprehensive and specific information has been urgently needed. In addition to implementation of modern analytical methods for assessment of lipids qualitative parameters, we also were concerned with introduction of novel non-target screening strategies enabling lipids authentication. This presentation involves several case studies documents challenges in lipids analysis: (i) Simple and fast sample preparation strategy based on partition in ternary solvent system enabling, in a single step, fractionation of fish lipid classes according their polarity; (ii) application of an ambient high resolution mass spectrometry (HRMS) employing direct Analysis in Real Time (DART) ion source for an assessment of lipids quality with regards to content of primary and secondary oxidation products; (iii) Authentication of food lipids using fingerprinting / profiling strategy based on DART-HRMS; (iv) Employing supercritical fluid chromatography (SFC) coupled with high definition mass spectrometry (involving ion mobility, IM) for analysis of frying oils and lipids isolated from human/ animal adipose tissue. The performance parameters achievable by the above analytical strategies are critically assessed.

***Keywords**: lipids, oxidized lipids, supercritical fluid chromatography, high resolution mass spectrometry, ion mobility, fingerprinting, authentication*

## PL-02
## COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY AS A POWERFUL TOOL FOR FOOD APPLICATIONS

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Food products are very complex mixtures containing many nutrients of organic and inorganic nature. Their analysis may be directed to the assessment of food safety and authenticity, the control of a technological process, the determination of nutritional values as well as the detection of molecules with a possible beneficial or a toxic effect on human health.
One-dimensional liquid chromatography coupled to photodiode array (PDA) and mass spectrometry (MS) detection is one of the most widely applied analytical techniques for the analysis of food samples. Although such a method often provides rewarding analytical results, the complexity of many naturally occurring real-world samples exceeds the capacity of any single separation system. As a consequence, the use of multidimensional chromatography (MD-LC) becomes mandatory whenever the complexity of the sample overwhelm the resolution capability of any one-dimensional separation technique. To this regard, comprehensive (2D) techniques in which the whole eluate from the first dimension ($^1$D) is fractionated onto the second dimension ($^2$D) column may be a valuable tool through careful selection of independent (orthogonal) separation modes. Moreover, from a detection viewpoint, MS and tandem MS techniques could be successfully employed for detailed structure elucidation through characteristic fragmentation pattern. A brief overview on method development methodologies as well as selected applications in the field of food analysis are here provided.

## PL-03
## INVESTIGATION OF METABOLITES/TRANSFORMATION PRODUCTS OF EMERGING CONTAMINANTS IN THE AQUATIC ENVIRONMENT BY HRMS

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The investigation of metabolites/transformation products (TPs) of different organic contaminants in waters is a current topic of concern because of these compounds are commonly present in the aquatic environment at concentrations even higher than the parent compound. This is an analytical challenge due to the huge number of metabolites/TPs that may be present in waters. In addition, many of these compounds are still unknown and/or no commercial reference standards are available yet. The role of modern analytical chemistry is not only the detection and identification of a large number of TPs but also the discovering of new compounds still unreported.

The use of LC-HRMS appears nowadays as the most efficient way to investigate TPs in the aquatic environment. Accurate-mass full-spectrum acquisition measurements and the reasonable sensitivity provided by HRMS are the key of the strong potential of this technique to this aim. This fact together with the robustness, versatility and compatibility of LC for determination of polar, even ionic, compounds, made that LC-HRMS is at present the preferred technique to investigate metabolites/TPs (commonly of medium-high polarity) of organic pollutants/residues in different applied fields, such as environmental pollution, toxicology or food-safety, among others.

In this conference, different strategies are presented to investigate organic contaminants' TPs in waters by HRMS. Examples given are focused on emerging contaminants (pharmaceuticals and drugs of abuse) and on the use of QTOF MS. Several situations will be considered: 1) detection and identification of known metabolites/TPs, already reported in the bibliography; 2) investigation of unknown metabolites/TPs, where the use of "fragmentation-degradation" relationship between the parent compound and TPs may allow discovering new/unexpected compounds; 3) prediction of possible TPs using computational (in-silico) prediction tools; 4) use of laboratory experiments under controlled conditions, which allows to identify compounds that may be expected in the aquatic environment for selected contaminants (after the sample being subjected to different processes, such as hydrolysis, photodegradation, chlorination, biodegradation, etc).

The methodology applied also depends in a great deal on the availability of reference standards. Obviously, reference standards make the research of any compound much easier and are compulsory for confirmation of the identity of the compound detected. But in HRMS is possible to perform this research without standards being available, as tentative identification is feasible thanks to the great amount of information provided by this powerful technique.

## KN-01
## STRATEGIES AND TECHNIQUES FOR IDENTIFYING UNKNOWN COMPOUNDS IN ENVIRONMENTAL SAMPLES

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There are approximately 100,000 industrial chemicals or chemicals of commerce used currently, but only a small fraction are monitored routinely. The Stockholm Convention on persistent organic pollutants (POPs) targets 24 halogenated organic compounds or compound classes. Environmental samples can contain thousands of non-targeted compounds, some of which may pose a risk to the environment or human health. Their identification can be very challenging because most routine analytical techniques are blind to non-targeted compounds.

(Ultra)High resolution mass spectrometry (MS) and multidimensional gas chromatography (GCxGC) are complementary analytical techniques for non-targeted analysis of environmental samples. This contribution deals with the strategies implemented by our group to identify halogenated compounds in a range of challenging environmental samples.

Multidimensional chromatography offers unparalleled separating power which often displays thousands of peaks and the task of reviewing and interpreting such enormous data sets can be extremely laborious and time-consuming. One strategy is to use automated software (scripts) to rapidly and automatically identify halogenated compounds in complex GCxGC-TOF chromatograms using isotope patterns of Cl and Br.

Another approach for the identification of halogenated compounds exploits the negative mass defects of F, Cl and Br. (Ultra)high resolution mass spectra of environmental samples often display thousands of peaks. Distinguishing halogenated and non-halogenated peaks is can be a significant challenge, but is made straightforward by constructing a mass defect plot: the nominal mass of each mass spectral peak is graphed vs. the corresponding mass defect. Examples of complex environmental samples that have been characterized using one or a combination of the approaches described above will be presented.

## KN-02
## NEW ANALYTICAL STRATEGIES FOR SORPTION-BASED METHODS

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In the past two decades, the sorption-based methods have played an important role in analytical chemistry, by proposing miniaturized devices with convenient design, sorbent coatings with selective phases, easy manipulation of the analytical procedures, as well as, low sample volume requirements and negligible or even the absence of organic solvents, in compliance with the green analytical chemistry principles [1].
Some of the currently well-established, successfully or more accepted sorbent-based methodologies use the passive or static sampling mode, in which apart from other possibilities, solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) are actually the most applied in many scientific areas. For trace analysis in particular, both are effective, very easy to implement and have demonstrating high-throughput to enhance selectivity and sensitivity prior the combination with chromatographic or hyphenated systems. Moreover, these techniques can use the headspace and immersion sampling modes for the enrichment proposes depending on the type of analytes involved, *i.e.* volatile, semi-volatile or non-volatile compounds. Besides SPME allows selecting the right sorbent phase polarity, presents in many cases low capacity in particular for ultra-trace analysis, the fibbers are expensive and is an approach more indicated for gas chromatography analysis. On the other hand, SBSE uses almost exclusively the nonpolar polydimethylsiloxane phase and, although present much higher capacity than SPME, presents lack of selectivity if polar targets are involved [2].
In the recent past, we introduced a new technique having bar-shaped geometry, *i.e.* bar adsorptive microextraction (BAμE), that operates under the “floating sampling technology” and uses a different and more advanced enrichment mode [3]. During the static operation, the analytes migrating by diffusion from the sample bulk to the sorbent phase that coats the analytical device, under a free-floating process. Several sorbent phases have already been tested with remarkable performance, including many types of activated carbons, polymers, mixtures, etc. Furthermore, this new microextraction technique has been successfully applied in the analysis of several classes of polar compounds in many types of real matrices.
More recently, we have been involved in the use of polyurethanes as a new sorbent matrix, since this type of polymers can ideally combine the sorption and mechanical properties for both microextraction and back-extraction steps, respectively [4]. On the other hand, this approach reduces the manipulation, the overall time required for sample preparation, and is also indicated to be combined with the great sensitivity of the modern instrumental systems. The present contribution is an overview on the state-of-the-art and novel trends for sorption-based microextraction techniques, including the discussion of advantages, practical manipulation, cost, selectivity, sensitivity, as well as, the best strategy for trace analysis of priority compounds in complex samples.

## KN-03
## ANALYTICAL DEVELOPMENTS AND BIOMEDICAL APPLICATIONS OF CAPILLARY ELECTROPHORESIS IN NON-TARGETED METABOLOMICS

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The comprehensive measurement of the metabolic response of an organism to a stimuli (disease, diet, drug,…) without a priori hypothesis is the aim of non-targeted metabolomics. This global approach is really promising in a discovery phase, as unexpected or never thought results can be obtained; however obtaining a really global metabolic profile of a biological sample is challenging, because there are many different classes of physicochemical properties and ranges of concentrations that one single technique is not able to get them all.

Metabolite coverage is important in a fishing experiment, because otherwise results are biased by the technique. In that context, analytical techniques with different selectivity such as capillary electrophoresis play a key role, despite of its drawbacks, which are also well known.

Metabolomics studies at CEMBIO go usually through a multiplatform approach including LC-MS, GC-MS and CE-MS. The latter being especially suited for ionic or polar compounds in aqueous or highly ionic media, such as urine or cell culture media. In addition to those samples, an appropriate pre-treatment can make CE-MS useful for other matrices such as plasma, parasites, cells or tissue extracts. Moreover, we have developed the tools to improve identification capabilities in CE-TOF-MS or even semiquantification of co-eluting compounds.

CE-MS was the driver when explaining the mechanism of action of rosemary extracts in an animal model of oxidative stress in urine [1]; resistance to antimonium in leishmania parasites [2]; ventilator induced lung injury in mice [3], among others and results will be discussed.

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## O-01
## MULTI-COMPOUND GC-MS ANALYSIS OF ORGANIC TRACERS IN ATMOSPHERIC SAMPLES

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Atmospheric aerosols influence climate and air quality, and contain significant but variable fractions of organic material. The combination of gaseous phase and particle size filter sampling techniques coupled to gas chromatography-mass spectrometry (GC-MS) allows in-deep organic tracer speciation that is useful to re-construct the emissions from multiple sources and to get insight into the formation processes of Secondary Organic Aerosols (SOA) after oxidation of volatile organic compounds (VOC) (Alier et al., 2013; Fontal et al. 2015; van Drooge et al. 2015).

This work presents analytical methodologies to detect and quantify organic tracer compounds in gaseous and particulate phase samples of the atmosphere, in order to chemically characterize the size distribution of the organic aerosol (OA) at urban and rural sites in the Western Mediterranean Basin (WMB).

Urban areas in the WMB are characterized by highest vehicle and population densities. Moreover, its geographical position favors photo-chemical reactions of VOC and accumulation of SOA. Rural sites, in forested areas, are exposed to biomass burning in winter for domestic heating, while biogenic VOC emissions in summer generate SOA.

The applied methodologies allow the quantification of organic compounds over a wide range of polarity, including acids, sugars and lipids. Analysis of more than 100 organic tracer compounds showed that the composition of submicron particulate matter (PM<1) is more uniform than the larger size PM, and consisted mainly of combustion products and compounds that are photo-chemically formed (SOA), either from anthropogenic or biogenic VOC precursors. Larger PM was composed of mixed sources from combustion processes, vegetation emissions, soil re-suspension, road dust and urban life-style activities (Primary OA). Important seasonal differences were observed in rural sites, with predominance of biomass burning in the cold period and photo-chemically transformed biogenic organic compounds in the warm period.

Chemometric tools (MCR-ALS) have been applied on the data for source apportionment purposes. Figure 1 shows an example of this data analysis between an Urban and a Rural site, where the analyzed organic tracer compounds essentially originated from six source components. Four of them reflected primary emissions related with either natural products, e.g. vegetation emissions and up whirled soil dust, or anthropogenic contributions, e.g. combustion products and compounds related with urban lifestyle activities, mainly vehicular exhausts and tobacco smoking. Two secondary organic aerosol components were identified. They accumulated in the smallest (<0.5 µm) or in the larger fractions (>0.5 µm) and involved strong or mild photo-chemical transformations of vegetation precursor molecules, respectively (Figure 1).

Toxicologically relevant information was also disclosed with the present approach. The strong predominance of biomass burning residues in the rural site during the cold period involved atmospheric concentrations of polycyclic aromatic hydrocarbons that were three times higher than in the urban sites and benzo[a]pyrene concentrations above legal recommendations.

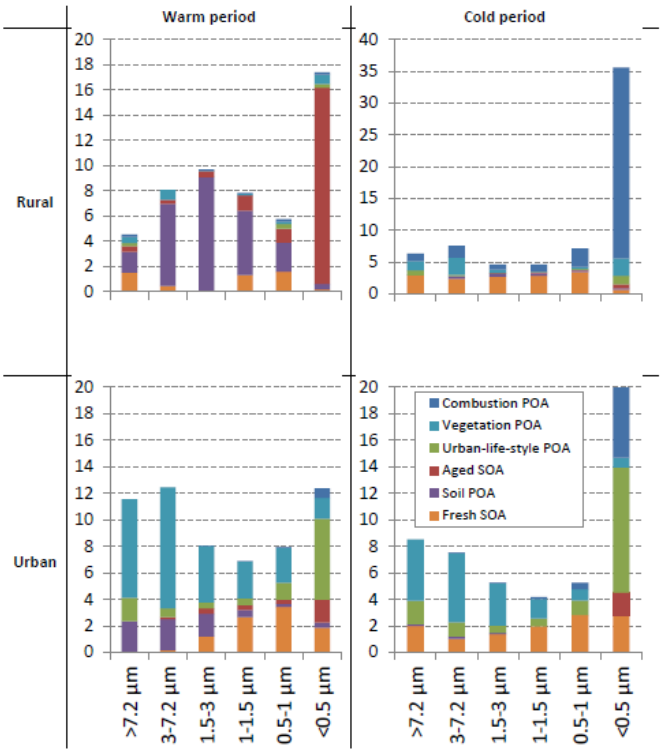


Figure 1. average ∑score-values from the MCR-ALS

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## O-02
## DETERMINATION OF GASOLINA RANGE ORGANICS IN WATER BY HEAD SPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Pollution of surface and underground waters by petroleum hydrocarbons, as well as in soils, is a very common problem in Spain and in the world. It is usually generated by spillage of fuel at petrol stations, during the refining of crude oil in oil industries, as well as during fractionation and distribution of fuels and lubricating oils. This type of pollution can alter the organoleptic characteristics of water sources by inducing rejection of consumers and represent a risk for the health and the ecosystem. Markers of possible water contaminations are gasoline range organics (GRO) which correspond to the range of alkanes from $C_6$ to $C_{10}$ and covering a boiling point range of approximately 60 ºC to 170ºC.

A quantitative method for the determination of the different fractions of the gasoline in function of the number of carbons has been developed, validated and accredited. The procedure is based on solid phase microextraction from the sample headspace followed by determination by gas chromatography mass spectrometry (HS-SPME-GC-MS). Extraction was carried out using 10 ml of water sample in a 20 ml vial, adding 25 % of NaCl, with magnetic stirring, and a Polydimethylsiloxane 100 µm fiber for 30 minutes at a 40ºC. Chromatographic determination was carried out by GC-MS under full scan mode.

The analyzed fractions of gasoline were: Aliphatic Hydrocarbon $C_5$-$C_6$, Aliphatic Hydrocarbon >$C_6$-$C_8$, Aliphatic Hydrocarbon > $C_8$-$C_{10}$, Aromatic Hydrocarbon $C_6$-$C_7$, Aromatic Hydrocarbon > $C_7$-$C_8$ and Aromatic Hydrocarbon > $C_8$-$C_{10}$. The sum of these six fractions gave us the total organic gasoline range (GRO). Method performance is comparable to that of current protocols for GRO determination and validated further by comparisons to certified standards, interlaboratory proficiency tests and in house standards. This procedure was accredited by the Spanish National Accreditation Body (ENAC, Entidad Nacional de Acreditación) according to the requirements of UNE-EN-ISO/IEC 17025.

The method validation was performed in drinking, continental, sea and waste water at three different concentration levels. The quantification detection limit (LOQ) of the different fractions varied between 2.0 and 5 µg $L^{-1}$ and the LOQ for GRO was 50 µg $L^{-1}$. The precision and the accuracy obtained were in all cases around 10 % and 90% respectively.

## O-03
## ANALYSIS OF FULLERENES IN THE ENVIRONMENT BY LC-APPI-HRMS AND PRELIMINARY METABOLOMIC STUDIES

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The occurrence, fate and behaviour of nanomaterials (NMs) in aquatic environments, their potential uptake by primary producers and posterior scaling up through the trophic chain are different aspects that remains relatively little understood. To tackle these issues mesocosms experiments are required as first step.
Here, we present the assessment of seven fullerenes (C60 and C70 fullerenes and 5 functionalised fullerenes, N-methylfulleropyrrolidine, [6,6]-phenyl C61 butyric acid methyl ester, [6,6]-thienyl C61 butyric acid methyl ester, C60 pyrrolidinetris-acid ethyl ester and [6,6]-phenyl C71 butyric acid methyl ester) in environmental samples (river water and sediments, wastewater effluents and soils) using an analytical method based on liquid chromatography (LC), using a pyrenylpropyl group bonded silica based column, coupled to a high-resolution mass spectrometer (HRMS), using a dual ion source, Atmospheric Pressure Photoionisation / Atmospheric Pressure Chemical Ionisation source (APCI/APPI).
In addition, to assess potential damages in receptor environments, we have performed a series of experiments based on marine mesocosms to assess the uptake of carbon-based NMs by primary producers and their potential scale-up by filter-feeders (Mytilus galloprovincialis). In this work, we have also conducted preliminary metabolomic studies to evaluate the sub-lethal toxicity of carbon based NMs to filter-feeding organisms. Primary results confirmed the uptake and scaling up of NMs through the aquatic trophic chain as well as different changes in essential amino acid with specific effects on feed consumption and weight gain, especially in the initial stage of animal production.

## O-04
## EVALUATION OF RETENTION MECHANISMS OF BENZOTRIAZOLES, BENZOTHIAZOLES AND BENZENESULFONAMIDES ON SOLID-PHASE EXTRACTION SORBENTS WITH STRONG CATIONIC OR ANIONIC MIXED-MODE PROPERTIES

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Benzotriazoles (BTRs), benzothiazoles (BTs) and benzesulfonamides (BSAs) are high production volume substances with a wide range of industrial applications which can reach environmental waters becoming emerging contaminants [1,2]. Most of the analytical methods developed to determine these compounds include solid-phase extraction (SPE) followed by liquid chromatography (LC) coupled to mass spectrometry in tandem (MS/MS) or high resolution mass spectrometry (HRMS). Due to the particular chemical structure of the analytes, the retention of the BTRs, BTs and BSAs was studied on strong cationic and anionic exchange mixed-mode sorbents (Oasis MCX and MAX) to selectively extract these analytes. Their chemical structure do not possess ionic groups which could be retained by ionic interaction but in this study we confirmed that BTRs and BTs were successfully retained in the sorbents even when a wash step with methanol was included, which confirms the retention by ionic interaction. Therefore, we studied the mechanism of retention in depth and it seems that ionic interactions responsible of this retention behavior might be induced by the strong character of the quaternary amine and sulfonic acid groups of the sorbents. The BTRs and BTs have a delocalized electronic density that might be polarized and stabilized by the charges of the sorbent creating densities with opposite charges in the molecules allowing their interaction even when they are actually in their neutral state.

The methods using Oasis MAX and Oasis MCX were developed and compared when river and sewage waters were analyzed. The matrix effect was satisfactory in both methods (< 20 % in all the matrices) thanks to the possibility of adding efficient washing steps in the SPE procedure. The application of the methods to samples from the Ebre River and effluent and influent waters from a sewage treatment plant in Tarragona showed that several compounds of the target list were quantified and the concentrations obtained for both polymers showed no significant differences.

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## O-05
## APPLICATION OF HR-MS (Q-EXACTIVE-MS) FOR THE EVALUATION OF THE FATE OF DRUG GLUCURONIDES IN WASTEWATER TREATMENT PLANTS

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Pharmaceuticals are biotransformed in humans which lead to metabolites with different chemical structures and physico-chemical properties than their parent compounds. They are metabolized by a collection of drug-metabolizing enzymes such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and glutathione S-transferases which are present in the human liver at high abundance. After the excretion of pharmaceuticals in conjunction with their human metabolites they reach the wastewaters (WW). Little attention has been paid to the glucuronides of pharmaceuticals in WW. Only few works analyzed these conjugates in samples from wastewater treatment plants (WWTP) mainly using low-resolution mass spectrometry (MS) applying a target approach which involves the use of reference standards to quantify a list of target analytes. By contrast, the use of high-resolution MS platforms has laid the groundwork for a successful non-targeted analysis. For instance, suspect screening, in which one has information of the analytes but lacks reference standards, in combination with new developed software provides a more comprehensive overview of the contamination in any environmental sample of great complexity. Suspect screening has been used for the detection of pharmaceuticals and their human metabolites but it can also be used for the evaluation of the fate of drug glucuronides in WW samples. Drugs and their metabolites such as glucuronides can be also biotransformed by secondary treatments in WWTP. Some authors have proposed that drug glucuronides can be cleaved in the WWTP treatment. However, little evidence has been published as regards metabolic pathways of drug glucuronides in complex microbial communities like those encountered in the aeration tank of the activated sludge treatment. In our previous work[1], we accidentially detected non-predicted transformation products of lamotrigine glucuronide in wastewater effluent using FISH (an application from Mass Frontiers program, Thermofisher). Against this background, the present study aimed at investigating the fate of glucuronides in WWTPs through their detection and the evaluation of their biodegradation in WW samples. Selected glucuronides (lamotrigine-N2-glucuronide, sulfamethoxazole-N1-glucuronide, propranolol-O-glucuronide, tamazepam-O-glucuronide, diclofenac-acyl-glucuronide and atorvastatin-acyl-glucuronide) were degraded under controlled laboratory settings in order to gain further insight into the biodegradability and metabolic pathways of the selected compounds. The samples from the biodegradation studies were screened for the presence of stable intermediates and these were characterized by Q-exactive-Orbitrap-MS. In this study, several samples from influent and effluent WWTP samples were taken and analyzed. In this samples, it was observed differences in occurrence patterns of several glucuronides of pharmaceuticals. Further examination of mass spectral data of biodegradation of N-glucuronides has revealed the presence of closely related compounds in biorecators and also in real WWTP samples.

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## O-06
## UNVEILING POST MORTEM CHANGES IN MOUSE BRAIN BY A MULTIPLATFORM NON-TARGETED METABOLOMIC STUDY

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Metabolomics, the last of the omics sciences, studies the metabolites in biological samples, such as biological fluids or tissues. Non-targeted metabolomics is the global unbiased quantitative and qualitative analysis of all possible small molecules that constitute the entire metaboloma. The global throughput approach is based mainly on NMR or a combination of different separation techniques coupled to mass spectrometry [1].

Measurement of metabolites in precisely dissected regions of autopsied brain can provide useful information as to the biochemical bases of neurological and psychiatric disorders [2]. There are previous applications to study brain tissue for different purposes. [3]. Compared to biofluids, tissue collection and preservation generates intrinsic challenges in proteins and metabolites, especially with brain tissue because of its heterogeneity. In most cases the time necessary to separate different parts is not short and therefore a major problem here is to determine whether or not biochemical findings in autopsied brain are reasonably representative of those which prevailed during life.

The hippocampus is a mammalian brain structure which a fundamental role in cognitive functions. It is very vulnerable to damage and one of the earliest to be affected in age-related neurodegenerative pathologies, such as Alzheimer's disease and Parkinson. Metabolomic analysis was performed with hippocampus from left cerebral hemisphere of fifteen C57BL/6 mice. Samples were classified in three groups, differing in *post mortem* time: 30 min and 2 or 5 hours. For optimization of sample treatment, two extraction methods [4,5] were tested and best results were obtained using MeOH:$H_2O$ 50/50 (v/v) for homogenization and a combination of solvents MetOH:Methyl tert-butyl ether 320/80 (v/v/v) for extraction. With only 30 mg of tissue a multiplatform non-targeted metabolomic study based on UHPLC-ESI-QTOF-MS and GC-EI-MS-Q was performed. After analysis and data treatment, univariate and multivariate statistical analysis were applied for selecting statistically significant variables. Identification of metabolites was based on home-made & commercial libraries.

When comparing findings from different *post mortem* times as a proof of concept, different metabolites including amino acids, biogenic amines, carbohydrates, lipids and nucleosides resulted altered *post mortem* due to degradation of cellular structures, eventually cell lyses, and catabolic processes. Recommendations based on our findings will be presented.

Non-targeted multiplatform metabolomic approaches offer a powerful tool to study neurodegenerative and psychiatric diseases.

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## O-07
## UPLC-QTOF MS METABOLOMICS FOR BIOMARKER DISCOVERY IN CHEMICALLY NON-DIAGNOSED DISEASES: FETAL ALCOHOL SPECTRUM DISORDER

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Untargeted metabolomics have opened new ways of research in several fields. In the clinical field, this powerful approach allows for the discovery of new markers for accurate diagnostics and/or helps in highlighting the metabolic effects that treatments have on patients. For these reasons, the importance of metabolomics diseases has substantially increased. The combination of state-of-the art high resolution mass spectrometers in both positive and negative ionization mode with complementary chromatographic systems (RP and HILIC) is the option of choice since it provides a sensitive and comprehensive tool to achieve this challenge.
Fetal Alcohol Syndrome (FAS) is the principal preventable cause of mental retardation. The clinical features of FAS can be broadly divided into: growth retardation, craniofacial malformations and Central Nervous System impairment. Individuals with all of these categories of defects are at the most severely affected level of alcohol teratogenicity. The term Fetal Alcohol Spectrum Disorder (FASD) is used to describe the majority of FAS-related phenotypes. The objective of the study is to identify candidate biomarkers that contribute to identify children prenatally exposed to alcohol and reveal some metabolic pathways involved in the disease profile observed in FAS children. With this aim, we obtained blood samples from: children no-exposed (prenatally) to ethanol (control group; N=9), children diagnosed with partial FAS (N=6) and complete FAS (N=9). Briefly, after centrifugation, serum samples were analyzed by UHPLC-ESI-QTOF MS.
Samples were diluted with acetonitrile (1:4), centrifuged for protein precipitation and the supernatant was divided in two aliquots. The first one was directly injected in the system with a HILIC column. The other was dried and reconstituted with mobile phase before injection in a RP column. The injection in both chromatographic conditions allowed for maximizing the compound covering. Also for this purpose, samples were injected in both positive and negative ionization modes. Reliable peaks were extracted with XCMS (R free package), aligned and normalized. Partial Least Square – Discriminant Analysis (PLS-DA) was used to observe the behavior between the groups and the correct normalization by observing the QC grouping. Finally, Orthogonal PLS-DA was employed to find the most significant biomarkers. MS/MS experiments from about 40 highlighted compounds will support in the elucidation to study their relevance in feasible biological processes related to FAS disease will be studied. Some of these chemicals could become an important tool to diagnose or understand the processes affected by their disorder.

## O-08
## UNTARGETED DETECTION OF UNALTERED GLUCURONOCONJUGATED METABOLITES OF METANDIENONE IN SPORTS DRUG TESTING BY LC-MS/MS

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Metandienone (MTD), a synthetic anabolic androgenic steroid (AAS), is one of the most frequently detected in sports drug testing. MTD is extensively metabolized and excreted in urine as phase I and phase II metabolites, mainly as glucuronoconjugates. Traditionally, these metabolites are indirectly measured by the detection of the steroid released after enzymatic hydrolysis with β-glucuronidase. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) allows for the direct detection of phase II metabolites. The objective of the study was to evaluate the untargeted detection of unaltered glucuronide metabolites of MTD by LC-MS/MS.

Urine samples collected after administration of a single oral dose of MTD to a healthy volunteer were analyzed. The method consisted of a solid-phase extraction followed by LC-MS/MS analysis using a triple quadrupole instrument and electrospray ionization. Based on the common ionization and fragmentation for steroid glucuronides, three alternative strategies were designed for detecting them: (a) Precursor Ion (PI) scan methods of m/z 75, 85 and 113 in negative mode and, m/z 141, 159 and 177 in positive mode; (b) neutral loss (NL) scan methods of 176 and 194 Da for glucuronides that ionized as $[M+H]^+$ and, NL of 193, 211 and 229 Da for glucuronide metabolites that ionized as $[M+NH_4]^+$; and, (c) theoretical selected reaction monitoring (SRM) method for the detection of potential metabolites calculated for 35 different combination of phase I metabolic reactions. With open scan methods (both PI and NL) no significant differences were observed between pre- and post-administration samples, probably due to the low sensitivity. However, SRM methods allowed for the detection of 13 glucuronide metabolites in post-administration samples.

Some of the metabolites detected were previously reported after enzymatic hydrolysis. Among all 13 metabolites, two of them were identified by comparison with an standard synthesized in our laboratory (17-epimetendiol 3-glucuronide and 17,17-dimethyl-18-nor-5β-androsta-1,13-dien-3α-glucuronide). The feasible structure of several metabolites was proposed based on the molecular mass and the mass spectrometric data. The detection times were measured by analysis of samples collected after oral administration of MTD to four healthy volunteers. The analysis was performed using a SRM method including three characteristic ion transitions for each metabolite. Detection times were between one and 22 days. One of the glucuronides detected was resistant to β-glucuronidase, however it could only be detected in urine for up to two days after administration.

The strategy has demonstrated to be useful for detecting new glucuronide metabolites of AAS. Among all mass spectrometric methods used, the SRM approach resulted in a more sensitive methodology to detect potential metabolites when compared with open scan methods.

## O-09
## CYCLOBENZAPRINE MULTI-TARGETED MECHANISM AGAINST LEISHMANIA, METABOLOMIC AND FUNCIONAL STUDY FOR A DRUG REPURPOSING CANDIDATE

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The long and expensive drug development process has been an aggravating factor to neglected diseases. For example, since 1912, leishmanisis has been treated with very toxic, costly and long duration therapy besides alarming resistance. As alternative, the repurposing of available drugs to treat neglected diseases has proven to be convenient. It offers cost-effective, rapid means and clinical safety aspects. Interestingly, we found that cyclobenzaprine, an oral skeletal muscle relaxant has leishmanicidal activity. The cyclobenzaprine mechanisms involved in the death process in *Leishmania* are completely unknown. In humans, studies indicate that the blockade of serotonin 5-HT receptors at the brainstem level causes the relief of muscle spasm [1]. Above all, there is no description for those serotonergic receptors in *Leishmania.* To fulfill such gap, classical assays added to multi-analytical platform (CE-MS, GC-MS, LC-MS) untargeted metabolomics approaches were used.

Interestingly, cyclobenzaprine was not able to permeabilize the plasmatic membrane but it altered structural composition and fluidity. Lipids were the main biochemical class (70%) affected by the treatment, particularly glycerophospholipids (38%) and fatty acids (18%). The robust multi-analytical platform could identify almost all metabolites of polyunsaturated fatty acids (PUFA) biosynthesis pathway changed. *Leishmania* is the unique trypanosomid parasite to synthesize long PUFA such as C22:6 [2]. Also, ergosterol levels were found depleted. Ergosterol is a known sterol parasite-specific drug target in *Leishmania*. Furthermore, a depolarization of the plasma membrane was measured. The plasma membrane potential, with involvement of ATP-dependent pumps has been considered the driving force behind the maintenance of intracellular amino acids pool [3]. Once many amino acid transporters are located the plasma membrane it was not surprising that amino acids were the second biochemical class most affected. These membrane disturbances were followed by a rapid and consistent drop in intracellular ATP levels. More specifically, we found that the ATPase, the complex V in electron transport chain in the mitochondria, was blocked by cyclobenzaprine. In conclusion, cyclobenzaprine seems a multi-target leishmanicidal drug. It affects composition and fluidity of membranes, causes an active uptake of amino acids and blocks the ATP synthesis at mitochondrial level.

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## O-10
## COMMON ION LOSS SCAN FOR THE UNTARGETED DETECTION OF BIS-SULFATE METABOLITES

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Detection of untargeted metabolites is a common challenge in different application fields. MS based approaches are the gold standard for this purpose. Several strategies have been reported for metabolic studies. Thus, full scan acquisition with accurate mass measurements allows for the detection of all ionizable metabolites without any restriction but with limited structural information. On the other hand, neutral loss and precursor ion scan methods allow for the untargeted detection of metabolites sharing a specific structure. The development of additional open screening methods can help in the detection of previously reported metabolites.

In this study, we present a common ion loss strategy for the detection of bis-conjugated metabolites. Bis-sulfates of androgenic steroids have been selected as model compounds. Several bis-sulfates were synthesized and their MS behavior was studied. The chromatographic conditions were optimized in oder to have an adequate peak shape and separation between isomers. The ionization in negative mode of the model compounds showed an abundant $[M\text{-}2H]^{2-}$ together with the $[M\text{-}H]^{-}$. When selecting the $[M\text{-}2H]^{2-}$ as precursor ion, the CID spectra exhibited a common loss of $HSO_4^-$ (*m/z* 97) for all model compounds. These losses generated product ions with higher *m/z* than the precursor ion.

The low natural abundance of double charged metabolites and the use of ion transitions with an increase of *m/z* made the monitoring of these ion losses a very specific strategy for the target detection of bis-sulfates. For this reason, a common ion loss scan approach for the open screening of bis-sulfate metabolites was developed.

The suitability of the developed method was confirmed by the untargeted detection of several bis-sulfate metabolites in the analysis of real urine samples.

## O-11
## LC-MS/MS AND LC-HRMS TARGETED AND UNTARGETED APPROACHES FOR THE CHARACTERIZATION, CLASSIFICATION AND AUTHENTICATION OF NATURAL PRODUCTS

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Since several years ago, society is becoming very interested in the quality of food products, which are very complex mixtures consisting of naturally occurring compounds and other substances generally coming from technological processes, agrochemical treatments, or packaging materials. Although consumer preferences regarding food products are often influenced by organoleptic and socioeconomic factors, people are increasingly more interested in the presence of some specific compounds with health beneficial properties, thereby giving rise to the production of functional foods. Polyphenols, aromatic secondary metabolites ubiquitously spread through the plant kingdom, comprise more than 8,000 bioactive substances that caught the attention of consumers. The main reasons for that interest are the recognition of their antioxidant properties, their great abundance in our diet, and their probable role in the prevention of various diseases. However, nowadays there is a great concern about the possibility of frauds regarding the classification and authentication of, for instance, fruit processed products and pharmaceuticals. Typical applications deal with classification of wines according to their geographical origin and grape varieties, or authentication of pharmaceutical preparations claiming to contain specific polyphenols from some fruits with health beneficial properties.

The aim of this work is to present several LC-MS/MS and LC-HRMS targeted and non-targeted approaches to achieve the characterization, classification and authentication of natural products. On untargeted approaches, LC-HRMS full scan MS raw data was employed as metabolic fingerprint to be treated by principal components analysis (PCA). Examples of the application of this approach to the classification of wine samples depending on their geographical origin, and to the characterization of several mycorrhizal and no-mycorrhizal Rosemary plants will be discussed. On the targeted approach, LC-HRMS data was processed by ExactFinder v2.0 software by applying a customized target database list of more than 400 polyphenols and treated by PCA. This approach was applied to the classification of Spanish red wines and to the characterization and authentication of cranberry-based natural and pharmaceutical preparations. Moreover, the application of LC-API-MS/MS methods (using both ESI and APPI sources) by targeting 29 polyphenols to the characterization and authentication of cranberry-based foodstuffs will also be presented.

## O-12
## DIRECT ANALYSIS IN REAL TIME - HIGH RESOLUTION MASS SPECTROMETRY AS A VALUABLE TOOL FOR POLYPHENOLS PROFILING IN OLIVE OIL

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Polyphenols are one type of the numerous health-protective antioxidants in extra virgin olive oil (EVOO) [1, 2]. The European Food Safety Authority (EFSA) allows to establish health claims in relation to polyphenols in olive and protection of LDL particles from oxidative damage, maintenance of normal blood HDL-cholesterol concentrations, maintenance normal blood pressure, anti-inflammatory properties. An EVOO contains a large number of phenolic compounds that belong to the following classes: tyrosol and derivatives, derivatives of 4-hydroxybenzoic, 4-hydroxyphenylacetic, and 4-hydroxycinnamic acids, lignans, and flavonoids.
The objective of this study was to explore the potential of DART ionization coupled with high resolution mass spectrometry (DART–HRMS) in rapid identification of polyphenols in olive oil. The multivariate data compiled from DART-mass spectra were processed with principal component analysis (PCA) and linear discriminant analysis (LDA) to critically assess their discrimination potential. Results obtained using DART were compared to those obtained applying ultra-high pressure liquid chromatography (UHPLC)-LTQ Orbitrap-mass spectrometry (MS). To our knowledge, this is the first application of both DART source and LTQ Orbitrap to determine polyphenols in olive oil.
Simple, rapid and cost effective small-scale liquid-liquid extraction method based on dissolving the olive oil in hexane and extracting polyphenols with a mixture methanol-water provided the best results. A total of 32 compounds were recognised from MS and MS/MS dataset of LLE sample. These compounds were identified based on the empirical formula and literature data. A good performance, assessed in terms of repeatability, linearity, and sensitivity, was achieved. The validated method was applied to the qualitative determination phenolic compounds in collected EVOO extracts. A further great advantage of the method is the low solvent consumption making this technology environmentally friendly. The proposed method for the determination of polyphenols in olive oils makes use of a very simple system requiring small scale LLE.

[1] E. Scoditti, C. Capurso, A. Capurso, M. Massaro, *Vascular Pharmacology*, 63 (2014) 127-134.
[2] S. Lockyer, I. Rowland, 10 - Authorised EU health claims for polyphenols in olive oil, in: M.J. Sadler (Ed.) Foods, Nutrients and Food Ingredients with Authorised Eu Health Claims, Woodhead Publishing, 2014, pp. 212-228.

## O-13
## VALORIZATION OF FRUIT AND OLIVE PROCESSING BYPRODUCTS

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Fruit processing generates large amounts of wastes causing important environmental pollution and health risks. In other occasions, these byproducts are processed into low market-valued products such as animal feed. In order to contribute to environmental sustainability it is necessary to develop new approaches to reuse these wastes and to look for new opportunities of application [1].

Stones from olive (*Olea europeae*) and stone fruits (*Prunus* spp) such as peach, apricot, cherry, and plum have high protein contents. These seeds are cheap sources of proteins that are underused and undervalued. The aim of this work was to evaluate the presence of proteins and peptides with therapeutic properties in these byproducts as a strategy for their valorization.

Extraction of proteins from olive and fruit (peach, plum, apricot, and cherry) stones was optimized being possible to obtain seed protein isolates with protein contents higher than 95%. Gastrointestinal digestion of isolated proteins showed hydrolysates with moderate antihypertensive and antioxidant properties and no hypocholesterolemic capacity. Hydrolysis of extracted proteins using different enzymes (Alcalase, Thermolysine, Flavourzyme, etc.), under optimized conditions, enabled to obtain peptides with significant hypocholesterolemic, antioxidant, and antihypertensive properties. In general, hydrolysates showing the highest antihypertensive capacity were obtained using Thermolysin while hydrolysates yielding the highest antioxidant and hypocholesterolemic capacities were obtained with Alcalase. Further purification of most active hydrolysates enabled to isolate peptides with high antihypertensive capacity from the fruit stones and with very high hypocholesterolemic capacity from the olive stone. Peptides in most active fractions were identified by mass spectrometry and *de novo* sequencing. Most active peptides were submitted to studies to demonstrate their bioavailability.

[1] G. Deng, C. Shen, X. Xu, R Kuang, Y. Guo, L. Zeng, & H. Li, *International Journal of Molecular Sciences* **13** (2012) 8308-8323.

## O-14
## DETERMINACIÓN ULTRARRÁPIDA DE RESIDUOS DE PLAGUICIDAS MEDIANTE CROMATOGRAFIA DE GASES ACOPLADA A ESPECTROMETRÍA DE MASAS TRIPLE CUADRUPOLO EN MATRICES VEGETALES

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Se ha desarrollado un método ultrarrápido para la determinación de residuos de plaguicidas en matrices vegetales mediante el sistema Bruker EVOQ GC-MS TQ con el objetivo de reducir los costes y el tiempo total de análisis en laboratorios de rutina.

El tiempo total de adquisición cromatográfica resultó ser de 12.5 minutos para la cuantificación simultánea de 270 plaguicidas, utilizando al menos un ion de cuantificación y otro de confirmación para cada uno de ellos. Los límites de detección alcanzados fueron del orden de las sub-ppb para la gran mayoría de los compuestos bajo estudio, lo que lo hace un método ideal para análisis de alimentos ecológicos y destinados a alimentación infantil.

Se comprobó la robustez del método mediante la repetición de más de 100 análisis consecutivos sin realizar ningún tipo de operación sobre el equipo. Para lo cual se utilizó una disolución de un extracto QuEChERS de cebolla fortificado a un nivel de 10 ng/mL con todos los compuestos. Los valores de desviación estándar relativa en el área de los mismos fueron inferiores al 15% para prácticamente todos los analitos.

En todas las matrices se utilizó un extracto vegetal en acetonitrilo, tal y como se obtiene en el último paso de una extracción QuEChERS, evitando de esta forma la evaporación y reconstitución en un disolvente apolar, ya que esto supone el cuello de botella en la productividad analítica de los laboratorios de rutina en cuanto a la metodología GC-MS TQ se refiere.

## O-15
## IMPLEMENTATION OF METHODS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING SERIALLY-COUPLED COLUMNS

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A simple solution to expand the possibilities of high-performance liquid chromatography (HPLC) is to serially connect two or more columns containing different stationary phases. Nearly continuous transition of selectivity is yielded, which allows fine-tuning for each particular separation. Additionally, the analysis time is decreased making gradient elution less necessary. The effect of serially combining columns containing different stationary phases is comparable to multiplying the number of columns in the laboratory to hundreds, thousands or even millions, each of them behaving as a new column. The probability of getting the ideal column for a particular separation is highly increased. However, being the combination of different columns a highly powerful tool to resolve complex samples, its successful development and widespread application required solving some technical problems.

First, chromatographic columns should be connected without adding extra dead volumes between columns and without affecting the stationary phase nature near the column connections. We have solved these problems by connecting conventional chromatographic columns through zero dead volume fingertight couplers, which are screwed directly to the columns [1]. This is also a universal and reliable system which allows coupling of columns from different manufacturers.

Second, a system was needed to find the correct combination and optimize its performance. For this purpose, we adapted some tools developed in our laboratory for the optimization of single columns [1-4]. Finally, adequate software to properly exploit all the potential that the separation system offers should be developed.

This work summarizes the developments carried out to implement methods in HPLC using serially-coupled columns. The capabilities of the serial connection of columns is demonstrated taking as example the separation of a mixture of 15 sulfonamides considering five stationary phases of different nature (a conventional C18 column, C18 with increased carbon load, C18 with embedded polar groups, a phenyl phase and a cyano phase). All these columns showed insufficient performance for the separation of the probe compounds when used as single columns. However, with an optimized combination of columns, excellent separation in terms of resolution and analysis time was obtained. Similar improved separations are expected with other complex samples.

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[2] C. Ortiz-Bolsico, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, *J. Chromatogr. A* **1317** (2013) 39-48.
[3] C. Ortiz-Bolsico, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, *J. Chromatogr. A* **1350** (2014) 51-60.
[4] C. Ortiz-Bolsico, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, *J. Chromatogr. A* **1373** (2014) 51-60.

## O-16
## DETECTION OF NATURAL ADDITIVES IN A POLYLACTIC BASED POLYMER (PLA) USING PYROLYSIS COMPOUND SPECIFIC ISOTOPE ANALYSIS (Py-CSIA)

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Isotope ratio mass spectrometry (IRMS) is being extensively used to enlighten relevant scientific and technical questions important for science and the industry i.e. global element cycles, past climatic conditions, paleo diets, trace food sources/webs, polymer signatures/traceability, etc. Thus, isotopic analysis has become a key tool for scientists in many disciplines and the practical applications of the technique are continuously growing.

Compound-specific isotope analysis (CSIA) usually requires intermediate preparative procedures prior to chromatographic analysis to isolate analytes from bulk geological, biological or synthetic materials. In addition, non-volatile compounds must be made amenable to GC by derivatization or treated before chromatographic separation adding complication to the analytical process. Analytical pyrolysis is a long established technique that can help overcome preparative manipulation of samples. The sample is heated up in an inert atmosphere (usually He) to decompose into smaller units (pyrolysate) which are transferred for chromatographic separation to a GC connected to an appropriate detector.

In this communication we describe the results obtained by hyphenating analytical pyrolysis (Py-GC) with carbon IRMS for the analysis of a polylactic acid (PLA) based film extruded with variable quantities of natural plant extracts or essential oils for use in active food packaging.

Chemical structural information of pyrolysates was first determined by conventional analytical pyrolysis (Py-GC/MS). Bulk $\delta^{13}C$ measures were performed for each material by EA-IRMS. The direct study of $\delta^{13}C$ carbon isotopic signature in specific compounds was done by coupling a pyrolysis unit to a gas chromatograph connected (via Thermo Scientific GC-Isolink System) to a continuous flow IRMS unit (Py-GC-(FID)-EA-IRMS) [3]. Using this Py-CSIA device it was possible to trace natural additives with light $\delta^{13}C$ signatures derived from C3 photosystem vegetation, from the heavier bio-plastic backbone usually derived from corn (C4 vegetation) starch. Finally the results are discussed in terms of the potential of this new chromatographic application for food traceability and security.

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## O-17
## IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES BASED ON THE COMPLEMENTARY USE OF SPECTROMETRIC TECHNIQUES

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The increase in the number, type and availability of new psychoactive substances (NPS) on the European market with possible health and social risks is an issue of alarming concern [1]. NPS often have slight modifications to chemical structures of controlled substances and many of them are designed and intended as legal replacement of conventional illicit drugs like cocaine, cannabis, and amphetamines. During 2014 a total of 101 NPS were reported for the first time in Europe. Synthetic cannabinoids and cathinones account for almost 70% of the total number of seizures and have been introduced as legal alternatives to cannabis and stimulants such as amphetamine and MDMA [1]. Synthetic cannabinoids are mainly being sold mixed with herbs, but can also be bought in resin-like material, as powder, and even in e-cigarette refills. New legislation in some countries banned the so-called third generation of cannabinoid receptor agonists. The banned compounds were removed from the market by the sellers on the day the ban was in place prompting new compounds to be marketed, the fourth generation. These new substances were unknown to both forensic and clinical toxicologists and could be missed in routine analysis.

In this work, we surveyed the "legal highs" market looking for new substances and for the fourth generation of synthetic cannabinoids. Gas chromatography coupled to mass spectrometry (GC-EI-MS) and liquid chromatography coupled to quadrupole time of flight mass spectrometry (LC-QTOF MS) were used to detect and elucidate NPS based on their fragmentation pattern/common structures and by accurate-mass full-spectrum measurements. The tentative identification was feasible even when reference standards were not available. Twenty-six new synthetic cannabinoids were found during different sampling campaigns between December 2012 and February 2015. In one occasion, we received an unknown white powder from the UK market labeled as "idanyl-biphenyl-amninone", intercepted by customs at the island of Jersey. Elucidation of this unknown was troublesome and the combined use of different spectroscopic techniques was needed, *i.e.* GC-EI-MS, LC-QTOF MS, nuclear magnetic resonance (NMR) and finally X-ray crystallography. The information obtained on the identity of these NPS is of outstanding relevance for toxicology, forensic and clinical laboratories, and allows tracking of possible further spreading of these NPS worldwide.

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[2] L. A. King, A.T. Kicman, *Drug Testing and Analysis* **3** (2011) 401-403.

## O-18
## POTENTIAL OF GAS CHROMATOGRAPHY COUPLED WITH ORBITRAB-BASED MASS SPECTROMETRY FOR THE ANALYSIS OF HALOGENATED PERSISTENT ORGANIC POLLUTANTS IN FOOD AND ENVIRONMENTAL SAMPLES

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Persistent organic pollutants (POPs) comprise a variety of organic chemicals that persist in the environment and bioaccumulate in biological systems posing environmental risks to humans and ecosystems. Amongst POPs, halogenated compounds such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are of particular importance due to their extreme toxicity. Exposure to POPs can induce adverse health effects including certain cancers, birth defects, dysfunctional immune and reproductive systems [1].

Detection, confirmation and quantification of POPs in food and environment is important and strict regulations and guidelines regarding the analytical methodologies used exist in most countries around the globe [2], [3]. Typically, the analytical methods of choice for routine POPs quantification and confirmation include GC-MS, GC-MS/MS and GC-high resolution magnetic sector mass spectrometry. These instrumentation are well characterised for routine analysis of POPs and offer excellent levels of sensitivity when operated in a targeted way using selected reaction monitoring (SRM) or selected ion monitoring (SIM). However, when targeting only a specific class of POPs one can overlook possible metabolites of POPs and/or other emerging or unknown contaminants, compounds critical for risk assessment of human exposure to POPs. Screening for these compounds using low resolution instrumentation or high resolution with low scan speed and/or limited sensitivity can result in ambiguous results due to potential interferences that are not resolved. Hence, given the complexity of the matrices to be assessed, a comprehensive screening of samples for their POPs content requires analytical instrumentation that can deliver fast, full scan data acquisition, with high level of sensitivity and selectivity. A sensitive and selective full scan accurate mass acquisition will allow for a retrospective analysis of the data and could potentially lead to the discovery of new pollutants and help finding evidences of eventual synergies between the contaminants present in the samples.

The objective of this study was a preliminary evaluation of the utility of Orbitrap based GC-MS technology for the analysis of halogenated organic pollutants in the environmental and food matrices. Here it is demonstrated that the GC-Orbitrap mass spectrometer is a unique tool that can be used for accurate mass screening of POPs in a wide range of samples. The outstanding mass accuracy obtained allows for unambiguous identification and elemental composition confirmation of halogenated POPs whereas routine high resolving power allows for excellent selectivity in difficult matrices.

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[3] Stockholm Convention on Persistent Organic Pollutants Stockholm, 22 May 2001.

## O-19
## AUTENTICIDAD DEL ACEITE DE OLIVA: PERFILADO DE ACEITE DE OLIVA EXTRA VIRGEN USANDO GC-QTOF MS, DESARROLLO DE UN MODELO DE PREDICCIÓN GEOGRÁFICA PARA DETERMINACIÓN DE ADULTERACIONES.

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La demanda del aceite de oliva está creciendo a nivel mundial, a la vez que lo hacen en general los alimentos de origen Mediterráneo por sus beneficios sobre la salud. El aceite de oliva es reconocido como uno de los responsables sobre la longevidad de la población que se presentan en los países del sur de Europa. De echo la FDA reconoce a los ácidos grasos mono insaturados del aceite de oliva como reductores del riesgo coronario. Recientes estudios atribuyen efectos antiinflamatorios a los compuestos contenidos en el aceite de oliva vingen extra (EVOO) de primera presión, por la cantidad de antioxidantes presentes en el mismo.
El COI y la USDA han establecido estándares para la clasificación de EVOO, incluidos, test sensoriales, paneles de catas y test químicos. Sin embargo, estos test sensoriales son caros y subjetivos.
Esta aplicación presentada, demuestra la posibilidad de desarrollar un modelo de predicción que pueda predecir cuando un aceite pasará los test sensoriales de una manera no subjetiva. Para ellos se usa una aproximación de un analisis no dirigido de componentes presentes en el aceite mediante cromatografía de gases y con acople a espectrometría de masas de alta resolución, al igual que se ha realizado ya en clasificaciones en el mundo del vino.
Los datos se obtuvieron en modos de ionización por impacto de electrones (EI) como en ionización química (CI), usando un GC de Agilent 7890B acoplado a un espectrómetro de masas de masa exacta Agilent 7200B. Se realizó la deconvolución cromatográfica de todos los componentes presentes en las muestras, y posterior procesado para la construcción de la clasificación del modelo de predicción con el software estadístico Mass Profiler Professional (MPP) de Agilent.
Las conclusiones del presente estudio fueron: Usando la masa exacta en modo EI y CI genera datos suficientes para deconvolucionar los compuestos que pueden ser específicos para determinar no solo la estacionalidad, localización sino incluso la posibilidad de desechar muestras que presenten adulteraciones.

## O-20
## DIRECT CHARACTERIZATION OF VOLATILE COMPOUNDS BY 2D NON-CHROMATOGRAPHIC SEPARATION BY A HYBRID PLANAR DIFFERENTIAL MOBILITY ANALYZER AND MASS SPECTROMETRY

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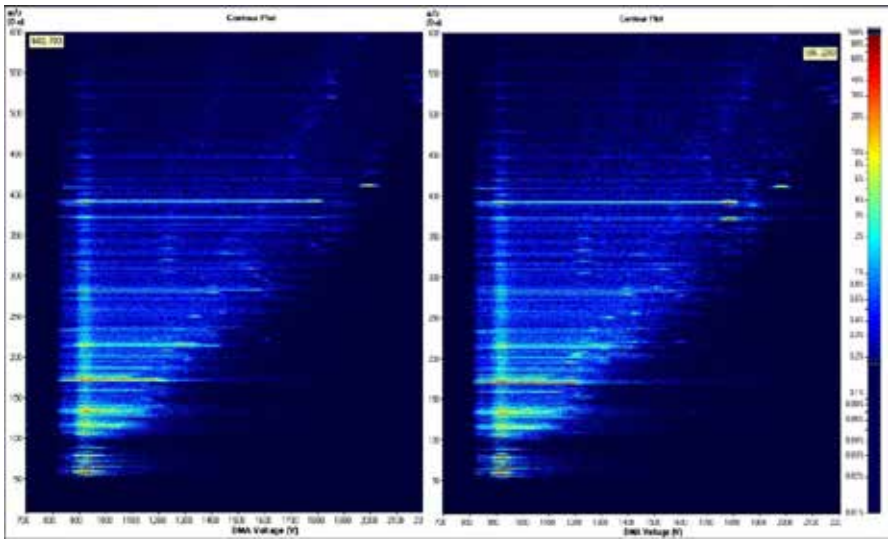
Direct classification of volatile compounds can be performed by differential mobility analyzers (DMA). When such devices are coupled to mass spectrometrs through atmospheric pressure interfaces, the hybrid system (DMA-MS) turns into a powerful analytical tool capable of providing information related to aerodynamic size and composition of the analyzed compounds. Such dual information can be succesfully used in applications related to ternary structures in molecules or biochemical activity or as an additional element to remove interferences from the final spectra.

The instrument described in the present communication is based on a planar DMA architecture that includes an atmospheric pressure ionizar by low-flow electrospray. The ionized compounds are sorted by size based on their electrical mobility (U) and guided to the mass spectrometer where theuy experience the separation based on their mass-to-charge ratio (m/z). The system allows multiple operational modes that will be described.

The communication will be illustrated with different examples in the fileld of food analysis, environment or homeland security. As an example of the analytical potential of the system, the attached figure shows the direct analysis of the headspace of two vials containing different premium gins. The m/z was scanned from 40 to 600 Th while the DMA was scanned from 700 to 2200 V.



## O-21
## POTENTIAL OF ORBITRAP GC-MS FOR TARGETED AND UNKNOWN COMPOUNDS ANALYSIS IN FOODS

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Coupling Orbitrap analyzer is the next step in Full Scan GC-MS evolution. It integrates highly successful technologies like modular capillary GC, removable ion source, quadrupole mass filter and latest Orbitrap analyzer. This study explains first the design of such technologies and summarizes the performance of such instrument. [1]. [3].

GC-MS Food Analysis is really demanding for an Orbitrap analyzer. Is it fast enough? What resolution do we need? How good is the mass accuracy? Is it sensitive enough? How about screening/quantitation? We use pesticide analysis in food for testing and answer these questions. In collaboration with Prof. Hans Mol at RIKILT we screen 55 pesticides of different classes in different food matrices. The results are presented in order to demonstrate performance and suitability of this technology for GC-MS Food Analysis.

Automatic screening and identification of unknown compounds requires new data processing workflows and new tools for extracting and filtering the data. We present a new workflow for automated accurate mass confirmation. Data processing comprises spectral deconvolution of EI HRAM data, Spectral Library search and matching with normal and high resolution score for filtering results and compound identification, and finally perform automated quantitation of positive hits against standards. In order to show an example of the use of these tools for unknown compounds screen and analysis of foods we use Whisky. A profiling of different types of Whisky is used as an example of profile study in order to look for the differences between the Whisky types using statistical tools included in differential expression software SIEVE 2.2. Using software tools including NIST libraries, deconvolution software and elemental composition and fragment matching software it is possible to identify what these difference are. High sensitivity in Full Scan mode and ultrahigh resolution are very helpful to get high quality data much easier to interpret. [2].

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## YS-01
## PRELIMINARY BIODEGRADATION STUDIES OF DIFFERENT COMMERCIAL HOMO-POLYMERS BY MALDI-TOF MS TECHNIQUE AND THEIR APPLICATION TO THE EVALUATION OF RIVER METABOLISM

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), first developed to study peptides and proteins, has also been applied to synthetic polymers analysis[1] . This technique requires that the polymer has a relatively narrow molecular weight distribution [1250-10000].

MALDI-TOF is ideally suited for polymer analysis because of simple acquisition of the mass spectra which show mainly single-charged quasi-molecular ions with hardly any fragmentation of the polymer. Simple sample preparation, fast analysis times, the variety of available matrices, low sample consumption, and particularly the formation of singly charged ions, are other advantages of the MALDI technique[2].

The present study aims at developing an analytical quantitative method for the study of biodegradation of homo-polymer to be used as a probe for the evaluation of the so called “river metabolism”.

The method was tested in the Ebro River basin in 11 sites characterized by different environmental conditions. Progress of biodegradation after 97 days of exposure wasreflected on the changes in the mass spectra corresponding to variations the chain length distribution pattern as well as in the loss of weight. Results were tentatively interpreted using different statistical methods (ANOVA, hierarchical analysis and principal component analysis) and discussed in comparison with other standard methods commonly used in fresh water ecology to estimate river metabolism[3].

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## YS-02
## ULTRASENSITIVE EMERGING ILLICIT DRUGS PROFILING IN WASTEWATER USING A HYBRID QUADRUPOLE TIME-OF-FLIGHT TANDEM MASS SPECTROMETRY (TRIPLE TOF 5600)

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Since 2005, there is a growing interest in determining illicit drugs and/or their metabolites in influent wastewater as an additional tool to assess their use in communities and populations in a direct, quick and objective way. The aim of this study was to screen and quantify 46 drugs of abuse and metabolites of wastewater samples using a hybrid quadrupole time-of-flight tandem mass spectrometry and furthermore carry out a post-target screening to identify additional compounds present in the water samples.

Wastewater samples were collected from the influent of three wastewater treatment plants (WWTPs) in Valencia (Pinedo I, Pinedo II and Quart-Benàger). Illicit drugs were extracted by solid-phase extraction (SPE) using Phenomenex Strata-X cartridges. Samples (250mL) were trapped through the cartridges under vacuum at a flow rate of 10 mL $min^{-1}$. Analytes were eluted with methanol, evaporated and reconstituted to water-methanol (9:1). The chromatography was performed with an Agilent 1260 Infinity ultra high performance liquid chromatography (UHPLC) using a Poroshell 12 D EC-C18 column (50 mm × 30 mm internal diameter, 2.7 µm). A constant flow rate of 0.2 mL $min^{-1}$ was used. The UHPLC system was coupled to a hybrid quadrupole time-of-flight ABSciex Triple TOFTM 5600. All analytes were analyzed in positive and negative ionization mode. Acquiring full scan MS data was employed for quantification of drugs of abuse, and automatic data dependent information product ion spectra (IDA-MS/MS) was checked for identifying emerging illicit drugs and other compounds in wastewater samples. The use of a database containing 1212 compounds achieved high confidence results for a wide number of contaminants.

In the present study, the presence of compounds that belong to amphetamines group (amphetamine, methamphetamine, ephedrine and MDMA), arylcyclohexylamines (ketamine and 4-methoxyphencyclidine), tryptamines (bufotenine), cocainics (cocaine, benzoylecgonine and ecgonine methyl ester) and morphine derivatives (codeine, morphine and methadone) were detected in the influent of the selected WWTP. These compounds were quantified, reaching cocainics and morphine derivates the highest values. Regarding post-target screening approach, more than 20 contaminants, mostly pharmaceuticals, but also mycotoxins and polyphenols were unambiguously identified in influent wastewater samples. This new approach to data evaluation by non-target screening analyses opens the possibility of various other applications, for example in open and groundwater or for monitoring natural attenuation.

## YS-03
## PREDICTION OF CHROMATOGRAPHIC RETENTION TIME USING ARTIFICIAL NEURAL NETWORKS FOR THE IDENTIFICATION OF EMERGING CONTAMINANTS IN ENVIRONMENTAL WATERS

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The recent development of wide-scope screening methods based on the use of high resolution mass spectrometry has resulted in a much improved capability for new compound identification in environmental samples. However, positive identification at the trace levels commonly seen still requires the purchase of analytical reference standards. With instrumentation improvements, research has begun to focus on the detection and identification of metabolites and transformation products, for which standards are sometimes unavailable. Chromatographic retention time prediction can help increase the confidence of such compounds, as well as reduce tedious data processing when several peaks appear in extracted ion chromatograms.

The current work explores the use of artificial neural networks (ANNs) for retention time prediction in gradient reversed-phase liquid chromatography coupled to high resolution MS applied to the screening of emerging contaminants in waste and surface water samples. From an initial database of more than 500 compounds, three subsets for analysis were made: training, verification and blind sets. A ±2 minute window for the predicted vs experimental retention time was used an indicator for the fitness of the model within the sets, with 85% of all compounds in training set, 90% in the verification set and 95% in the blind set being within this window. With such a high success rate for the blind set, this method was applied for the tentative identification of metabolites and transformation products (TPs).

A total of ten pharmaceutical and illicit drug metabolites and TPs (for three of which standards were available), were able to be tentatively identified in environmental water samples, including 10, 11-dihydroxy carbamazepine, carboxy losartan, 4-desmethoxy omeprazole and *O*-desmethylvenlafaxine.

## YS-04
## CLASIFICATION AND CHARACTERIZATION OF MYCORRHIZAL ROSEMARY PLANTS BY UHPLC-HRMS COMPOSITIONAL PROFILES AND CHEMOMETRICS

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Rosemary (*Rosmarinus officinalis*) is an aromatic shrub herb that grows wild in the Mediterranean basin. This plant is cultivated worldwide due to its diverse uses as household culinary spice for flavoring, preparation of cosmetic fragrances and in phytotherapy. Furthermore, the antimicrobial and antioxidant properties of rosemary essential oil are of great interest in food, cosmetic and pharmaceutical industries. In addition, long-term studies have shown that scrubland and shrubs contribute to reduction of erosion and improvement of soil quality in Mediterranean environment, and plants with economic potential, such as rosemary, could have an important role in soil rehabilitation and remediation.

*R. officinalis* colonized with several arbuscular mycorrhizal (AM) fungi have been used for the reclamation of low-nutrient-content soils [1]. These studies revealed that inoculation with AM fungi positively affect rosemary plant growth helping in a better capacity to compete for light with the spontaneous vegetation. Moreover, it is believe that the symbiosis between plant and fungi could alter the composition of plant metabolome, particularly of polyphenols which are the main bioactive compounds in rosemary.

The aim of this work is to explore a suitable methodology to assess the differences between non-inoculated and AM fungi inoculated *R. Officinalis* plants. The characterization and classification can be tackled from compositional profiles as a source of analytical information [2]. For this purpose, a UHPLC-HRMS and UHPLC-MS/HRMS (Q-Orbitrap) using C18 reversed-phase separation has been proposed for the analysis of 10 non-inoculated (control samples) and 50 mycorrhizal rosemary plants (inoculated with 5 different fungi isolates, 10 rosemary plants per fungi). Full scan MS raw data were employed as metabolic fingerprints to be treated by principal components analysis (PCA), and an interesting pattern distribution regarding the different mycorrhizal and no-mycorrhizal plants was observed. Furthermore, polyphenolic profiles were also employed for rosemary characterization. Thus, MS data was processed by Exact Finder 2.0 software (Thermo Fischer Scientific) by applying a target home-made database with more than 400 polyphenols. Retention time, accurate mass measurements, isotopic pattern fit and product ion scan spectra were used to identify and confirm the compounds when necessary. Finally, the most remarkable polyphenols that allowed the classification were identified and selected to achieve the *R. Officialus* characterization.

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## YS-05
## FINGERPRINTING ANALYSIS OF EXTRACTS OF LICORICE BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAHY

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Licorice (*Glycyrrhiza glabra*) is an herbaceous perennial plant, belonging to the Leguminosae family and it is one of the oldest and most popular herbal medicines in the world. A wide array of biological activities have been related to this plant, including antiulceric, anti-inflammatory, antispasmodic, expectorant, antiallergic, antidepressive, antiviral and antioxidant activities. Besides, licorice has been used in the food industry as a sweetener and a flavor enhancer.
*Glycyrrhiza glabra* is native to central and south-western Asia and the Mediterranean region, but the licorice from the region of Calabria (Italy) has been described as one those with highest quality. In order to avoid possible adulterations, it is important to search metabolomic markers that may allow the correct identification of licorice species and varieties. In this regard, the content on secondary metabolites could be employed for the geographical identification of licorice due to fact that the composition of the secondary metabolites in this plant may significantly vary depending on the geographical area of origin. These metabolites present in licorice are mainly triterpene saponins and phenolic compounds including flavanones, chalcones, flavones, isoflavones and isoprenylated flavonoids.
Due to the complex composition of this matrix, chromatographic techniques with high separation power are needed in order to obtain the maximum separation of the compounds.
Therefore, the approach carried out in this work was the development of a comprehensive two-dimensional liquid chromatography method coupled to mass spectrometry (LC x LC-MS IT) for the analysis of licorice extracts from Iran, China and Azerbaijan, as well as two Italian licorices from the region of Calabria, combining ZIC-HILIC and $C_{18}$ columns in the first and second dimension, respectively. The objective was to establish specific compounds that contribute to the differentiation between samples of different geographical origins.
The results of this work revealed that each sample presented several unique compounds in its metabolic profile. The Chinese licorice was the most different of the analyzed samples, followed by the Azerbaijani and the Iranian samples. While the two Italian licorices were the most similar samples. In conclusion LC x LC has been shown to be able to provide complex metabolic prolife useful to reveal specific unique compounds from each sample that could be effectively used as metabolic markers to identify the licorice origin.

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## YS-06
## CAPILLARY ELECTROPHORESIS TANDEM MASS SPECTROMETRY AS A SIMPLE ALTERNATIVE FOR THE DETERMINATION OF AMINOGLYCOSIDES IN HONEY USING MOLECULARLY IMPRINTED POLYMERS FOR SAMPLE TREATMENT

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Aminoglycoside (AG) antibiotics are widely used in veterinary medicine to treat foulbrood infection (caused by bacteria), and Nosema disease (caused by protozoa). The use of these compounds is regulated by European Union (EU) and maximum residue limits (MRLs) have been established in different edible parts or products derived from animals, such as muscle, milk, and eggs (EU regulation No 37/2010). However, EU does not authorize the use of antibiotics, including AGs, in beekeeping and MRLs have not been established for these compounds in honey, applying the so-called “zero tolerance”. In spite of that, honey and products derived from bees coming from non-EU countries, appears sometime contaminated with AG residues, which is a major concern in the honey trade. Some studies have revealed that a substantial part of the currently marketed honey contains residues of antibiotics and since 2003, the EU’s Rapid Alert System for Food and Feed (RASFF) has regularly alerted Member States about this fact.
Considering the determination of AGs, the high polarity of these antibiotics is a drawback for their analysis by liquid chromatography (LC), as they are not retained in reverse phase columns. Hydrophilic interaction chromatography (HILIC) has been recently proposed for the analysis of AGs in honey by LC-MS. However, in this methodology, a high concentration of salts in the mobile phase is usually needed which can be detrimental for MS detection. As an alternative to LC, in this work we propose a new analytical method based on capillary zone electrophoresis-tandem mass spectrometry (CZE-MS/MS) for the identification and simultaneous quantification of nine AGs in honey samples. Detection using an ion trap analyzer was used. In order to achieve a high selectivity in the sample treatment, a commercially available molecularly imprinted polymer has been used for the solid phase extraction (MISPE) of the analytes. The method was optimized and validated using multi-flower honey as representative matrix. Good linearity was obtained ($R2 > 0.993$ for all AGs) and recoveries for fortified samples ranged from 88.2% to 99.8%, with relative standard deviations lower than 8%. The limits of detection ranged from 1.4 to 94.8 μg $kg^{-1}$, demonstrating the sensitivity and applicability of this simple method. Thus, the proposed MISPE-CZE-MS/MS method can be considered an alternative to the established methods based on LC for the determination of AGs.

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## YS-07
## PHOTOCHEMICAL TRANSFORMATION OF COSMETIC PRESERVATIVES INTO 2,8-DICHLORODIBENZO-p-DIOXIN AND OTHER UNWANTED PHOTOPRODUCTS IN TOPICAL CREAM APPLIED ONTO ARTIFICIAL SKIN

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The antioxidant BHT or the antimicrobials triclosan (TCS) and phenyl benzoate (PhBz) are frequently found ingredients in personal care products (PCPs), which exhibit chemical structures capable of absorbing UV-light, thereby undergoing photochemical reactions [1]. Upon UV exposure, reactive intermediates of photounstable ingredients may behave as photo-oxidants, and even promote phototoxic or photoallergic contact dermatitis. The interaction of these photoproducts with cosmetic excipients or skin components, like sebum, may lead to the formation of new molecules with unknown toxicological properties.[2].
In this study, the photochemical behavior of BHT, TCS and PhBz in an artificial skin model was investigated through two sets of photodegradation experiments: (i) UV-Irradiation (8W, 254 nm) of artificial skin directly spiked with the target preservatives, (ii) UV-irradiation of artificial skin after the application of a cosmetic cream fortified with the target compounds. Subsequently, pressurized liquid extraction (PLE) was used to isolate the target preservatives and their transformation products. The follow-up of the photodegradation kinetics of the parent preservatives, the identification of the arising by-products (TPs), and the monitorization of their kinetic profile was performed by gas chromatography-mass spectrometry (GC-MS).
The photochemical transformation of triclosan into 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD) and other dioxin-like photoproducts has been demonstrated in this work. Furthermore, seven BHT photoproducts, and three benzophenones as PhBz by-products, most of them with unknown toxicological properties, have also been identified. It is worthy to notice that this is the first time that the phototransformation of cosmetic ingredients applied onto an artificial skin model has been investigated, which represents a valuable contribution to the requirements of public institutions like the Federal Food and Drug Administration (FDA) in the field of cosmetics safety evaluation [3].

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## YS-08
## LIQUID CROMATOGRAPHY- AND CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY MULTIPLATFORM FOR BROAD METABOLOMIC ANALYSIS OF DIETARY POLYPHENOLS EFFECT ON COLON CANCER USING IN VIVO MODELS

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Although several dietary polyphenols has shown promising anticancer activity, little is known about their activity at molecular level. In this context, metabolomics is a valuable tool to understand the underlying mechanisms of these compounds against cancer. In this work, an analytical multiplatform is used for broad metabolomics of the effect of these bioactive compounds against colon cancer using in vivo models. Capillary electrophoresis (CE) and ultra-high pressure liquid chromatography (UHPLC) coupled to time-of-flight (TOF) MS were combined to achieve a metabolomic examination of the effect of a polyphenol-enriched rosemary extract supplemented in the diet of xenograft mice inoculated with HT-29 human colon cancer cells. Inhibitory effect of the polyphenols on tumor growth and differences in metabolite profiles from liver samples were obtained by CE-MS and UHPLC-MS. The power of non-targeted metabolomics to reveal novel biological information on the effect of dietary polyphenols in in vivo models is demonstrated.

## YS-09
## QUALITY CLASSIFICATION OF OLIVE OILS BY GAS CHROMATOGRAPHY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION AND QUADRUPOLE TIME OF FLIGHT MASS SPECTROMETRY USING A METABOLOMICS-BASED STATISTICAL APPROACH

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Food quality is a matter of concern for consumers and producers. This quality is in most cases intimately connected to the origin of the products, as it is the case of French wines or Spanish ham, which contributes to the rise of their prizes. In this way, the determination of volatile organic compounds (VOCs) related to the characterization of volatile profiles in agricultural by-products or feeding, among others, is a particular work field to demonstrate the food quality [1]isocyanato- and isothiocyanatocyclohexane, have been included for the first time as target compounds due to their high occurrence in air samples. A dynamic air sampling method to trap gas and vapor on multi-sorbent tubes using portable pump equipment has been also developed. Sorbent tubes were filled with Carbotrap (70mg. One of the most interesting fields is the determination of olive oil quality, as the only way to classify their qualities is based on the use of a tasters panel test, a group of trained persons that decide if an olive oil must be tagged as extra virgin, virgin or lampante (not recommended for consumption) [2]. One interesting approach to face this problem is the use of metabolomics, defined as “the unbiased, global screening approach to classify samples based on metabolite patterns or fingerprints that change in response to disease, environmental or genetic perturbations with the ultimate goal to identify discriminating metabolites”[3].

The novel atmospheric pressure chemical ionization (APCI) [4] source has been used in combination with gas chromatography (GC) coupled to hybrid quadrupole time-of-flight (QTOF) mass spectrometer (MS), using a metabolomic approach for the classification of olive oil samples according to their quality. The accurate-mass full-spectrum acquisition data has allowed the detection of VOCs responsible of quality of olive oil to distinguish between extra virgin, virgin and lampante qualities. Analytes were extracted from the oil samples by a dynamic headspace purge and trap method and eluted from the SPE cartridges prior to GC-QTOF MS analysis. The metabolomic strategy consisted in three different steps: mass spectral alignment of GC-MS data, multivariate analysis and creation of the statistical model. The mentioned strategy was applied to process 120 extra virgin, 120 virgin and 60 lampante olive oil samples in order to create the classification model. Finally, the whole developed method was correctly validated by analyzing 125 blind samples, obtaining an accuracy in oil classification of 70 % when compared to the official established method, “PANEL TEST”.

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## YS-10
## COMPREHENSIVE STUDY OF THE ANTIPROLIFERATIVE ACTIVITY OF ROSEMARY POLYPHENOLS ON COLON CANCER CELLS USING NANO-LIQUID CHROMATOGRAPHY-ORBITRAP MS/MS-BASED PROTEOMICS

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Recent studies have demonstrated the anti-proliferative effect of a polyphenol-enriched supercritical rosemary extract (SC-RE) against HT-29 colon cancer cells [1]. This effect is attributable to its two major compounds, carnosic acid (CA) and carnosol (CS) [2], but the molecular mechanism of action has not yet been clarified. To improve our knowledge on the mechanisms underlying the antiproliferative activity of these polyphenols, a comprehensive proteomic study of the effect of SC-RE, CA and CS on HT-29 cells has been carried out using shotgun mass spectrometry based on a stable isotope dimethyl labeling. To attain this, cells were exposed to growth inhibitory and cytotoxic concentrations of the compounds for different time points (2-24 h). After incubation, proteins were extracted, digested with trypsin, and labeled using stable isotope dimethyl reagents. Equal amount of control and treated were mixed together, cleaned up using C18 SPE columns and redissolved in 0.1% formic acid prior to nanoLC−Orbitrap MS/MS analysis. The separation was carried out on a 150 mm × 75 μm analytical column packed in-house with Reprosil-Pur C18-AQ, 3 μm resin. To handle the large amount of data generated by this methodology, two different open-access bioinformatics tools have been applied. Initially, MaxQuant software was used for protein identification and quantification, and the generated protein data sets were subsequently processed by the Perseus software [3]. In general, the effect of polyphenol treatment on protein expression was more pronounced at longer incubation times. Following this strategy, the relative expression of a number of proteins, most of them related with antioxidant and detoxifying capacity, was typically found altered in all the treatments. In addition, different protein expression patterns were found to be polyphenol-specific. This methodology demonstrated to be suitable for the investigation of global changes in the Proteome induced by dietary antioxidants using *in vitro* cell models.

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## YS-11
## GENERAL APPROACH FOR DETERMINATION OF PHARMACEUTICALS, ILLICIT DRUGS AND PERSONAL CARE PRODUCTS IN WATER AND SEDIMENTS.

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Nowadays, emerging pollutants are being studied extensively by a large number of researchers [1]. This term stands for the substances that are released in the environment for which currently no regulations are established for their environmental monitoring. Their occurrence is reported worldwide in a range of aquatic environments, such as lakes, rivers, freshwater catchments, estuaries, reservoirs and marine waters. However, robust analytical methods are not fully developed yet, particularly for non-aqueous matrices as sediments.

This study is aimed at optimize a method for determine PPCPs and one illicit drug and their metabolite – 28 pharmaceuticals (28 in positive ion mode and 14 in negative ion mode), illicit drugs (THC and their metabolite THCCOOH) and personal care products (6 kinds all of them in negative ion mode) –. These substances were determined with an Agilent Technologies HPLC linked with a Triple Quad LC/MS in positive and negative ion mode using for compound separation a Kinetex C18 analytical column of 2.1 x 50 mm and 3.5 µm particle diameter from Phenomenex. Several mobile phases were tested to select the most appropriate. The analytes were extracted from 250 mL of water by solid-phase extraction using Strata-X cartridges, eluted with methanol, evaporated to dryness and dissolved in 250 µL of methanol:water (30:70) [2]. For sediments, before SPE, 5ml of methanol and 5ml of MacIlvaine-EDTA buffer were added to 1g of sediment. Then, the mixture was sonicated during 20 min and centrifuged 6 min at 3000 rpm and the supernatant collected in a different tube. The supernatant was added to 250ml of distilled water for their extraction through SPE. This approach provides acceptable recoveries (70% - 115% for water and 52% - 90% for sediments) and relative standard deviation (RSDs < 20 %) at the limits of quantification (from 0.1ng/L to 20ng/L in water and from 0.5 to 50 ng/L for sediments), which are in the low ppb range ensuring sensitivity enough.

The method was applied to the Turia River basin (water, sediments and soils), the Waste Water Treatment Plants that collect the wastewater of Valencia city, and drinking waters (tap and mineral water).

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## YS-12
## ANALYTICAL STRATEGY DEVELOPED TO EVALUATE THE EFFICACY OF DIFFERENT TREATMENTS FOR REMOVING ODOURS FROM DISHWASHERS

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The use of home appliances has inherent problems like the apparition of bad odours. In the case of dishwashers, odours can be generated in two different situations: on the one hand the degradation of leftover food inner of the dishwasher due to oxidation of food or to apparition of different microorganisms, on the other hand when a dishwasher has been washed but it remains closed during a long period of time some unpleasant odors could appear. Previous studies have identified responsible compounds for bad odours of dishwasher. These compounds belong to different chemical families: sulphur compounds, ketones, esters, terpenes, alcohols, phenols, amines…

An odorous atmosphere with some of the identified compounds wanted to be created to evaluate different treatments of removing odours. For that reason, an aqueous solution was placed in a thermostatized bath inside of a dishwasher and it was closed during 24 hours. The air of the home appliance was analyzed at 30 minutes, 6 hours and 24 hours after dishwasher was closed. Seven different treatments were applied to the odorous atmosphere during 24 hours.

A quantitative and a sensory analysis of the air were made. In the first case, tubes with Bond Elut ENV and Carboxen 1000 was used to trap volatile compounds and analyzed by Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS). In the second case, air was extracted with syringes of 20 mL and it was evaluated by a sensory panel of, at least, 8 judges. They should assess intensity and hedonic tone of the air. An ANOVA test and a Duncan test were applied to results.

Most of the compounds had a relative standard deviation (%RSD) less than 15% and good values were got for sensory analysis too. Treatments were effective in order to reduce odour except for hedonic tone at 6 hours. Statistical tests allowed us to sort efficacy of different treatments and there was a good coherence between results from quantitative analysis and sensory analysis. Therefore, TD-GC-MS is a useful method to predict sensory result of applying treatments to remove odours.




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## P-01
## DESIGN AND CONSTRUCTION OF A LIGHT-EMITTING DIODE INDUCED FLUORESCENCE (LEDIF) DETECTOR FOR MICROCHIP CAPILLARY ELECTROPHORESIS DEVICES

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Capillary Electrophoresis (CE) and Microchip Capillary Electrophoresis (MCE) analysis of most of the biomarkers in body fluids requires the use of a very sensitive detection method such as Fluorescence Detection (FD). Lasers are the most popular excitation sources for Fluorescence Detection (Laser-Induced Fluorescence, LIF). However, high brightness Light-Emitting Diodes (LEDs) present now a very competitive alternative to lasers in LIF detection, resulting in LED-Induced Fluorescence (LEDIF) detection approach. LEDs are inexpensive, consume less energy and are more stable than lasers. In addition, LED sources allow a wider range of wavelengths to better match the excitation maximum for the fluorescence of the target analyte [1]. These advantages of LEDs, combined with their very compact dimensions, make them suitable for integration into miniaturized systems such as microfluidic devices [2].

The aim of this work was the design and construction of a LEDIF detector for microchip capillary electrophoresis (MCE) devices.

Among the typical configurations used in LEDIF detection systems (collinear type, right-angle type, and optical-fiber FD [3]), collinear configuration was chosen. In general, this configuration is simple, and has low baseline noise, and high fluorescence collection efficiency [3]. The LEDIF detector developed consisted of 1) a LED source, 2) a power supply and cooling system for the LED source, 3) a system for focusing and optical filtering the excitation light on the detection spot, 4) a system to collect the fluorescence emitted by the sample at the detection spot, 5) a photosensor, and 6) an analogue-to-digital converter controlled by a computer to record and process the output signal. Different components and settings of the LEDIF detection system such as LED source, power supply and cooling system for the LED, focusing lenses (initial lens and microscope objective), optical filters (including a fluorescence filter cube), and polarization voltage of the photosensor were selected. With this setup, a limit of detection of $5x10^{-10}$ M for fluorescein in a channel of a polymethylmethacrylate (PMMA) microchip was obtained.

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## P-02
## DEVELOPMENT OF MONOLITHIC COLUMNS IN PTFE TUBES FOR HPLC

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Monolithic columns are highly permeable and uniform stationary phases, with a low back-pressure, enhanced diffusional mass transfer and easily tuneable surface chemistries. They are prepared from a monomer (or a mixture of them), a crosslinker, a porogenic solvent and a radical initiator. Polymerization is usually initiated by heating or with UV radiation.

Silica capillary tubes have been traditionally employed as physical supports in the preparation of monolithic columns. However, capillaries are too narrow for the flow-rate ranges employed in conventional high-performance liquid chromatography (HPLC). Hence, there is interest in developing monolithic columns confined in tubes different from silica capillaries (today available up to 0.5 mm i.d.), such as in stainless steel, PEEK or PTFE tubes of at least 0.75 mm i.d. Above this diameter, a column can be used in conventional HPLC and in other high flow-rate systems, such as in SPE devices for on-line sample preparation.

In this work, PTFE tubes of 0.8 mm i.d. were used. Since PTFE is a very inert material, chemical modification of the tube inner wall is necessary to assure covalent attachment of the polymer. For this purpose, the tubes were treated with several strongly oxidizing solutions. The objective was to create reactive groups on the PTFE surface. These groups were subsequently modified to obtain spacer arms with a terminal double bond. These double bonds performed as linking points during monolith polymerization.

An additional problem was that of monolith shrinkage during polymerization. The forces put to play by longitudinal and radial shrinkage of the polymer were strong enough to extensively breakdown the monolith-tube anchorage. The unwanted effects of shrinking are negligible in capillaries, but important in larger diameter tubes. These effects were diminished by using a mixture of monomers providing a polymer of increased flexibility. Further stability of the polymer-tube linkage was gained by allowing new polymerization mixture to enter into the two ends of the PTFE tube during thermal polymerization.

The resulting monoliths were inserted in a conventional HPLC system and tested with a mixture of alkylbenzenes. After optimization of both the polymerization mixture and thermal curing of the columns, chromatograms showing fair resolutions between the six successive alkylbenzenes of the test mixture were achieved. In addition to PTFE, work to construct monolithic columns with tubes of other fluorocarbon materials is in progress.

## P-03
## RAPID DETERMINATION OF PHARMACEUTICALS RESIDUES IN SURFACE WATER BY ON LINE SOLID-PHASE EXTRACTION COUPLED TO LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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The presence of pharmaceutical residues, in the environment has been considered a concern. Up to 95% of the administered dose of human or veterinary drugs can be excreted and discharged into the environment. However, most of the pharmaceuticals are not completely removed during wastewater treatment due to their high stability, and in consequence, these have also been detected in surface water, or in drinking water at trace levels. Regarding European legislation, there are no regulations that define maximum allowable concentrations of these pharmaceuticals. However, the Commission Implementing Decision (EU) 2015/495 in accordance with Directive 2008/105/EC, establishes a method detection limit for diclofenac in water at 10 mg/L [1]. Although there are some studies that determine different organic compounds in water, only a few of them analyze pharmaceuticals with enough sensitivity and precision to minimize the limit of detection.

A method was developed to determine carbamazepine, diclofenac, dimethylxanthine, lincomycin, naproxen, paracetamol and ibuprofen in surface waters. For the extraction, an on-line solid-phase extraction (SPE) was proposed. This has advantages such as, less sample volume and the cartridge could be reusable. For the determination of these drugs liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was used. Applying this methodology, suitable recoveries were obtained (71–104 %). Precision values were below 14 % and the limit of detection and quantification were below 10 and 30 ng/L respectively.

The validated method was applied to real samples from Almería finding positives in all of them, being ibuprofen the compound detected at the highest concentration (42.06 µg/L).

### Acknowledgements

The authors acknowledge Andalusian Regional Government (Regional Ministry of Innovation, Science, and Enterprise) and FEDER (Project Ref. P12-FQM-1838) for financial support.

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## P-04
## 3D PRINTED COLUMNS FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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The development of 3D printing in the last years has modified our concept of life. In 1983 Charles Hull invented the solid imaging process popularly known as3D Printing. Hull coined the term “stereolithography” in his U.S. Patent 4,575,330 entitled “Apparatus for Production of Three-Dimensional Objects by Stereolithography”. S. Scott Crump in the late 1980s developed the fused deposition modelling (FDM). But it was not until recently, with the expiration of both patents, when the revolution of 3D printing was born. In this work, the development of new chromatographic columns for high performance liquid chromatography (HPLC) is explored. As far as we know, this is the first time that the FDM technology is applied to the construction of HPLC columns. Several materials (acrylonitrile butadiene styrene, polylactic acid, high impact polystyrene and polysulfone) and different designs have been tried. The columns were inserted into a conventional HPLC system and eluted with hydro-alcoholic mobile phases. The problems associated with the design of columns using commercial 3D printing software, the restrictions and difficulties of the FDM technology and that of the experimental execution, are discussed. Several compounds (sodium chloride, benzyl amine, benzoic acid at different pHs) were selected for testing. Van Deemter curves for the 3D printed columns were constructed.

## P-05
## PRECONCENTRATION OF PROTEINS BY USING A POLYMER SOLID-PHASE EXTRACTION MONOLITH MODIFIED WITH GOLD NANOPARTICLES

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A novel solid-phase extraction sorbent has been developed to extract and preconcentrate proteins from vegetal extracts. The polymer was modified by attaching gold nanoparticles (AuNPs), since they confer a unique selectivity to the monolithic column for its use in protein and peptide preconcentration. For this purpose, monolithic columns were prepared from a polymerization mixture containing 20 wt% glycidyl methacrylate as monomer, which contained an epoxy group that can be easily functionalized, ethylene dimethacrylate (5 wt%), and a binary pore-forming solvent constituted by cyclohexanol (70 wt%) and 1-dodecanol (5 wt%). Azo-bisisobutyronitrile (1 wt% with respect to the monomers) was added as polymerization initiator [1]. After the amination of the epoxy group, AuNPs with a diameter of 10 nm were attached to the monolith surface. The bulk monolith was placed in an SPE cartridge, and extraction method was optimized in terms of sorbent amount, sample buffer composition and composition of the eluent and elution temperature. To perform the optimization, BSA and Cytochrome C were selected as test analytes since they shown different pI values (4.7 and 10, respectively). Desorption of the retained proteins was achieved using a phosphate buffer at pH 12, and the regeneration of the adsorption capacity of sorbent was done by washing the column at 80 °C for 2 h with water. In order to quantify the amount of proteins recovered from the column, a Bradford assay was used. The reproducibility and usability of sorbent was also examined. Satisfactory results were obtained for the loading capacity and protein recovery. Finally, the developed method was applied to real samples, specifically vegetal extracts, and the obtained eluates were subjected to SDS-PAGE analysis to establish possible differences related to the retention selectivity.

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## P-06
## A NOVEL CAPILLARY ELECTROCHROMATOGRAPHY METHOD FOR THE ANALYSIS OF BENZIMIDAZOLE RESIDUES IN WATER SAMPLES

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Benzimidazoles (BZs) are veterinary drugs widely used in the prevention and treatment of parasitic infections in agriculture and aquaculture. Some BZs are used as fungicidal agents in the control of spoilage of crops during storage and transport [1]. Several methods have been proposed for the determination of these BZs, though they are mainly analyzed by High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE). A very interesting alternative could be the use of Capillary Electrochromatography (CEC). This approach is considered to be a hybrid method which couples the high separation efficiency of Capillary Zone Electrophoresis (CZE) with the selectivity of HPLC. CEC uses an electric field rather than hydraulic pressure to propel the mobile phase through a packed bed into a capillary. With this technique, it is possible to use small diameter packings and thereby to achieve very high efficiencies because there is no back pressure [2].

In this work a simple and inexpensive methodology has been proposed for producing C18 packed capillaries. C18 particles (5 µm, non-encapped) were packed in a fused silica capillary (25 cm effective length and 75 µm i.d) using a compact steel unit (SP-400 NanobaumeTM) designed for packing capillary columns. The packing unit was connected to a high-pressure pump in order to propel a particle-transporting solvent through the capillary. Acetone was employed as solvent to carry the particles through the capillary and pack it under a pressure of 42 MPa. Outlet and inlet frits were made for retaining the particles inside the capillary. The packed capillary was heated with a metallic strip (80% Ni-20% Cr, 28 cm×2 mm×0.2 mm, electric resistance 1.3 Ω) connected to a 7 V AC power supply where frits must be located. The frit length was around two millimeters.

With the aim of achieving a good analytical performance, variables such as mobile phase composition (acetonitrile/buffer ammonium acetate, pH 6.5, 1mM; 60:40), separation voltage (25 kV) and temperature (25°C) were optimized. In addition, a combined hydrodynamic-electrokinetic injection mode was considered and samples were injected for 75 s under a voltage of 12.5 kV and a pressure of 50 mbar. The determination of benzimidazoles in water samples has been accomplished by CEC using dispersive liquid-liquid microextraction (DLLME). This methodology has been applied to different types of water (fish farm water, well water and spring water) obtaining satisfactory results in terms of linearity (R2≥0.990), repeatability (RSD≤1.2%), reproducibility (RSD≤2.2%) and recoveries (R≥87.7%). Detection limits were lower than 2.8 µg L-1 for all studied analytes.

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## P-07
## POTENTIAL OF GC-APCI-MS/MS FOR THE QUALITATIVE SCREENING OF URINARY EXOGENOUS ANDROGENIC ANABOLIC STEROIDS. COMPARISON WITH TRADITIONAL GC-EI-MS/MS

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Androgenic anabolic steroids (AAS) are the most frequently reported substances by doping control laboratories. The detection of AAS misuse is a constant analytical challenge for several reasons. The most important one is related with the fact that AAS are prohibited at all times i.e. in and out of competition. This prohibition makes that any evidence of AAS misuse (e.g. the mere presence of traces of the AAS and/or its metabolites) is sufficient for reporting an adverse analytical finding. Thus, sensitivity of the detection methods is a key factor.

Nowadays, the detection of urinary AAS in doping control analysis is achieved by the combination of two methodologies. Both liquid and gas chromatography (LC, GC) coupled to tandem mass spectrometry (MS/MS) in selected reaction monitoring (SRM) mode are used due to their inherent sensitivity and selectivity. On one hand, LC-MS/MS methods allow for the detection of thermolabile compounds with minimum sample treatment. On the other hand, GC-MS/MS allow for the detection of fully reduced metabolites. These GC-MS/MS based methods are normally developed with electron ionization (EI) source, which present high fragmentation. This elevated fragmentation could affect in the adequate selection of precursor ions for the SRM transitions decreasing the sensitivity of the method. The use of softer sources could minimize this drawback improving the sensitivity and specificity of these methods. In this work, the potential of an atmospheric pressure chemical ionization source (APCI) developed for GC has been evaluated for the detection of 16 exogenous AAS in urine. This interface promotes soft ionization generating, mainly, $[M+H]^+$ or $M^{+\bullet}$ ions as the base peak of the spectrum.

Qualitative methods are needed to report an adverse analytical finding in case of exogenous AAS. In the present work, a method has been qualitatively validated at two concentration levels. Adequate precision (RSD normally below 25 % at the low concentration level and below 15 % at the high) and suitable limits of detection (LOD) (below 0.5 ng/mL) were obtained. Validation results were compared with the commonly used method based on the EI source. Slightly better repeatability was found in EI analysis whereas lower LODs were found for APCI. The applicability of the method has been tested in samples collected after administration of 4-chloromethandienone. The highest sensitivity of the APCI method allowed to increase the period in which the administration can be detected.

## P-08
## TRACE DETERMINATION OF TETRACYCLINES IN ENVIRONMENTAL WATERS BY CAPILLARY ZONE ELECTROPHORESIS USING LARGE VOLUME SAMPLE STACKING WITH POLARITY SWITCHING

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Tetracyclines (TCs) are broad-spectrum antibacterials widely used in animal husbandry for the prevention, control and treatment of infections. The presence of TC residues in environmental waters is worthy of concern regarding their potentially harmful effects on drinking water quality. A sensitive method using capillary zone electrophoresis with UV-diode array detection (CZE-DAD) has been proposed for trace determination of five TCs (oxytetracycline, OTC; methacycline, MTC; tetracycline, TC; chlortetracycline, CTC and doxycycline, DC) in environmental waters.
The CZE separation was been accomplished using 75 mM bicarbonate buffer (pH 10.0), applying a voltage of 25 kV at 25°C. In order to increase the sensitivity in the monitoring of these residues in waters, an on-line preconcentration procedure, named large volume sample stacking (LVSS) with polarity switching has been applied. This methodology is based on the hydrodynamic injection of a large volume of sample in a solution of low conductivity in the capillary, change to negative polarity in order to focus the analytes in a part of the capillary, removing part of the matrix sample and change to positive polarity for CE separation. In this case sample solution containing the TCs was loaded (5 bar for 1 min), so the whole capillary column was filled. After sample injection, a negative voltage of−25 kV was applied, and the sample stacking started. Sample matrix removal from the capillary was indicated by monitoring the electric current, which progressively increased to its normal value as the low-conductivity injected zone was eliminated from the capillary. At this stage, the stacking process could be considered complete. The voltage was then switched to separate the compounds. Around 40-fold improvement of sensitivity was achieved when comparing to the standard injection (30 mbar for 10 s in water).
A solid-phase extraction (SPE) method using HLB cartridges was applied for off-line preconcentration and sample cleanup. The combination of both off-line and on-line preconcentration procedures provided a significant improvement in sensitivity, obtaining detection limits from 0.07 to 0.17 μg $L^{-1}$. The precision (intra- and inter-day), expressed as relative standard deviation (%,RSD), at three concentration levels (0.2, 1.0 and 2.5 μg $L^{-1}$) was below 12%. Recoveries obtained from river, spring and ground waters, at three concentration levels, ranged from 87 to 96%. Thus, the SPE-LVSS-CZE-DAD procedure is suitable for monitoring these compounds in water samples.

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## P-09
## MONOLITHS FROM POLY(ETHYLENE GLYCOL) DIACRYLATE FOR CAPILLARY ELECTROCHROMATOGRAPHY

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Poly(ethylene glycol) diacrylates (PEGDA) rigid monoliths have been used previously for capillary liquid chromatography for protein separation[1]. In this work, we have synthetized PEGDA monolith columns for capillary electrochromatography. For this purpose, monoliths were synthesized using as monomer and cross-linker PEGDA with different ethylene glycol chain lengths by one-step UV initiated polymerization. Methanol and ethyl ether were used as porogens for the PEGDA monoliths. 2,2-Dimethoxy-2-phenylacetophenone was added as polymerization photoinitiator and [2-(methacryloyloxy)ethyl]trimethylammonium chloride (META) was used to provide column with electroosmotic flow. Effects of poly(ethylene glycol) chain length, porogen ratios and reaction temperature on monolith morphology were investigated. Polymerization of monoliths was conducted at a 0 ◦C and room temperature, which produced significant differences in monolith morphology and permeability. Alkylbenzenes and organophosphorus compounds were selected as test analytes. Columns were characterized by constructing Van Deemter curves for each polymerization mixture and by scanning electron microscope (SEM).

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## P-10
## FAST DETERMINATION OF FLONICAMID AND ITS METABOLITES IN ORANGE SAMPLES BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - TANDEM MASS SPECTROMETRY

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Pesticides are regularly used throughout the world to increase production and quality of agricultural commodities. In consequence, pesticide and/or metabolite residues should be analysed in all agricultural commodities to improve food safety. Among these pesticides flonicamid, N-cyanomethyl-4-trifluoromethylnicotinamide, is a relatively novel selective systemic pesticide, highly effective against aphids and other insects. This compound has a minimal cross-resistance with no negative impact on beneficial arthropods. Thus, flonicamid is widely used in pest management programs. However, the maximum residue limits (MRLs) of flonicamid in foods have been regulated by government authorities such as the European Commission [1]. These MRLs for flonicamid have been set as the sum of residues of the parent compound (flonicamid) plus its two major metabolites, TFNA (4-trifluoromethylnicotinic acid) and TFNG (N-(4-trifluoromethylnicotinoyl) glycine). Therefore, specific and sensitive methods for the identification and quantification of flonicamid and its metabolites, TFNA and TFNG, in foods are required.

The aim of this study is the development and validation of a method to determine flonicamid, TFNA and TFNG in orange samples, using a simple and efficient extraction procedure and reliable quantification applying ultra high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Thus, a fast and simple extraction procedure with acidified methanol has been used. The methodology was validated, checking specificity, recoveries, precision, limits of detection (LODs) and limits of quantification (LOQs). The recoveries were between 75 and 120%, while precision values were lower than 17%. Finally, LODs and LOQs were lower than or equal to 3 and 10 μg $kg^{-1}$ respectively. The validated method was applied to the analysis of real samples, and no positive residues were detected.

[1] European Union: http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm.

## P-11
## ANALYSIS OF BENZOPHENONE-UV FILTERS IN HUMAN MILK SAMPLES USING ULTRASOUND-ASSISTED EXTRACTION AND CLEAN-UP WITH DISPERSIVE SORBENTS FOLLOWED BY UHPLC–MS/MS ANALYSIS

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Endocrine disrupting chemicals (EDCs) are a group of natural and synthetic chemicals that may interfere with the normal function of the endocrine system in animals and humans, benzophenones UV-filters (BPs) belong to this group of compounds [1, 2]. There is increasing evidence that BPs are able to interfere with the endocrine system. *In vitro* studies have shown that BPs stimulates the proliferation of the breast cancer cell line MCF-7 due to their estrogenic activity and that these compounds have also antiandrogenic activity [3]. Carcinogenesis and reproductive organ malformations have been also reported in rodents after exposure to BP-UV filters [4].

In this work, a new sample preparation method for the determination of 5 BP-UV filters in human milk is presented. The procedure involves the lyophilization of the sample prior to the extraction by ultrasound sonication with acetonitrile. In order to reduce matrix effects produced by coextracted milk components, mainly proteins, sugars and lipids, a further clean-up step with a mixture of dispersive-SPE sorbents, C18 and PSA was applied. Extraction parameters were optimized using experimental design based optimization techniques, and the compounds were detected and quantified by ultrahigh performance liquid–chromatography tandem mass spectrometry (LC–MS/MS) in positive ESI mode. Analytes were separated in 10 min. BP-$d_{10}$ was used as internal standard. The limits of quantification (LOQs) were between 0.3 to 0.6 ng $mL^{-1}$ for the studied analytes. The inter– and intra–day variability was < 12% in all cases. The method was validated using matrix–matched calibration and recovery assays with spiked samples. Recovery rates were between 90.9% and 109.5%. The method was successfully applied for the determination of these compounds in human milk samples collected from volunteers lactating mothers with no known occupational exposure to these compounds who live in the province of Granada (Spain). The analytical method developed may be useful for the development of more in–depth studies on the prenatal exposure and biomonitoring of these commonly used UV-filters.

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## P-12
## DETERMINATION OF HONEY MONOTERPENES USING HEADSPACE SORPTIVE EXTRACTION COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Monoterpenes are common in essentials oils obtained from plants, where they form the major volatile fraction in flowers. These compounds have interesting properties, since they act as wound healing, analgesic, anti-inflammatory, antibacterial and antifungal agents. The frequent intake of honey provides benefits for human health as result of its monoterpene content among many other components [1]ketone, acid, alcohol, hydrocarbon, norisoprenoids, terpenes and benzene compounds and their derivatives, furan and pyran derivatives. They represent a fingerprint of a specific honey and therefore could be used to differentiate between monofloral honeys from different floral sources, thus providing valuable information concerning the honey's botanical and geographical origin. However, only plant derived compounds and their metabolites (terpenes, norisoprenoids and benzene compounds and their derivatives. A method based in headspace sorptive extraction (HSSE) in combination with gas chromatography and mass spectrometry (GC-MS) has been developed for the determination of seven monoterpenes (eucalyptol, linalool, menthol, geraniol, carvacrol, thymol and eugenol) in honey samples.

Three commercially available coatings for the HSSE stir bars, namely polydimethylpolisiloxane (PDMS), polyacrilate (PA) and ethylenglycol-Silicone (EG-Silicone), were evaluated. These new commercially available polar polymeric coatings have been not yet applied to HSSE technique [2]. The influential parameters both in the adsorption and the thermal desorption steps were optimized for each coating, due to their different nature. PDMS provided the best sensitivity for linalool, geraniol, menthol and eucalyptol, whereas EG-Silicone was best for extracting the phenolic monoterpenes studied. Considering the average from all compounds, PDMS pointed as the best option. Despite EG-Silicone and PA polar nature, they did not show any improvement over PDMS, at least for the extraction of the evaluated volatile compounds.

The analytical characteristics for the HSSE-GC-MS method using this coating were obtained. Quantification of the samples was carried out by matrix-matched calibration using a synthetic honey. Detection limits ranged between 7 and 32 pg $g^{-1}$, depending on the compound. Twelve honey samples of different floral origins were analyzed using the HSSE-GC-MS method, the analytes being detected at concentrations up to 64 ng $g^{-1}$, depending on the compound.

This first application of HSSE-TD-GC-MS for the determination of volatile monoterpenes in honey samples showed this technique as a simple and solvent free method for the reliable control and quantification of these compounds.

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## P-13
## PRECONCENTRATION OF SPIROCYCLIC TETRONIC/TETRAMIC ACID DERIVATIVES AND NEONICOTINOID INSECTICIDES USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR THEIR DETERMINATION IN VEGETABLES BY LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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Insecticides are widely used to destroy and mitigate insect pests, permitting to obtain high production and quality of food. Nevertheless, their use is not free of drawbacks, considering their toxicity to humans and other species [1]. Spirocyclic tetronic/tetramic acid derivatives and neonicotinoids, two of the most extensively used insecticides for the control of important agricultural crop tests [2], act as lipid biosynthesis inhibitors and antagonists of the nicotinic acetylcholine receptor, respectively.

The present communication deals with the quantification of five neonicotinoid insecticides (thiamethoxam, clothianidin imidacloprid, thiacloprid and acetamiprid) and three spirocyclic tetronic/tetramic acid derivatives (spirodiclofen, spiromesifen and spirotetramat) in different fruits and vegetables using dispersive liquid-liquid microextraction (DLLME) combined with liquid chromatography and tandem mass spectrometry (LC-$MS^2$), with electrospray ionization and a triple quatrupole as analyser, in the multiple reaction monitoring (MRM) mode.

The analytes are released from the solid matrix samples by single-phase extraction in acetonitrile, using sample masses of 3 g. The different parameters affecting the extraction efficiency in DLLME were optimized, being 3 mL of the acetonitrile extract (disperser solvent) rapidly injected along with 100 µL of $CHCl_3$ (extractant solvent) into 10 mL of a 1% m/v sodium salt aqueous solution, the selected conditions. The enriched extract was evaporated and reconstituted with 25 µL acetonitrile, 10 µL being injected in the LC system. A gradient elution programme using formic acid 0.1% and acetonitrile provided a good resolution of the analytes, being eluted with retention times in the 6.8-19.4 min by using a $C_{18}$ stationary phase. A total of fifteen samples were analysed (tomato, pepper, lemon, orange and grapefruit) and, all of them showed to be free from the insecticides, at least above the corresponding detection limits.

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## P-14
## MULTIRESIDUE DETERMINATION OF PESTICIDES IN OLIVES AND OLIVE OIL BY UHPLC-MS/MS AND ACETONITRILE PARTITIONING WITH DIFFERENT CLEAN-UP STEPS USING ZIRCONIUM DIOXIDE-BASED SORBENTS

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The determination of pesticide residues in fatty matrices such as edible oils or fatty vegetables constitute a challenging application, provided the high percentage of lipid components that may be coextracted during the sample treatment stage using generic multi-residue methods. The more common sample treatment approach used nowadays is QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) adapted for fatty matrices [1], followed by analysis using GC-MS/MS and UHPLC-MS/MS. Recently, the use of zirconium dioxide-based sorbents has been found useful for the removal of coextractants in order to minimized matrix effects during pesticide determination, particularly for vegetable matrices with high fat content such as avocado, almonds and even olive oil [2-4]. In this study, two commercially available sorbents using silica beads functionalized with zirconium dioxide or zirconium dioxide and C18 groups (Supelco Z-Sep and Z-Sep+ respectively) have been used (in combination with primary secondary amine (PSA) or alone) to improve the sample treatment for pesticide multiresidue methods for olives and olive oil. A method including over 80 multiclass pesticides typically used in olive groves based on the use of ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-ESI-MSMS) (Thermo TSQ Endura) was developed and applied for the assessment of the four different cleanup steps assayed (Z-Sep, Z-Sep+PSA, Z-Sep(+) and Z-Sep(+)+PSA.

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## P-15
## HOW TO AVOID AGGLOMERATION OF MAGNETIC BEADS FOR IMMUNO-RECOGNITION AND ANALYSIS OF GLYCOPROTEINS IN CAPILLARY ELECTROPHORESIS SYSTEMS.

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The recognition of a specific protein by an antibody (Ab) developed for this purpose allows the protein to be selectively isolated from a complex mixture. Performing this antigen-antibody recognition process using Abs bound to magnetic beads (MBs) makes it possible to simultaneously purify and concentrate the protein of interest. For in-line coupling of immuno-recognition and capillary electrophoresis of proteins, the MBs with Ab bound to them (Ab-MBs) can be retained at the capillary inlet by using magnets.

In our laboratory we are interested in binding the disease biomarker alfa 1-acid glycoprotein (AGP) to antiAGP-MBs for a further in-capillary CE analysis of isoforms of the purified AGP. To achieve this, commercial polyclonal antiAGP was bound to MBs with nominal particle size of 1.08 mm. Lack of repeatability in the CE results led us to suspect that MBs could be agglomerating.

The effect of ultrasound (US) treatment on MB size has been studied by dynamic light scattering (DLS). The influence of the suspension media, the suspension concentration, and the US treatment time to decrease agglomeration has been studied.

The selected conditions allowed us to obtain particle size for Ab-MBs very close to the nominal size of MBs. Dilution of Ab-MBs to 0.1 mg/mL in water and ultrasound treatment with a UP400S Hielscher US probe with the sonotrode H7 for 30 min, with pulses of US every 0.5 s, a potency of 12.1 ± 2.7 W and an amplitude of 20%, led to a particle size of 1.20 ± 0.07 mm that was stable for at least two months.

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## P-16
## OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION OF *Mentha* sp. BIOACTIVES

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Despite the existence of a wide variety of food preservation techniques, the microbial contamination continues to be an important problem that affects not only food quality but also food security [1]. Diverse formulations based on plant extracts/essential oils have been developed as a healthier alternative to synthetic antimicrobials [1, 2]. Among them, those arising from different *Mentha* species have been reported as highly effective [3].
Microwave assisted extraction (MAE) is a fast and efficient emergent technique which has gained a great acceptance for the extraction of plant bioactives [4]. To the best of our knowledge, MAE has not yet been applied for obtaining antimicrobials from *Mentha* sp.
After selection of optimal extraction solvent, the effect of different factors (sample weight: 0.75-1.5 g, extraction temperature: 50-100 °C, and extraction time: 5-30 min) on recovery of compounds with antimicrobial activity was evaluated by a Box-Behnken experimental design. Prior to quantitation, extracts were comprehensively characterized by using different techniques: (i) Extracts were directly analyzed or derivatized (trimethylsilyl oximes) for their Gas Chromatography-Mass Spectrometry (GC-MS) analysis using a methyl polysiloxane column (30m x 0.25mm x 0.25µm). (ii) After filtration, extracts were analyzed by Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry (LC-QToF MS), using a reversed phase $C_{18}$ column (100 x 2 mm, 3 µm, 100 Å) and a binary gradient of methanol and water, both phases acidulated with 0.1% acetic acid. The optimized MAE method was applied for extraction of bioactives from different *Mentha* species (*M. spicata*, *M. rotundifolia*, *M. piperita*, *M. pulegium*, etc).
MAE is shown as an advantageous technique for obtaining extracts of *Mentha* sp. with antimicrobial activity of application in the food industry.

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## P-17
## DYNAMIC FABRIC PHASE SORPTIVE EXTRACTION FOR A GROUP PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

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There are several analytical methods developed for the determination of pharmaceuticals and personal care products (PPCPs) in environmental water samples. Most of them are based on a extraction techniques, mainly solid-phase extraction (SPE) or stir bar sorptive extraction (SBSE) [1,2] followed by liquid chromatography with mass spectrometry in tandem (LC-MS/MS).

A novel sorptive extraction technique, fabric phase sorptive extraction technique (FPSE) has been introduced [3]. The fabric is coated with different material chemistries using sol-gel technology. The sorbent material is uniformly distributed on the cellulose/polyester fabric substrate and which is 10 times more than SBSE. There are different available sorbents for FPSE covering different polarities. FPSE was already evaluated for the extraction of a group of PPCPs followed by LC-MS/MS with suitable results in terms of recovery [4]. However, the main drawback was the extraction time (up to four hours) to reach the equilibrium.

In this work, we have evaluated a new design of this technique, the dynamic fabric phase sorptive extraction (DFPSE), in which the sample is percolated through the fabric sorbent material using the filtration assembly. Then, the retained analytes were eluted by passing the solvent through the same assembly. Using the DFPSE technique we have developed a new method to determine a similar group of PPCPs from environmental water samples. In this study, different parameters affecting the extraction were optimized for the sorbent material in order to increase the extraction recovery of the analytes and results were compared with conventional FPSE.

The method based on DFPSE/LC-MS/MS was validated and applied to analyze different surface and also influent and effluent samples from wastewater treatment plants located in Tarragona area.

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## P-18
## SUSTAINABLE PROTEIN PURIFICATION/ENRICHMENT BASED ON CARBOXYLATE-TERMINATED CARBOSILANE DENDRIMERS-PROTEIN INTERACTIONS

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Protein sample preparation involves the extraction, isolation, purification, enrichment, etc of proteins present in a sample. This step usually requires the use of tedious methods that employ high amounts of solvents. The development of new materials offers new opportunities for a sustainable protein sample preparation [1].

Dendrimers are nanostructured hyperbranched macromolecules whose structure, constituted by layers called generations, has the capability of be functionalized by groups or ligands with biological activities. Carbosilane dendrimers show improved properties attributable to their strong silicon-carbon skeleton which make them to show high thermodynamic, kinetic and hydrolytic stability, biocompatibility, and water-solubility. They have been employed in many biological applications but they have never been used in protein sample preparation [2].

The aim of this work was to explore the nature and characteristics affecting interactions between carboxylate-terminated carbosilane dendrimers and proteins and to evaluate their potential in protein purification/enrichment.

Three different generations of carboxylate-terminated carbosilane dendrimers at different pHs and dendrimer concentrations were tried against three standard proteins with different isoelectric points and molecular weights. Studies on protein-dendrimer binding were based in the variation of fluorescence intensity and emission wavelength of proteins when adding the dendrimer. Both parameters resulted affected when dendrimers were added observing most significant changes at acidic pH. Carboxylate dendrimers at acid pH were proposed for the purification/enrichment of proteins extracted from a food matrix.

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## P-19
## PRELIMINARY STUDY OF PROTEIN INTERACTIONS WITH SULPHONATE TERMINATED CARBOSILANE DENDRON-GOLD NANOPARTICLES AND THEIR APPLICATION IN PROTEIN SAMPLE PREPARATION

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Gold nanoparticles (GNPs) show attractive physical and chemical properties and they have been used for protein enrichment in proteomics. Specifically, gold nanoparticles have been used to preconcentrate proteins from urine as an alternative strategy to trichloroacetic acid precipitation [1-3]. The GNP core can be easily modified by carbosilane dendrons which can be functionalized by different functional groups, increasing nanoparticles stability, improving size control, and making them more suitable for the interaction with biological molecules. The aim of this work has been to study the interaction of proteins with sulphonate-terminated carbosilane-GNPs and to evaluate their potential use in protein sample preparation.

GNPs modified with sulphonate-carbosilane dendrons (first, second, and third generation) showing 597, 378, and 200 kDa molecular weights, respectively, were used. Bovine serum albumin, myoglobin, and lysozyme were employed as standard proteins. The influence of different parameters (protein:GNP ratio, pH, polarity, and dendrimer generation) on the protein-GNP interaction was investigated. Protein-GNP interaction was studied by monitoring the fluorescence signal of proteins and by the evaluation of its deactivation in presence of dendrimer-GNP under different conditions. To further support the interpretation of protein interaction, RP-HPLC and gel electrophoresis were employed.

Fluorescence quenching was observed by the addition of dendrimer-GNP at different concentrations. These results were compared with commercial citrate stabilized non dendrimer-GNP, observing highest quenching reduction in dendrimer-GNP samples. Most suitable conditions (dendrimer-GNP, pH buffer, and concentration) were employed to extract proteins from a complex food matrix.

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## P-20
## IMPROVED RECOVERY, REPRODUCIBILITY AND MATRIX EFFECTS WITH AN ADVANCED TECHNOLOGY IN SOLID PHASE EXTRACTION (SPE)

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A novel reversed-phase solid phase extraction (SPE) sorbent has been developed to enable simpler and faster SPE protocols, while at the same time generating cleaner extracts than other sample preparation methods. In this application, a 3 step load-wash-elute SPE protocol, eliminating conditioning and equilibration, was successfully employed to extract 22 synthetic cannabinoids and metabolites from whole blood samples using this novel reversed phase SPE plate. Superior analyte recoveries, low %RSDs and modest matrix effects (ME) were achieved across the entire panel of compounds. At the same time, parallel extractions were conducted with other reversed phase (RP) SPE devices using recommended 5 step SPE protocols. Lower recoveries, higher %RSDs and higher matrix effects were obtained. Besides, This new SPE sorbent removes 90% more phospholipids when compared to the other RP SPE devices.

## P-21
## NON-TARGETED UHPLC-HRMS BASED METABOLOMICS FOR THE IDENTIFICATION OF NUTRITIONAL VARIATIONS IN FISH SERUM

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The high power of new analytical techniques hold their expansion to new application fields as health research [1], nutrition [2] or food safety, extremely interesting for human welfare. This widening in analytical chemistry has been supported with new non-targeted approaches as "-omics" techniques. The relevance of these techniques encourages opening new perspectives in other subjects as the nutrition of farmed animals. The assessment of nutrients and energy sources that animals need to grow ensures a correct life quality for both animals and consumers. In order to ensure this quality, metabolomics are being currently explored.

On the last years, NMR based metabolomics have been typically used because of its versatility, robustness, universality and elucidation power. On the other hand, their high price and maintenance cost together with a low sensitivity have raised the search for alternative methods like LC-HRMS. This hyphenated technique is considered "ideal" for this kind of research thanks to its full spectrum acquisition with high sensitivity (around ppb) from small sample quantities (microliters) of animal bio-fluids (blood, urine…).

In our case, juveniles of gilthead sea bream, one of the most important fish species in European aquaculture, were fed with four different diets. In these diets, marine derived ingredients were partially substituted by alternative plant materials in order to have more sustainable diets for fish farming. On the other hand, the potential introduction of mineral additives might also induce extraordinary nutritional-dependent effects on animals [3].

Serum samples were treated with acetonitrile followed by centrifugation. An aliquot was directly injected in HILIC chromatography and the rest was dried and reconstituted for RP chromatography with $H_2O$:MeOH (90:10). UHPLC-HRMS data was extracted, aligned and normalized with XCMS package and finally analyzed by multivariate analysis. Some potential biomarkers from the different experiences highlighted different metabolic pathways relevant to the diet intake and composition.

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## P-22
## A TARGETED METABOLOMICS APPROACH FOR QUANTIFICATION OF AMINE COMPOUNDS BY LIQUID CHROMATOGRAPHY / MASS SPECTROMETRY

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Metabolomics is the scientific study of the metabolites present within an organism, cell, body fluids and tissues. Metabolomics approaches combine strategies for identification and quantification of metabolites using advanced analytical technologies. These analytical methodologies are used as a tool to support the development of diagnostic and pharmaceutical interventions at the initial stages of disease. Analyzing metabolites can also be used to obtain insights in underlying processes and connectivity between organs and the dynamics at molecular levels to better understand pathological processes.
An important group of metabolites are the amines, which include amino acids and biogenic amines. These molecules are present in many of the metabolic pathways and are present in different matrixes including: plasma, urine, tissues, cerebrospinal fluid [1] and cells. Elucidation of amine profiles is important for understanding their homeostatic roles in the body, as well as their roles in disease development and/or progression. Thus it is critical to extend the scope of these analyses, adapting the sample preparation to different types of biological matrixes.
Here we describe a fast, sensitive, targeted UHPLC-MS/MS metabolomics method enabling the simultaneous determination of 74 compounds including amino acids and biogenic amines, employing an AccQ-Tag derivatization strategy. Performance characteristics of the method are reported which is based on a reverse phase separation using a Waters BEH column and a Waters Acquity UHPLC coupled with a triple quadrupole Mass Spectrometer (Xevo TQMS) using Multiple Reaction Monitoring (MRM).
Application of this method for the quantitative determination of amine levels in plasma, urine and tissue samples are given. In addition, a strategy which uses an adapted sample preparation method is presented resulting in better sensitivity for low concentration samples. This fast methodology shows good resolution for the 74 compounds enabling high throughput screening of biological samples with a high dynamic range.

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## P-23
## IDENTIFICATION OF PLUM AND PEACH SEEDS PROTEINS BY nLC-MS/MS VIA COMBINATORIAL PEPTIDE LIGAND LIBRARIES

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Plum (*Prunus domestica* L.) and peach (*Prunus persica* (L.) Batsch) seeds are common residues generated during fruit processing that, in most cases, are irretrievably lost. Recent studies have demonstrated that these byproducts possess high protein contents and could be useful to obtain high-valuable compounds such as bioactive peptides [1-3]. Therefore, the comprehensive identification of plum and peach seed proteins is of great importance.

A crucial step in proteomic analysis is sample preparation. The detection and identification of low abundance proteins is usually limited by the presence of high abundance ones. The problem is even more significant when the sample contains high levels of proteases and non-protein compounds, as in the case of vegetable samples. Combinatorial peptide ligand libraries (CPLLs) technology emerged, a few years ago, as an excellent tool to reduce the dynamic concentration range of proteins and to allow their comprehensive identification [4]. The aim of this work was to comprehensively identify proteins in plum and peach seeds using CPLLs technology and to evaluate their potential to obtain bioactive peptides.

Plum and peach seeds were milled and defatted with hexane. Native and denaturing extracting buffers were employed. Obtained proteins were precipitated with acetone, redissolved and adjusted to pH 7.4 and 2.2, optimal conditions for commercial ProteoMiner (PM-CPLLs) and home-made CPLLs (HM-CPLLs), respectively. After incubation with CPLLs and elution, proteins were analyzed by gel electrophoresis. The use of native extracting conditions and HM-CPLLs at acidic pH enabled a significant increase in the intensity of low abundance protein bands in comparison with the control. Protein identification was carried out by nLC-MS/MS. Comparing MS data against general vegetable database and specific for the *Prunus* genus allowed the identification of 141 and 97 proteins for plum and peach seeds, respectively. Among them, it could be identified many proteins from the *Prunus* genus, as well as several sequence which have been attributed by homology with other plant species. It has been also demonstrated that some potential antioxidant and antihypertensive peptides previously identified are within the sequence of identified proteins.

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## P-24
## EXPLORING THE COMPLEXITY OF YEAST METABOLOME USING ENHANCED CHROMATOGRAPHIC AND MASS SPECTRAL RESOLUTION

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Yeast has been used since ancient times for the production of bread and wine. In modern times, yeast is used as a "cellular factory" to manufacture bioethanol and organic acids. Yeast metabolism must be clearly understood to facilitate its bioengineering and improve yields of these valuable products. Gas chromatography - time of flight mass spectrometry, a "Gold Standard" for metabolomic studies, has been successfully implemented for the study of blood, plasma, urine, and plant materials. The next generation instrument for metabolomic exploration combines the power of GCxGC with high resolution time-of-flight mass spectrometry (GCxGC-HRT). The GCxGC-HRT greatly exceeds LC-based chromatographic capabilities and was successfully utilized for identification of acids, diacids, amino acids, sugars, nucleosides, nucleotides and phosphorylated metabolites in yeast.

Yeast powder was spiked with twenty three D and $^{13}C$ labeled standards. It was extracted with 1:1 methanol/chloroform and filtered into 2mL vials. Sample components were derivatized using a two-step procedure: 1) Treatment with methoxylamine hydrochloride and 2) MSTFA. They were analyzed using a combination of EI and CI, GCxGC-HRT. Compounds were separated using an R*xi*-5MS column in the first dimension and Rxi-17MS in the 2$^{nd}$ dimension and detected with the HRT operating at a resolution of 25,000 (m/z =219). System performance was monitored using internal standards (e.g., octafluoronaphthalene and fatty acid methyl esters). Confident characterization of metabolites was facilitated through spectral deconvolution, database searches combined with accurate mass formula generation.

The study resulted in the confident identification of hundreds of compounds in yeast. Compound characterization was facilitated through effective peak deconvolution of data which was critical for identification of coeluting labeled and native components. This was clearly evident from the ability of software to separate and provide deconvoluted spectra for coeluting $D_5$, $^{13}C_{11}$ and native tryptophan (3TMS). Native compounds were matched to spectra in large, well-established databases (NIST, Wiley, etc.) and accurate mass molecular, adduct and fragment ions were leveraged to confirm the identity of metabolites through formula determination. For example, the average mass accuracy and spectral similarity values for a representative set of derivatized amino acids (Glycine, serine, methionine, 5-oxo-proline, aspartic acid, ornithine, phenylalanine, glutamic acid, asparagine, lysine, tyrosine and tryptophan) were 0.72 ppm and 916/1000 respectively. This enhanced chromatographic and mass spectral resolution was particularly useful for the identification of unknowns where characterization via retention indices and database searches were inconclusive.

## P-25
## IN-SOURCE FRAGMENTATION FOR METABOLITE IDENTIFICATION IN CE-TOF BASED METABOLOMICS

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Discovery based approaches are widely applied in many research areas bringing new possibilities, especially from the exploratory point of view. These approaches are very powerful in non-targeted metabolomics, since they open research into new and unexpected findings. However, its successfulness strongly depends on the possibility and correctness of metabolite identification.
Among different methods for metabolite identification or ID confirmation, tandem MS analysis plays a very important role. However, not all analysers are suitable for tandem MS measurement e.g. TOF-MS. Therefore, in combination with the lack of commercially available standards, fragmentation is the only way to obtain information about the structure of a molecule.
For this purpose we propose a novel use of induced in-source fragmentation, by enhancement of the fragmentor voltage, with the aim of obtaining information about the fragmentation pattern. In contrast to classical tandem MS analysis, there is no prior precursor ion selection and therefore all ions present in the source undergo fragmentation. This results in spectra containing large amounts of precursors and product ions. To assign product ions to their precursors, correlation analysis has been used, assuming that $r \geq 0.9$ is an assignation of a product ion belonging to the precursor.
This method along with the chosen cut-off has been tested on three different sample complexity levels: conducting the analysis on a single standard, mix of co-eluting standards and on a plasma sample.
The results obtained clearly prove the effectiveness of the proposed methodology for metabolite ID confirmation, even for such complex samples as biological fluids.
The proposed methodology was successfully used for quick differentiation between two metabolites with the same monoisotopic mass, for example valine and betaine (m/z 118.0863).
Moreover this methodology can be applied for semi-quantification of co-eluting metabolites with the same monoisotopic mass, which has been exemplified for differentiation between leucine and isoleucine.

## P-26
## VALIDATION OF A METHOD FOR THE DETERMINATION OF THIOPURINE METHYLTRANSFERASE ACTIVITY

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Thiopurine methyltransferase (TPMT) is a cytosolic enzyme involved in the metabolism of thiopurine drugs. A genetic polymorphism is responsible for large inter-individual differences observed in TPMT activity. The aim of this study was to configure a HPLC technique, based on the conversion of 6-mercaptopurine (6-MP) to 6-methylmercaptopurine (6-MMP) using S-adenosyl-L-methionine (SAM) as methyl donor in red blood cell (RBC) lysates.

We purchased 6–MP, 6-MMP and SAM from Sigma – Aldrich. The method was developed on an Agilent 1200 HPLC system equipped with a fluorescence detector (using an excitation and emission wavelength of 310 and 390 nm, respectively). Samples were injected onto a Mediterranea Sea column from Teknokroma, using a 20% methanol, 0,04 M PBS, pH=7.4 mobile phase. Hemoglobin concentration was measured on a Sysmex XT – 1000 analyzer and used for normalization purposes.

A concentration of 600 mM and 80 mM of 6-MP and SAM respectively allows incubations of up to 2 hours, without losing the linearity of enzymatic reaction. Precision was evaluated by measurement of 3 spiked pools (at 3 levels of concentration), being 4.9% the highest coefficient of variation obtained. An average recovery of 88% was obtained in three spiked pools, analyzed for 7 days (21 replicates). No deviation of linearity was detected up to 200 µg/L. There are no differences on slopes of calibration curves by standard addition and PBS solutions, assuming that no matrix effect is present. No significant carryover was observed at 600 µg/L.

The developed method shows good analytical performance and will be implemented in our routine clinical laboratory in the near future.

## P-27
## INVESTIGATION OF NEW PSYCHOACTIVE SUBSTANCES IN URINE SAMPLES BY UHPLC-QTOF MS

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Over the last decade, an increasing number of new psychoactive substances (NPS) have been introduced on the drug market. Recent reports on their adverse effects have produced an alarming social concern. Detection of such NPS remains a challenge, due to the quick introduction of new substances. It is even more difficult to identify their main metabolites to determine which should be the most appropriate biomarkers when investigating drug consumption or intoxications. To this aim, high resolution mass spectrometry is a valuable analytical tool, as it provides sensitive full spectrum MS data with high mass resolution and mass accuracy. This work shows the potential of UHPLC-QTOF MS for investigating NPS and their metabolites in urine samples, with several illustrative examples.

In the first case, a human in vivo metabolism study was carried out for mephedrone under controlled conditions. Urine samples collected at pre-dose (control sample) and 4 hours after drug administration were analyzed, facilitating the comparison of their full-spectrum mass data. The resulting metabolites were detected and tentatively identified making use of the accurate-mass information provided by QTOF MS. Six phase I metabolites and four phase II metabolites were identified, four of them not previously reported in the literature.

In a second study, the human in vivo metabolism of MDPV (also known as “cannibal drug”) was investigated using a patient’s urine sample. As no blank control sample was available, an alternative strategy based on common fragmentation pathway was applied. Assuming that most metabolites shared the same fragmentation pathway with the parent drug, specific narrow-mass window extracted ion chromatograms at the expected m/z of MDPV fragments were obtained from full-spectrum TOF MS acquisitions. The presence of chromatographic peaks at different retention times than the parent alert on the possible presence of potential metabolites. In this case, 10 phase I metabolites, together with some glucuronides and sulphates, were detected and tentative structures were proposed.

Finally, the third example dealt with a possible case of intoxication by burundanga. After injecting the urine sample in the UHPLC-QTOF MS, the drug present in the sample was not the expected scopolamine but scopoletin, a coumarin found in the root of some plants which in higher dose also has hallucinogenic and memory-inhibiting effects.

The information provided by HRMS on NPS metabolism was essential to establish target biomarkers that can be quantitatively determined in the future by, for example, LC-MS/MS to investigate the presence of these drugs in potential consumers.

## P-28
## IMPACT OF CAPILLARY CONDITIONING AND ELECTROLYTE COMPOSITION ON CAPILLARY ELECTROPHORESIS OF PROSTATE SPECIFIC ANTIGEN ISOFORMS

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New prostate cancer (PCa) biomarkers are needed to avoid the high number of false positives caused by the PCa marker usually employed, this is, the serum concentration of prostate specific antigen (PSA).

PSA is a glycoprotein which can experience glycosylation modifications as result of pathological situations. Detection of these changes can be useful as disease biomarkers. These modifications, that can alter size and/or charge of the glycoprotein, can be monitored by capillary zone electrophoresis (CZE).

CZE methods to analyze PSA isoforms have been developed [1, 2]. In this context an isoform is a CZE peak including one or more different molecules (glycoforms) of PSA. To use the CZE pattern of PSA isoforms as disease biomarker high precision in terms of peak migration and relative area as well as resolution of as many peaks as possible are desirable. The method previously developed in our laboratory performs appropriately for some capillary lots [2]. However, lack of repeatability among lots was observed and for some of them the PSA isoforms resolution was markedly decreased.

The objective of this work is to find a methodology for CZE analysis of PSA leading to repeatable migration, size, and resolution of a large number of PSA isoforms in different capillary lots.

To this aim two cooperative strategies have been followed. The effect of basic versus acidic conditioning of capillaries and the impact of the background electrolyte (BGE) pH and composition on precision and resolution have been studied.

As a result, conditioning of capillaries with HCl instead of NaOH has been chosen. BGE consisting of 2 mM decamethonium bromide, 10 mM sodium phosphate, 5mM sodium tetraborate, 3 M urea, pH 8.0 led to the best migration and peak size precision and to the resolution of the largest number of PSA peaks.

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## P-29
## MAKING FEASIBLE TO ANALYZE PROSTATE SPECIFIC ANTIGEN ISOFORMS IN SERUM BY CAPILLARY ELECTROPHORESIS

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Controversial results about prostate cancer (PCA) screening on saving lives are being published [1]. This screening is performed using the serum concentration of prostate specific antigen (PSA). The main drawback of this biomarker is the high number of false positive results because non-malignant prostate diseases can also increase the PSA level.

One approach to look for better PCA markers is to study changes in the capillary electrophoresis (CE) pattern of PSA caused by altered glycosylation of the protein. To investigate if this is a valid approach, free PSA (PSAf) needs to be isolated from biological fluids before being analyzed by CE.

PSAf accounts only for 5–30% of the total PSA in serum. The most abundant form of PSA in this fluid is a covalent complex of PSA with alpha-1-antichymotrypsin (ACT). Ethanolamine is usually employed to dissociate the ACT-PSA complex [2]. Preliminary assays seemed to indicate that combination of this treatment with immunopurification could alter the CE profile of PSAf isoforms precluding its study as prostate cancer marker [3].

In the present study a methodology that makes possible to dissociate and immunopurify PSAf from serum to increase its available concentration to be analyzed by CE is studied.

The method has been developed using commercial standard PSAf. Afterwards it has been applied to a serum sample from a prostate cancer patient with extremely high total PSA concentration (> 20.000 ng/mL). Polyacrylamide gel electrophoresis, circular dichroism and capillary zone electrophoresis have been used to control the protein changes in the different steps of the procedure.

We have proved that the shown methodology enables to extract PSAf from serum in such a way that CE separation of its isoforms is possible.

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## P-30
## EXPLORING POTENTIAL OF GAS CHROMATOGRAPHY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION AND TANDEM MASS SPECTROMETRY FOR SENSITIVE DETERMINATION OF ETHYL GLUCURONIDE IN HAIR

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The detection and quantification of alcohol consumption is of significance in both forensic and clinical settings and can have a large impact on future legal actions and/or healthcare decisions. A few examples include the legal decisions made concerning the custody of children or the removal of a patient from the liver transplant list, among others [1].

Ethyl glucuronide (EtG) is a minor metabolite of ethanol that accumulates in hair and has proved to be a specific and sensitive long-term biomarker for the detection of chronic and excessive alcohol consumption. Due to the typical wide detection window of the hair matrix and the possibility of segmentation, the evaluation of alcohol consumption in different periods is feasible which constitutes a substantial advantage from routine methods in blood and urine [2].

The metabolization of ethanol to EtG represents approximately 0.05% of the total alcohol elimination and it is excreted mainly in urine providing EtG concentrations in the lower picogram range: >30 pg/mg hair in alcohol-dependent individuals, between 7 and 30 pg/mg hair for moderate alcohol consumers, and <7 pg/mg hair for teetotalers. Sensitive analytical methods are thus required for the reliable determination of such low EtG concentrations.

The current methods offer limits of quantification (LOQs) varying between 2 and 5000 pg/mg with LOQs generally higher (>10 pg/mg hair) for liquid chromatography (LC) methods compared to gas chromatography (GC) methods (<10 pg/mg hair) being better by the use of negative ion chemical ionization (NICI) instead of electron impact (EI), mainly due to the high fragmentation degree of EtG in EI source.

The aim of this work is to explore the capabilities of the recently revived atmospheric pressure chemical ionization source (APCI) in combination with GC and triple quadrupole mass spectrometer for the sensitive quantification of EtG in hair samples after pentafluoropropionic anhydride (PFPA) derivatization. A higher sensitivity would allow a simpler and cheaper preparation step. Soft ionizaton of this source allowed to form [M+H]+ as the base peak of APCI mass spectra, giving the possibility of selecting it as a precursor ion for MS/MS experiments. Matrix matched calibration curve in the range of 1 pg/mg to 250 pg/mg hair was injected in order to check matrix effects and estimate a LOQ and LOD. Obtained results have been compared with GC-NCI-MS/MS.

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## P-31
## 2D-LC AS A SINGLE PLATFORM FOR THE ANALYSIS OF A mABS DIGEST, SEPARATION OF ENANTIOMERIC COMPOUNDS AND PROFILING OF IMPURITIES.

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The in-depth characterization of biopharmaceuticals and the analysis of impurities in drug product formulations are from increasing interest in the pharmaceutical environment. The analysis of biopharmaceuticals like monoclonal antibodies (mAbs) requires an analytical tool with highest separation power due to the complex nature of the sample.

Furthermore, the analysis of impurities in pharmaceutical drugs is of high importance to define the safety of the final product. For a reliable impurity profiling, selective and sensitive analytical methods are needed to separate coeluting compounds and detect low level impurities. Comprehensive and (multiple) heart-cutting 2D-LC is a powerful analytical tool for both tasks. This poster will describe the use of a single 2D-LC platform for the analysis of a complex mAbs sample and of impurities in pharmaceutical substances

## P-32
## ON-LINE SOLID PHASE EXTRACTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE ANALYSIS OF WATER FRAMEWORK DIRECTIVE (WFD) PRIORITY POLLUTANTS.

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In the field of water policy, the European Union (EU) adopted the Directive 2013/39/UE amending the Water Framework Directive (WFD) 2000/60/EC and Directive 2008/105/EC. Moreover, environmental quality standards were established in Directive 2008/105/EC in order to define the maximal concentrations of priority pollutants authorized in different types of waters. More recently, the Decision 2015/495 of the EU has published a watching list of some new priority pollutants including diclofenac, hormones, antibiotics or neonicotinoids that they should be monitored and considered in the future revisions. So, multiresidue methods that can analyze a large number of these substances with low detection limits are needed.

The present work describes the development of a reliable and fully automated multiresidue method, based on on-line solid phase extraction (SPE)- liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of 26 WFD priority pollutants, including pesticides, hormones, antibiotics and neonicotinoids in environmental waters. Eight different types of cartridges (HySphere C2-SE, HySphere C8 EC-SE, HySphere C18-HD, HySphere Resin GP, HySphere Resin SH, HySphere CN-SE, HySphere MM anion and HySphere MM cation) were tested for sample preconcentration. Chromatographic separation was performed with reversed-phase F5 (pentafluorophenyl) (100 x 4.6mm, 2.6 µm) core-shell column from Phenomenex. Several mobile phases were tested and finally 1 mM ammonium formate 0.1% formic acid / methanol and 0.2% ammonium hydroxide / methanol were used in positive and negative ionization mode, respectively. For MS/MS detection selected reaction monitoring (SRM) mode in both ionization modes was used. Quantification was performed by the isotopic dilution approach using the corresponding isotopically labelled standards of the analytes.

The method was validated in surface water and waste water according to ISO 17,025. Quality parameters including recoveries, linearity, precision (intra-day and inter-day), limits of quantification (LOQs) and limits of detection (LODs) have been established for all the compounds. Obtained recoveries (higher than 70%) were sufficient to achieve method LOQs between few µg/L and ng/L for all analytes and matrices. Finally, to demonstrate the applicability of the method, several environmental waters from Metropolitan Area of Barcelona (Catalonia, NE Spain) were analyzed.

## P-33
## DETERMINATION OF HUMAN-SPECIFIC BIOMARKERS IN WASTEWATER BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Sewage epidemiology is based on the principle that untreated wastewater can be considered as a huge urine pool because the parent compounds and/or metabolites of any substance from the human body are excreted by the urine that ends up in the municipal sewage system [1]. The data were, then, normalized for the local population size (number of people served by the treatment plant). Thus, population size is crucial when estimating population-normalized consumption from wastewater-based drug epidemiology. The aim of this study is to test a methodology to determine human-specific biomarkers as widely consumed pharmaceuticals (codeine, atenolol and carbamazepine), caffeine, cholesterol and its metabolite coprostatol, and the main metabolites of nicotine (cotinine and hydroxycotinine) in wastewater for estimating population size.

Wastewater samples were collected from the influent of three wastewater treatment plants (WWTPs) in Valencia (Pinedo I, Pinedo II and Quart-Benàger). Human-specific biomarkers were extracted by SPE using Phenomenex Strata-X cartridges (Torrance, Ca, USA). Samples (250mL) were trapped through the cartridges under vacuum at a flow rate of 10 mL $min^{-1}$. Analytes were eluted with 6 mL of methanol, evaporated and reconstituted to 1 mL of water-methanol (9:1). The samples were determined by liquid chromatography (Agilent Technologies 1260 HPLC) coupled to an Agilent Technologies 6410 triple quadrupole mass spectrometer with an electrospray ionization source working in the negative mode (ESI-) (LC-QqQ-MS/MS).

In the present study, the determination of human-specific biomarkers were used to estimate the population size and the results were compared to the population size estimated by hydrochemical parameters as biological oxygen demand (BOD) and chemical oxygen demand (COD) (one inhabitant is equivalent with 59 g $day^{-1}$ BOD, 128 g $day^{-1}$ COD). The two different populations estimated were applied to determine the consumption of selected drugs of abuse.

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## P-34
## DEVELOPMENT OF A METHOD FOR DETERMINATION OF ORGANOPHOSPHATE FLAME RETARDANTS IN WATER SAMPLES BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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In this study, an efficient sample preparation method is presented for the determination of 9 organophosphosphate flame retardants (PFRs) in water samples. Analytes were extracted using solid-phase extraction (SPE) technique. The influence of several variables (e.g. solid-phase cartridge, volume of the sample, elution solvent, etc.) on the performance of the sample preparation step was carefully evaluated. Phenomenex Strata-X Polymeric Reversed Phase 200 mg cartridges, 100 mL of water samples and 6 mL of dichloromethane: methanol (1:1 vol) as elution solvent were selected as the best options. PFRs were determined by ultra-high performance liquid chromatography (UHPLC) coupled to triple quadrupole (QqQ) Mass Spectrometer (MS/MS) with electrospray ionization (ESI). The mobile phases consisted of (A) water and (B) methanol, both containing 0.1% of formic acid. PFRs were separated with a Kinetex C18 (50 × 2.1 mm, 1.7 µm) from Phenomenex. Analysis was performed in positive ion mode. The following gradient was applied: 0 min (30% B), 0.5 min (30% B), 12 min (95% B), 18 min (98% B), 25 min (98% B), and return to the initial conditions, with an equilibration time of 12 min. Interferences caused by the ubiquity of PFRs is a challenge for the study of these substances. The use of a tramp column and procedural blanks has achieved to separate or eliminate these problems. The accuracy and precision of the method was validated using spiked samples of 3 replicates for water samples with the addition of internal standards. The method validation results showed good accuracy and precision with the exception of the most polar TCEP, with average recovery efficiencies >75% and relative standard deviations (RSDs) <10%. TCEP had recovery efficiencies of 50% (RSD, 13%). Matrix effects were assessed using different water samples: distilled, river samples, influent and effluent of wastewater treatment plants.

**Acknowledgements**

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## P-35
## NON-TARGET SCREENING OF HALOGENATED ORGANIC MICROCONTAMINANTS IN MEDITERRANEAN BLUEFIN TUNA SAMPLES BY GC×GC−TOF MS

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Comprehensive gas chromatography coupled to a time-of-flight mass spectrometer analyzer (GC×GC−TOF MS) is recognized as a powerful separation technique that, when combined with an appropriate (generic) sample preparation technique, can provide information simultaneously for targeted and non-targeted compounds [1]. In this type of study, the feasibility of the technique to generate structured chromatograms is an additional feature that contributes to the identification of analytes and families of analytes for which standards are not available and so to the (virtual) identification of known-unknown and unknown compounds when only mass spectral information is available. However, when analyzing complex extracts, the manual inspection of all compounds detected, even for a particular region of the contour plot, can be a tedious and laborious work. For these types of samples, when the interest focus on a particular category of compounds, the use of classification tools can effectively contribute to further reduce the initial data set through its automatic filtering on the base of, for example, specific structural characteristics.
In this work, classifications and scripts (mass spectral filtering) software was employed to process data generated using GC×GC−ToF MS for bluefin tuna muscles subjected to a rather generic sample preparation procedure, i.e. Soxhlet extraction and acidic fat removal [1]. The script function was based on that reported on [2] and described to recognize a generic halogenated isotope cluster pattern. Therefore, in practice, it allowed the automatic and simultaneous filtering and visualization on the contour plot of the detected chlorinated, brominated, or mixed Cl-Br compounds in the investigated samples.
Once optimized, the proposed scripting and classification tool allowed the successful classification of 96% of the halogenated compounds present in a standard mixture containing 114 compounds, including PCBs, organochlorinated pesticides, PBDEs, MeO-PBDES, PBBs, and other naturally occurring organobrominated compounds. When applied to the analysis of a real tune extract, it resulted in a significant reduction of the time required for data treatment by identifying as organohalogenated among only some 4-5% of the ca. 10000-5000 initially detected peaks. Manual inspection of this sub-data set revealed an average identification accuracy of ca. 75%. Up to know, 249 chlorinated-, brominated- and mixed Cl-Br- compounds belonging to 20 general classes were identified in the 10 Mediterranean bluefin tuna muscles investigated. Identified compounds included targeted and non-targeted legacy, emerging and naturally occurring compounds.

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## P-36
## SIMULTANEOUS DETERMINATION OF PYRETHROIDS AND PYRETHRINS IN WATER AND VEGETABLES BY LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

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Pyrethroids are synthetic derivates of pyrethrins, which are natural insecticides produced by certain species of chrysanthemum (Chrysanthemum cinerariaefolium). They are increasingly used in agriculture due to their broad biological activity and slow development of pest resistance. However, these compounds are considered hazardous to the environment and human health since they dispersed from their target application crops (vegetables, fruits and livestock), reaching aqueous environments. [1].
These substances, mostly determined by gas chromatography mass spectrometry (GC-MS) can be difficult to analyse due to their volatility and degradability. The purpose of this study is, as an alternative, to develop a fast and sensitive multi-residue method for the target analysis of 7 pyrethroids and the 6 natural pyrethrins currently used in water and vegetable samples by liquid chromatography tandem mass spectrometry (LC-MS/MS). The compounds included in the study were acrinathrin, etofenprox, cyfluthrin, esfenvalerate, cyhalothrin, cypermethrin and flumethrin as pyrethroids and a commercial mix of pyrethrins containing Cinerin I, Jasmolin I, pyrethrin I, cinerin II, jasmolin II, pyrethrins II in different percentages.
As a preliminary step, the ionization and fragmentation of the compounds were optimized injecting individual solutions of each analyte at 10 ppm in the system, using a gradient elution profile of water-methanol both with 10 mM ammonium formate. The ESI conditions were: capillary voltage 4000 V, nebulizer15 psi, source temperature 300◦C and gas flow 10 L min−1. [M+H]+, [M+Na]+ ,[M+NH3]+ ,[M+NH4+]+ were tested as precursor ions. The most intense signal was for ammonium adduct for all compounds. The optimal fragmentor range for product ions were between 20 to 80 ev and the collision energy ranged between 5 to 86 ev.
The efficiency of the method was tested in 20 fruits and vegetable samples without any known exposure to pyrethroids as well as in water samples from the Turia River. At least three of the seven pyrethroids were detected.

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## P-37
## AIR SAMPLING OF CURRENT USE PESTICIDES IN BANANA PRODUCTION AREA COSTA RICA

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Agricultural activities in Costa Rica are characterized by intensive soil use and depend on a high input of agrochemicals. Weekly aerial spraying of fungicides is a common procedure at banana plantations in Costa Rica, even though often situated very close to villages. Nematicides and herbicides are applied by ground application and banana bunches are protected with insecticide-treated bags.

As a part of a large-research project in which exposure and related health effects to infants are assessed in banana producing regions of Costa Rica, different sampling techniques were applied for evaluating environmental air concentrations of current use pesticides in the Matina county located at the Caribbean coast.

Air samples were collected for six months using passive and active sampling techniques based on polyurethane foam disks (PAS-PUF) and high volume air sampler with particle collection and XAD- foam cartridges (AAS-XAD). Pesticide deposition was collected by petri dishes.

PAS-PUF sampling chambers were located at primary schools in 12 villages of the Matina county, 10 immersed in banana plantations and 2 located at about 2 kilometers distance. The PUF disks were deployed between 27 and 55 days at each site and were changed four times between June and December 2010; a total of 52 air samples were collected. The 24 hour AAS-XAD samples were taken at the start and end of each PAS-PUF sampling. A total 16 samples were taken.

PUF disks were extracted with acetone-hexane mixture. Analyses were performed for pesticides currently used in banana using a GC -MS. in selected ion mode (SIM). Sampling rates for the PUF-passive samplers were determined from the loss of depuration compounds spiked on the disks prior to deployment. Chlorpyrifos D10 was used like internal and quantitation standards were made in the PUF matrix.

The insecticide chlorpyrifos was detected in all air samples with a mean of 11.2 ng/m$^3$. The other most frequent detected pesticides for both sampling techniques, in the gas phase, were the fungicides pyrimethanil, chlorothalonil and fenpropimorph, and the nematicides cadusafos, ethoprofos and terbufos. The fungicides epoxiconazole, spiroxamine and difenoconazole were detected mainly in the particulate fraction of the AAS-XAD. This behavior was confirmed by the deposition on petri dishes.

Pesticides used at banana plantations results in environmental air contamination in adjacent primary schools, forming potential health risks for children and school personnel. Passive air samplers can be effectively used to measure average concentrations of pesticides at low levels in a tropical agricultural area

## P-38
## INVESTIGATION OF EMERGING CONTAMINANTS IN SEWAGE SLUDGE

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Nowadays, there is an increasing concern over the presence of contaminants in the environment (mainly in the aquatic system) due to the high number of potential pollutants which might be present. Emerging contaminants include compounds such as pharmaceuticals, veterinary drugs, drugs of abuse or many metabolites/transformation products, which are not currently covered by existing water-quality regulations. Some of these contaminants can reach the environment after human exposure/consumption and excretion as unchanged compounds or metabolites, and subsequent incomplete elimination in the wastewater treatment plant. Although interesting data have been reported on the concentrations of these compounds in wastewater, less attention has been paid to sewage sludge.

In this work, we have investigated the degradation of selected emerging pollutants in the aqueous and solid phases of sewage sludge submitted to anaerobic bacteria digestion, using two different types of treatments (thermophilic and mesophilic). For this purpose, after solid phase extraction (aqueous phase) and solvent extraction (lyophilized solid phase), sample extracts were analyzed by UHPLC-QTOF MS. Several compounds were detected and identified, such as pesticides, pharmaceuticals and illicit drugs, including some metabolites.

In a second step, a target method based on LC-MS/MS with triple quadrupole was developed and validated for the 11 selected analytes, identified in the previous screening, at three concentration levels: 0.2, 2 and 20 μg $L^{-1}$, for the aqueous phase of the sludge, and 50, 500 and 2000 μg $kg^{-1}$ for the solid phase. Most of analytes were satisfactorily validated, showing good recoveries and precision. The limit of quantification (LOQ) showing, in general, LOQ values below 0.1 μg $L^{-1}$ and 50 μg $kg^{-1}$ in the aqueous and the solid phases, respectively.

Finally, the behaviour of the selected compounds based on its degradation efficiency and its distribution between both sludge phases was studied. Irbesartan and benzoylecgonine seemed to be degraded during the digestions in both phases of the sludge. Venlafaxine showed a significant concentration decrease in the aqueous phase in parallel to an increase in the solid phase, proving that this compound could be mainly adsorbed onto the solid phase during the treatment. The rest of the compounds showed an increase of their concentrations in both phases after the digestions. This phenomenon might be explained as a possible release from conjugated compounds present during anaerobic digestion. Most of the contaminants presented higher concentrations in the solid phase than in the aqueous phase, therefore being mostly adsorbed on the solid particles.

## P-39
## CHANGES IN THE TRITERPENE SIGNATURE OF SOIL LIPIDS ASSOCIATED TO VINEYARD MANAGEMENT IN CANARY ISLANDS

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The molecular composition of the soil lipid extracts provides valuable biogeochemical information on the dynamics of the soil trophic system. Thus, in the particular case of the triterpenes it has been found that their assemblages vary in terms of the different management practices. The aim of this work was to monitor changes in soil functioning following transformation of secondary forests into cultivated soils, by analyzing lipid composition in soil samples from Tenerife Island (Spain) using gas chromatography–mass spectrometry (GC-MS). The results were interpreted by multivariate chemometric data treatments.

The total soil lipids were extracted at room temperature by ultrasonic shaking with a mixture of dichloromethane-methanol 3:1 (v:v). Then, the lipid extracts were methylated with trimethylsilyldiazomethane and separated by GC-MS using an HP 5890 chromatograph connected to an HP 5971 mass detector (EI, 70 eV) provided with an 25-m, 0.22 mm i.d., cross-linked OV-1 column. Helium flow was adjusted to 1 $cm^3$ $min^{-1}$; the oven temperature was programmed from 70 °C to 220 °C at 4 °C $min^{-1}$ during the chromatographic run. Lipid compounds were identified from both their retention time and mass spectra stored in spectral databases.

The main lipid families identified consisted of *n*-alkanes and *n*-fatty acids, but conspicuous amounts of cyclic compounds, mainly triterpenes (lanostenol > ursenone > taraxerol > friedelanone > amyrin > lupanone) were found in the case of semi natural forest soils (Macaronesian laurel forest). Apart from these, other diagnostic compounds (e.g., diterpenes and a variety of steroids) were found depending on the vegetation type.

The results showed how the chromatographic signature of lipid compounds quantitatively reflects the impact of agricultural practices. After clearing the Laurel forest, an increase in the even-to-odd C-number preference of alkanes was observed. As regards the chain length, both in the case of alkanes and fatty acids, a dominance of short-chain length compounds was observed in cultivated soils. More interestingly, the lipids in cultivated soils displayed a significant decrease in the total amount of cyclic constituents, mainly triterpenes, which can be used as a semi-quantitative surrogate of the extent of the changes in land-use and its agroecological significance, and can be interpreted as a simplification in the soil trophic system.

## P-40
## CHROMATOGRAPHIC FINGERPRINT OF AROMATIC PYROLYSIS PRODUCTS. ITS USEFULNESS AS QUANTITATIVE PROXY TO ASSESS THE IMPACT OF TILLAGE SYSTEMS IN SEMIARID SOILS FROM CENTRAL SPAIN

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The effects on the structural features of humic acids (HA, the stabilized, alkali-soluble, acid-insoluble colloidal fraction of the soil organic matter) from a Calcic Haploxeralf in dryland farming system located in Toledo (Central Spain) managed with different tillage practices have been approached by analytical pyrolysis (Curie-point Py-GC/MS). The split plot field design established in 1987 includes conventional, conservation (i.e., minimum and no-tillage) as well as uncultivated plots. The isolated HA fractions showed significant differences in their pyrolytic patterns of alkyl compounds (fatty acids, alkenes and alkanes). In particular, the occurrence of very short-chain fatty acids ($C_5$ to $C_{11}$) in uncultivated plots could be indicative of constitutional alkyl structures in the relictual HA from undisturbed soil. As regards the effect of soil tillage, a substantial increase of the relative amounts of fatty acids in plots under conservation practices (mainly no-tillage) was observed.

The HA from uncultivated plots showed the greatest yields of alkanes and alkenes. This was associated to the increase of the proportions of even C-numbered alkene homologues from $C_{12}$ to $C_{18}$, which is interpreted as incorporation and stabilization into the HA structure of microbial compounds during the humification process. High yields of alkylbenzenes and catechols were also characteristic of the uncultivated plots. More interestingly, the increased yields of methoxyphenols, in special of the syringyl (dimethoxyphenyl) type, in plots subjected to conventional tillage pointed out to formation processes of HA based on progressive alteration of plant lignins. From the viewpoint of soil quality, the results suggest comparatively higher maturity of the HA from uncultivated plots. In this sense, conservation tillage practices apparently lead to increasing soil C levels at expenses of accumulation of raw organic materials of comparatively lower aromaticity derived either from altered lignins and microbial biomass.

## P-41
## ANALYSIS OF DIOXANES AND DIOXOLANES IN WATER BY SOLID PHASE EXTRACTION AND GAS CROMATOGRAPHY TANDEM MASS SPECTROMETRY (GC-MS/MS)

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Dioxanes and Dioxolanes are compounds that have been identified as the cause of several odor episodes in water in the Llobregat river basin over the last years. According to published studies, some of these episodes were caused not only by resins synthesis processes [1] [2] but also by industrial residues added to dehydrated sludge in Waste Water Treatment Plants in order to increase the efficiency of the biogas production [3].

As a routine analysis, an analytical method based on Closed Loop Stripping Analysis (CLSA) tandem GC-MS/MS was established in our laboratory in order to check the trend of these episodes. However, methodologies based on Solid Phase Extraction (SPE) provide better accuracy and robustness on their results and proved to be suitable for routine analysis [3,4,5].

The aim of this study is to prove the suitability of a SPE method tandem GC/MS-MS and to determine the concentration levels of these compounds in samples from the Drinking Water Treatment Plant (DWTP), in order to know in which steps of the DWTP process studied compounds are retained or eliminated, and prevent them from entering the DWTP.

Parameters were optimized for the determination of some Dioxanes and Dioxolanes responsible of odor episodes that have recently occurred in Llobregat river. Correlation coefficients obtained were greater than 0.99. Limit of quantitation was evaluated as well as recoveries between 0,010 and 1.0µg/L for dioxanes and Dioxolanes and 0,5-50µg/l for 1,4-Dioxane.

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## P-42
## STUDY OF GEL PERMEATION CHROMATOGRAPHY TO PURIFY FOOD EXTRACTS FOR DETERMINATION OF DECHLORANES

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Dechlorane Plus (DP) is a chlorinated flame retardant additive used in electrical and electronic applications such as wires, hard plastic connectors in televisions and monitors, and furniture. DP and other similar products, such as Dechlorane 602, 603, 604, have been used since the 1960s. Their production grew in the 1970s when Mirex was banned. Although they are not under the United Nations Stockholm Convention on Persistent Organic Pollutants, they have similar properties to other POPs: persistency, bioaccumulation, long-range transport and toxicity. Since 2006, when they were first detected, these compounds have been mainly studied in environmental matrices, but the dietary exposure to these chemicals has been hardly investigated. Thus, there is a need for the development of analytical methods to determine them in food matrices.

The analytical method for the determination of Dechlorane 602, 603 and 604, syn- and anti-DP consists of the following steps: (1) addition of $^{13}$C-labelled internal standards, (2) extraction, (3) clean-up, (4) concentration (5) instrumental determination by GC-HRMS and (6) quantitation.

In this work, we have focused on the clean-up step, based on gel permeation chromatography. In order to separate the analytes from the fat, Bio-Beads S-X3 resin was used since it is recommended for the separation of chlorinated pesticides from animal fat. Elution was performed with a mixture of dichloromethane and hexane (1:1). To study the elution profile of the analytes, a standard solution was loaded on the GPC column and then different fractions were collected and analysed separately by GC-μECD. In this case, the first 95 ml did not contain any of the analytes whereas, the following 90 ml contained all of them in the following elution order: (a) Dec 602, (b) Dec 603, (c) DP-syn and DP-anti, (d) Dec 604. Since the objective of the work was the clean-up of food extracts, the same study was carried out with a spiked sample of olive oil. In this case, the fractions were analysed by GC-HRMS. In those fractions containing the fat, the analysis of Dechlorane was not possible. So, the first 90 ml and the next 25 ml (containing fat) were removed and only the following 80 ml were analysed by GC-HRMS. Most Dechloranes were eluted in those 80 ml with high recovery (90-100%) and only Dec 602 showed some lower recovery.

This clean-up method was successfully applied to the analysis of different food samples: milk and dairy products, eggs and meat.

## P-43
## COMPARISON OF SOLID PHASE EXTRACTION AND HOLLOW FIBER LIQUID PHASE MICROEXTRACTION FOR THE DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS BY MASS SPECTROMETRY AND ISOTOPE PATTERN DECONVOLUTION

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Endocrine disrupting compounds (EDCs) are a group of exogenous organic compounds that are able to interfere with the normal function of the endocrine system. To get full scientific knowledge of EDCs impact on humans and wildlife, occurrence, transport, and fate of these compounds should be known. Analytical methodology involved should face very low concentrations in matrices of high complexity. Thus, developing reliable methods for EDCs quantification is still a challenge.

A very high number of papers have been published about EDCs determination. However, data comparability for these compounds is difficult. In a recent interlaboratory comparison [1] the need for robust, standardized methods to improve data quality is emphasized. This conclusion is based on the high degree of variability between methods and the high number of outliers observed, specifically for BPA, NP and OP, which are regarded as especially difficult to measure accurately.

In the present work two different extraction methodologies have been developed and validated for the determination of bisphenol A (BPA), t-octylphenol (OP) and the technical mixture of nonylphenol (NP) in water samples by isotope dilution mass spectrometry and isotope pattern deconvolution (IPD). Quantification was performed by ultra high pressure liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS). For this purpose two extraction methodologies were proposed and compared: a routine extraction methodology based on solid phase extraction (SPE), and a non-conventional technique based on hollow fiber liquid phase microextraction (HF-LPME). The sought methodologies were satisfactorily validated in drinking water, surface water and effluent wastewater at two concentration levels, 0.1 (LOQ of the method) and 1 µg/L for alkylphenols and 0.5 (LOQ of the method) and 5 µg/L for BPA. Recoveries within 89-113% and 91-113% were obtained for HF-LPME and SPE respectively, with acceptable coefficients of variation in all cases according to SANCO guidelines. Besides, the use of IPD calculations permitted to obtain the concentration of each analyte without the need to perform any calibration curve. Finally, a thorough comparison of both methodologies was included, showing that while HF-LPME provides shorter sample preparation time and overall cost, SPE extraction and manipulation is highlighted by its simplicity.

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## P-44
## UHPLC–API–MS/MS FOR THE DETERMINATION OF POLYFLUORINATED COMPOUNDS

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Fluorotelomers are fluorocarbon–based oligomers partially saturated by fluoride ions which present hydrophilic groups (alcohol, sulfonamide and sulfonamido ethanol). The concern over these compounds has been increased because their widespread in consumer products and their facility to metabolize into the environmentally toxic and persistent perfluorinated carboxylic acid (PFOA) or sulfonates (PFOS) [1].

Gas chromatography coupled to mass spectrometry (GC–MS) is the analytical technique use to analyze fluorotelomers due to their volatile and neutral character, although electron ionization and chemical ionization show some sensitivity problems [2] when analyzing these compounds. Since PFOS and PFOA are ionic compounds, generally determined by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), it would be interesting to explore the possibility of analyzing fluorotelomers by LC–MS/MS, in order to make possible the simultaneous determination of the whole family compounds.

In this work, two UHPLC–MS/MS methods are developed using atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), since ESI was inefficient to ionize fluorotelomers. The UHPLC separation methods were carried out in a C18 column using different mobile phase gradients and the best mobile phase additives that favor the ionization in both APCI and APPI. From fragmentation studies the most intensive and characteristic product ions were selected for quantitative analysis and confirmation purposes when determining these compounds using MRM (multiple reaction monitoring) acquisition mode.

UHPLC–APPI–MS/MS with an acetonitrile–water gradient and post–column addition of toluene as dopant showed 5 times better limits of detection than UHPLC–APCI–MS/MS with a methanol–water gradient. Finally, the selective, sensitive and repetitive UHPLC–APPI–MS/MS method developed has been applied to the analysis of different water samples to evaluate their applicability for environmental monitoring purposes.

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## P-45
## MULTI-CLASS DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS, PESTICIDES AND POLYCHLORINATED BIPHENYLS IN AQUACULTURE SAMPLES BY GAS CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION

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The increasing demand of aquatic products during the last decade has accelerated the growth of aquaculture industry. However, the availability of marine feed ingredients is considered a limiting factor for the sustainability and profitability of this industry. For this reason, marine components in feeds have been replaced by vegetal ingredients. This change has led to the emergence or increased levels of organic pollutants like polycyclic aromatic hydrocarbons (PAHs) in feeds and fish [1].

The analysis of PAHs has been traditionally performed by gas chromatography coupled to mass spectrometry with electron ionization (EI) source. However, in the last years the application of atmospheric pressure chemical ionization (APCI) in environmental analysis has led to a notable sensitivity and selectivity improvement in selected reaction monitoring (SRM) based methods. The improved performance of GC-APCI-MS/MS enables to reduce the matrix content in the final extract by simple dilution, minimizing the matrix effect and avoiding the need of using matrix-matched calibrations.

In this work, a multi-class method for the determination of 24 PAHs, 15 pesticides and 7 polychlorinated biphenyl (PCB) congeners in 25 different matrices, including fish tissues, feeds and feed components, has been developed based on gas chromatography coupled to triple quadrupole tandem mass spectrometry with APCI source (GC-APCI-QqQ MS/MS). The sample procedure was a modification of the unbuffered QuEChERS method, using freezing as an additional clean-up and diluting 10 times the final extract. Matrix effect was evaluated by comparing the calibration graphs obtained with standards in solvent and in matrix after applying different dilution factors. A ten-fold dilution of the sample extract was selected, as the relative error between the slopes of calibration in solvent and calibration in matrix was acceptable for most analyte/matrix combinations. The developed method was evaluated at 0.05 and 0.5 $mg \cdot kg^{-1}$ spiking levels. Lower concentrations could not be tested because “blank” samples contained, in most cases, concentrations around or above 0.005 $mg \cdot kg^{-1}$ of several target analytes, mostly PAHs. Isotopically labeled internal standards were applied to correct for remaining matrix effects and for recoveries.

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## P-46
## ANALYSIS OF SHORT-CHAIN CHLORINATED PARAFFINS AND DECHLORANE PLUS IN GULL EGGS BY GAS CHROMATOGRAPHY-NEGATIVE ION CHEMICAL IONISATION MASS SPECTROMETRY

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In the recent years, there has been a growing concern about the risks associated with the presence of persistent organic pollutants (POPs) in marine-terrestrial ecosystems. These compounds are toxics and ubiquitous pollutants in the environment and tend to be bioaccumulated into the fat tissues of living beings, and to be biomagnified through the food web. Among them, short-chain chlorinated paraffins (SCCPs) and Dechlorane plus (DP) constitute two new groups of emerging environmental pollutants that have recently attracted a special attention because they cause toxic effects on aquatic organisms and have been detected at relatively high concentrations in environmental matrices [1-3]. In addition, information about their occurrence, fate and behaviour is still limited compared to other POPs. Seabirds have been commonly used as sentinel species for monitoring environmental pollutants because of the high trophic position in the food chain and their widespread distribution around world. The use of gull eggs has been proven suitable for monitoring the levels of environmental pollutants because they reflect the contaminant burden of the female at the time of egg laying, that is accumulated through the diet.

The aim of the study was to develop an analytical method for the analysis of SCCPs, Dechlorane plus and analogues in gull eggs based on a selective pressurised liquid extraction combined with gas chromatography-mass spectrometry, operating in negative ion chemical ionisation (GC-NICI-MS). The developed method was validated and applied to the analysis of the target compounds in yellow-legged gull eggs (*Larus michahellis*) as bioindicators of environmental pollution from four Spanish natural parks during the period 2010-2014. The presence of SCCPs in gull eggs was detected at concentrations ranging from 1.7 to 24 $ng \cdot g^{-1}$ ww, while DP levels were between 118 and 771 $pg \cdot g^{-1}$ ww. Dechlorane 602 and 603 were the most abundant DP analogous and the levels found ranged from 452 $pg \cdot g^{-1}$ ww in Ebro Delta to 2,103 $pg \cdot g^{-1}$ ww in Atlantic Islands.

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## P-47
## OPTIMIZATION OF AN INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE SCREENING OF SULFONAMIDE ANTIBIOTICS IN THE CATALAN COAST

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Aquaculture activities have greatly increased during last decades reaching the 20% of the seafood production in Europe and the 50% of global production. The widespread of these activities supposes many inconvenients for the environment as is the case of chemical contamination. Many chemicals are constantly used in feeding, therapeutic treatments and grown promotion of the seafood, causing the propagation along the aquatic environment and the food chain. Some of these chemicals can induce potential risks because its hazardous effects. In the case of antibiotics, one-third of total pharmaceutical antibiotics used in Europe are destined for veterinary purposes and a high proportion corresponds to sulfonamides [1]. Residues of these compounds are continually reaching the environment via run-off from farms, wastewaters and aquaculture, fact that has made these compounds being thought as pseudo-persistent chemicals. Moreover, trace quantities of these compounds have been associated to the mechanisms that lead bacterial resistance [2]. An accurate and efficient monitoring of these compounds is required in order to control and enhance the quality of the production as well as the preservation of the surrounding ecosystems. Biosensors are sensitive and cost effective tools of fast detection used as analytical methods to measure the presece of deteminated compound in real time.

A competitive indirect enzym-linked immunosorbent assay (ELISA) has been optimized to determine simultaneously the presence of seven of the most frequently used sulfonamide antibiotics -sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPY), sulfadimethazine (SDZ), sulfamethoxy-pirydazine (SMP) and sulfaquinoxaline (SQ)- in seawater. Selective rabbit polyclonal antibodies were raised against 5-[6-(4-amino-benzenesulfonylamino)-pyridin-3-yl]-2-methyl-pentanoic acid obtained in a previous work [3]. The optimized immunoassay presented an EC50 of 1.051± 0.384 µg/L and a limit of detection of 0.060 ± 0.041 µg/mL in seawater. The cross reactivity of the assay with different sulfonamides and their degradation products have been studied as well as matrix effects.
A previous step of pre-concentration based on solid phase extraction was included in order to increase the sensitivity. The method was validated with blind and real spiked samples and compared with a method based in HPLC/HRMS in an Orbitrap Q-exactive. A total of 20 samples collected along the Catalan coast from May to June 2015 at surface levels were assessed by the optimized ELISA and compared with the HLPC/HRMS method.
The main objective of this optimization was the selection of the proper parameters to tranfer to a biosensor that is being developed under the frame of the Sea-on-a-chip project (FP7-KBBE-OCEAN2013.1).

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## P-48
## CONTROL OF 2,4,6 TRICHLOROANISOLE IN AIR QUALITY BY TENAX/LIQUID DESORPTION

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Cork taint is a set of very undesirable flavors in wine mainly caused by 2,4,6-trichloroanisole (2,4,6-TCA) so the air control of this compound is necessary to assure the quality of cork production.
In this study a methodology to research the presence of 2,4,6 TCA was made by using SKC Tenax 150/50 cartridges and desorption by hexane. In order to study the desorption efficiency and the breakthrough volume, both 100 ng and 200 ng 246 TCA were added and desorbed with 10 ml Hexane at several air volumes sampled: 10, 20 y 60 L. Active sampling was made by a SKC Universal Pump model TX8 Deluxe. 246 TCA was analyzed in triplicate by GC/ECD

| 100 ng/L 2,4,6-TCA | | | | | | | | 200 ng/L 2,4,6-TCA | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Air Volume (l) | 2,4,6 TCA (ng) | | | Recovery (%) | | | | Air Volume (l) | 2,4,6 TCA (ng) | | | Recovery (%) | | |
| 10 | 10.9 | ± | 3.1 | 109.0 | ± | 31.4 | | 10 | 10.7 | ± | 2.8 | 53.5 | ± | 13.8 |
| 30 | 11.3 | ± | 2.1 | 112.7 | ± | 21.0 | | 30 | 11.9 | ± | 2.3 | 59.7 | ± | 11.3 |
| 60 | 9.8 | ± | 1.8 | 98.0 | ± | 17.6 | | 60 | 9.9 | ± | 0.5 | 49.7 | ± | 2.3 |

The desorption efficiency average is acceptable for a level of 100 ng. The odor detection threshold for most people to detect TCA is 4 ng/L (120 ng for a sampling volume of 30 L).

In conclusion, analysis by Tenax cartridges and liquid desorption by hexane can give an acceptable recovery in average at range of 10 ppb in active sampling in order to control the presence of 2,4,6 TCA for air quality in cork sector. However, this methodology has risks of analyte losses due to volatility of target compound and sorbent, besides the several steps in the analysis.

**Keywords:** cork, TCA, air, quality

## P-49
## LEVELS AND TEMPORAL TRENDS OF PHTHALATE METABOLITES IN HUMAN SPANISH URINE SAMPLES FROM 2012 TO 2015

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Phthalates (diesters of 1,2-benzenedicarboxylic acid), also known as phthalic acid esters (PAEs), are synthetic organic compounds that are used in a broad spectrum of industrial and commercial applications, like toys, food packaging, lubricants, vinyl flooring, adhesives, detergents, hair spray, shampoo, etc. [1] . They have endocrine disrupting potency and have become nearly ubiquitous. For the general population, the human exposure to phthalates is through ingestion, inhalation, dermal absorption, and/or contact with medical devices [2]. Once they are ingested, PAEs are rapidly metabolized to their respective monoesters in human body and some of these primary metabolites can be further metabolized to secondary metabolites and excreted by urine. All of these metabolites can be used as biomarkers of human exposure to phthalates [3].
The sample preparation and instrumental determination methods have been previously described elsewhere [4]. Briefly, the sample preparation consisted of an enzymatic deconjugation of the glucuronide phthalates followed by a purification step with OASIS© HLB SPE cartridge (Waters, MA, USA). The instrumental determination was carried out by using UPLC QqQ $MS^2$ (Xevo-TQS, Waters) working in MRM mode, with a BEH-phenyl column (50 mm x 2.1 mm, 1.7 µm),.
We present here, for the first time, the levels and temporal trends of 9 PAE metabolites (6 phthalate monoesters (monomethyl phthalate [MMP], monoethyl phthalate [MEP], mono-*iso*-buthyl phthalate [MiBP], mono-*n*-butyl phthalate [MBP], monobenzyl phthalate [MBzP] and monoethylhexyl phthalate [MEHP]) and 3 secondary metabolites of DEHP (5-OH-mono(2-ethylhexyl) phthalate [5-OH-MEHP], 5-oxo-mono(2-ethylhexyl) phthalate [5oxo-MEHP] and 5-carboxy-mono(2-ethylpentyl) phthalate [5cx-MEPP]) in human urine samples collected in 2012 and 2015 from 9 volunteers living in the Community of Madrid. Detectable amounts of the 9 MPAEs were obtained in all the studied samples, being the MPAE levels found in almost all collected in 2012 slightly lower than those found three years later (2015).

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## P-50
## USE OF Q-TOF GC/MS TO QUANTIFY SPMD EXTRACTS FROM RIVER WATER AND MARINE SEDIMENT

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Surface water quality is vitally important to our everyday life. Protecting such a valuable resource requires chemical testing typically involving mass spectrometric analysis. However, extracts from river water, waste water and marine sediments using GC/MS generates a large number of unknown compounds that need to be identified and subsequently quantified. In principle, MS can used to elucidate the de novo structure of unknown compounds beginning with the determination of molecular formula of the molecular ion, using accurate mass measurements that have errors less than a few parts per million (ppm). Mass measurements obtained with errors below 2 ppm combined with the isotopic distribution of the mono-isotopic peak can be used to eliminate many of the possible molecular formulae.

The complex organic matrix found in sediment samples makes the identification and quantification by low resolution MS difficult. This poster demonstrates the use of exact mass measurement using a Q-TOF GC/MS to identify and quantify several classes of pollutants commonly found in marine sediment and river water.

The identification and quantification will be demonstrated by means of high resolution and accurate mass measurements. The availability of standards is required to unequivocally confirm compound identities and to quantify. The combination of accurate mass (< 2 ppm mass accuracy), high resolution (>12,000 at *m/z* 200), and a large linear dynamic range allowed excellent quantification of typical pollutants found in these complex matrices. Accurate determination of method detection limits, limits of quantitation, and mass accuracy will require more extensive studies using matrix spiked standards. The use of MS/MS to further improve detection limits and reduce interferences will also be studied.

## P-51
## A SENSITIVE MULTI-RESIDUE METHOD FOR THE DETERMINATION OF 35 MICROPOLLUTANTS INCLUDING PHARMACEUTICALS, IODINATED CONTRAST MEDIA AND PESTICIDES IN WATERS

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A sensitive, multi-residue method using solid-phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed for the determination of a representative group of 35 analytes, including pharmaceuticals of different families, such as analgesic and anti-inflammatory drugs, five iodinated contrast media, and ß-blockers, corrosion inhibitors, pesticides and some of their metabolites and transformation products in water samples. Few other methods are capable of determining such a broad range of analytes.

The parameters for the extraction of the target analytes, including sorbent selection, and their chromatographic separation as well as ionisation sources (ESI and APCI) were studied. The use of Oasis HLB cartridge, which has a hydrophilic-lipophilic balanced reversed-phase sorbent at pH 7 was selected as the best compromise between the recoveries obtained for the different compounds. In order to correct matrix effects, a total of 20 surrogate/internal standards were used. ESI was found to have better sensitivity than APCI. Recoveries ranging from 79 % to 134 % for tap water and 66 % to 144 % for surface water were obtained. Intra-day precision calculated as relative standard deviation was below 34 % for tap water and below 21 % for surface water, groundwater and effluent wastewater. Method quantification limits (MQL) were in the low ng $L^{-1}$ range, except for the contrast agents iomeprol, amidotrizoic acid and iohexol (22, 25.5 and 17.9 ng $L^{-1}$, respectively). Finally, the method was applied to the analysis of 56 real water samples as part of the validation procedure. All of the compounds were detected in at least some of the water samples analysed.

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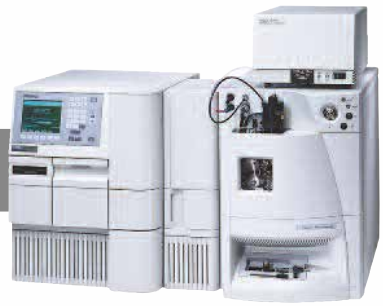

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## P-52
## USE OF GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR IDENTIFICATION AND QUANTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOY-BASED NUTRACEUTICAL PRODUCTS

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Nowadays, an increase in the consumption of soy-based nutraceutical products has occurred because the beneficial effects of these products. Although there are considered as natural products, they can be contaminated by toxic substances, such as polycyclic aromatic hydrocabons (PAHs) because they are ubiquitous and widespread compounds in the environment.

Only one maximum residue limit (MRL) has been defined by the European Union (Commission regulation (EC) No 208/2005) for benzo [a] pyrene (BaP) [1] but not in nutraceuticals. Therefore, PAHs detection studies in nutraceutical products are important, considering the lack of specific legislation. For that, the development of analytical methods that allow the detection and quantification of PAHs in these commodities are needed.

The aim of this work is the development and validation of a method to determine 13 PAHs in soy-based nutraceutical products, using an efficient extraction procedure and reliable quantification applying gas chromatography coupled to tandem mass spectrometry (GC-QqQ-MS/MS). Thus, a "dilute and shoot" procedure with acetone:*n*-hexane (1:1, v/v) has been proposed. Bearing in mind the complexity of these matrices, dispersive solid phase extraction (d-SPE), using a mixture of $C_{18}$ and Zr-Sep+, was used as clean-up stage. For quantification purposes, matrix-matched calibration was used.

The methodology was validated, checking recoveries, precision, limits of detection and limits of quantification. The recoveries were between 70 and 120%, while precision was lower than 23%. Finally, LODs and LOQs were 8 and 20 μg $kg^{-1}$ respectively. The validated method was applied to eleven soy-based nutraceutical products. Phenanthrene, pyrene, fluoranthene, fluorene and naphthalene were detected in this study, with concentrations between 4.1 to 18.5 μg $kg^{-1}$.

Acknowledgments

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[1] Regulation (EC) No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons.

## P-53
## DETERMINATION OF BIOACTIVE COMPOUNDS IN "GAZPACHO" BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY

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Processed tomato-based products as gazpacho are becoming very popular because the consumption of fruits and vegetables are associated with the prevention of cancer, cardiovascular problems and degenerative chronic diseases [1]. These beneficial effects are mainly correlated to their phenolic composition and content, which are secondary metabolites synthesized by plants. Despite the importance of these products there are scarce works that evaluate the presence of phenolic compounds in gazpacho [2].

The aim of this study has been the development of a fast method for a reliable determination of the main phenolic compounds present in gazpacho, using ultra high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS). The extraction method was optimized comparing the results provided by well-known extraction procedures as QuEChERS, rotary agitation and sonication. The best results were obtained with the last one using a mixture of methanol:water (80:20 v/v) as extraction solvent. Other extraction conditions as extraction time and number of extraction cycles were evaluated.

The developed method was validated obtaining recoveries that ranged from 90 to 107 % at the assayed concentrations (0.25, 1.00 and 5.00 mg/kg) with relative standard deviations lower than 7 % in most of the cases. The sensitivity of the method was suitable for the determination of the target compounds at the levels found in gazpacho.

The method was applied to commercial and home-made gazpachos detecting chlorogenic acid and quercetin-3-*O*-rutinoside at high concentrations (up to 11.9 and 17.0 mg/kg).

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## P-54
## UHPLC–MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF ANTIBIOTIC RESIDUES IN RAW COW MILK SAMPLES USING ULTRASOUND-ASSISTED EXTRACTION AND DISPERSIVE SOLID PHASE EXTRACTION

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Antibiotics are chemicals widely used in medicine that are able to inhibit the proliferation of bacteria. Since 2006, these substances are forbidden by the European Union (EU) to be used in sub-therapeutic doses for prophylaxis and as growth promoters in veterinary medicine [1]. In some cases the antimicrobials that are used in animals intended for food production are the same that those used in human medicine. Although the presence of residues of antibiotics in foodstuffs of animal origin could have several effects on human health, the most serious issue is the transference of resistant bacterial strains from animals to humans. In order to ensure the consumer health, the EU established the maximum residue limits (MRLs) for antibiotics in food by the Commission of the European Communities [2].

In this work, a sensitive, selective and accurate method to determine 17 quinolone and 14 β-lactam antibiotic residues in raw cow milk samples has been validated. Ultrasound-assisted extraction (UAE) followed by a clean-up step using a dispersive solid-phase extraction (d-SPE) sorbent is used. The sample treatment was optimized by experimental designs. After liquid chromatographic separation (the analytes were separated in 7 min), the MS conditions, in positive electrospray ionization (ESI) mode, were individually optimized for each analyte to obtain maximum sensitivity in the selected reaction monitoring (SRM) mode. Cincophen and piperacillin were used as surrogates for quinolones and β-lactams, respectively. The method was validated according to European Directive 2002/657/EC [3]. Recovery rates ranged from 96.0% to 104.5%. The limits of quantification ranged from 0.3 to 2.0 ng $g^{-1}$, while inter- and intra-day variability was under 7.1% in all cases. The method was applied for the determination of selected antibiotics in 28 raw cow milk samples, being observed that 28% of the samples were positive in any of the studied antibiotics. However, only 11% were considered non-compliant with the current European Union legislation, due to some milk samples corresponded to treated cows with these antibiotics.

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## P-55
## MUSK CONCENTRATIONS IN SEAFOOD ACROSS EUROPE. DIETARY INTAKE AND HEALTH RISK ASSESSMENT

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Synthetic musk compounds are cyclic emerging organic compounds used as fragrances in a wide variety of daily products such as personal care and household products. The widespread use of synthetic musk compounds in consumer products means that they are flushed down into sewage treatment plants. Due to their lipophilic characteristics and their slow biodegradation, a bioconcentration can be expected in biological aquatic system. These compounds can be divided according to their chemical structure, being classified into four classes: nitro, polycyclic, macrocyclic and alicyclic musks [1] [2].

Similarly to other environmental contaminants, such as persistent organic pollutants or heavy metals, diet may be an important route of exposure to musks. Unfortunately, there is a complete lack of knowledge regarding the role of this route in front of other potential exposure pathways. The main goal of this study was to determine the occurrence of 10 synthetic musks in fish and shellfish species from different locations across Europe. The concentrations of nitro and polycyclic musks, as well as HHCB-Lactone, a degradation product of galaxolide, were analyzed in mussels, flounders, mullet, clams and macroalgae from European hotspots. Musk levels were determined by QuEChERS and gas chromatography-ion trap-tandem mass spectrometry as separation and detection techniques [3].

For validation purposes, the analytical method was also applied to analyze musks in tissues from fish and shellfish species of high consumption in Tarragona country (Spain). Method detection limits ranged from 1 to 10 ng $g^{-1}$, while overall method recoveries (range: 47-117%) were similar between matrices. The results from European samples identified cashmeran, galaxolide, tonalide and HHCB-Lactone the main musk congeners in seafood species. HHCB-Lactone showed the highest concentration, being mackerel the specie with the greatest value (231 ng $g^{-1}$).

This information was used to assess the dietary exposure of the general population to these fragrances through seafood consumption and to characterize the human health risk.

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## P-56
## COMPREHENSIVE CHARACTERIZATION OF GOAT COLOSTRUM OLIGOSACCHARIDES BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Goat milk is a complex mixture of nutritive and bioactive components with reported health benefits such as carbohydrates, lipids and proteins. Although lactose is the main carbohydrate, presence of other oligosaccharides (OS) similar to those found in human milk, has been reported [1]. Some of these oligosaccharides, such as those containing fucosyl- or sialyl- groups have been described to have prebiotic and pathogen binding activities [2].

In recent years, different analytical techniques have demonstrated their potential in OS characterization. Nanoflow Liquid Chromatography-Quadrupole-TOF MS (Nano-LC-Chip-Q-TOF MS) shows a high sensitivity and capacity for OS compositional verification [3], whereas, Hydrophilic Interaction Liquid Chromatography (HILIC) is a powerful LC operation mode for quantitative analysis of OS, providing appropriate resolution and good peak shapes.

In this work, qualitative and quantitative analysis of goat colostrum oligosaccharides (GCO) has been carried out. Milk colostra, previously defatted and deproteinized, were treated by Size Exclusion Chromatography (SEC) to remove lactose.

Up to 78 oligosaccharides containing hexose, hexosamine, fucose, *N*-acetylneuraminic acid or *N*-glycolylneuraminic acid monomeric units were identified in the samples by Nano-LC-Chip-Q-TOF MS, some of them detected for the first time in goat colostra.

As a second step, a previously optimized HILIC-MS method [4] was used for the separation and quantitation of the main GCO, both acidic and neutral compounds. Values ranging from 140 to 315 mg $L^{-1}$ for neutral oligosaccharides and from 83 to 251 mg $L^{-1}$ for acidic oligosaccharides were found. The most abundant OS were galactosyl-lactoses (124.92-265.77 mg L-1), followed by 6'-sialyl-lactoseand sialyl-lactosamine.

To the best of our knowledge, this is the first time that a comprehensive characterization of GCO is carried out.

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## P-57
## THE VOLATILE COMPOUNDS PROFILE OF FOUR WILD EDIBLE MUSHROOMS

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Volatile organic compounds (VOCs) are low molecular weight compounds that easily evaporate at normal temperatures and pressure. Fungi VOCs include alcohols, aldehydes, ketones and hydrocarbons among others, which are derived from both primary and secondary metabolism. Many of these VOCs are involved in biological roles like fungal growth and development. The diverse function of fungal VOCs can be developed for use in biotechnological applications like biodiesel, biocontrol, mycofumigation, to monitor food spoilage and to detect plant and animal disease. In addition, fungal VOCs may be considered as "volatile biomarkers" for fungi species discrimination [1-3].

In the present work, the main objective was to investigate the volatile compounds profile of four wild mushroom species from Soria (*Boletus edulis, Lactarius deliciosus, Calocybe gambosa and Hygrophorus marzuolus)*. The volatile compounds composition was analyzed by the headspace (HS) technique by using a Turbomatrix 16 Headspace sampler (Perkin Elmer). The samples were thermostatized at 120C for 60 min. The separation of the VOCs released was performed by gas chromatography-mass spectrometry (GC-MS). The assignment of the peaks was established by comparison with the National Institute of Standards and Technology library (NIST) and the retention time of the standards.

A total of 25 volatile compounds were identified. In general, 1-octen-3-ol was the major constituent and its relative percentage was highest in *Hygrophorus marzuolus* (48.90%) as compared to 39.20% in *Boletus edulis*, 34.77% in *Lactarius deliciosus*, and the lowest concentration, 8.08% in *Calocybe gambosa*. Although 1-octen-3-ol is the most abundant and organoleptically important aroma component in all species, other important flavor-imparting compounds as 3-octanone (3.95% in *B. edulis*, 3.07% in *L. deliciosus*, 22.67% in *C. gambosa* and 3.71% in *H. marzuolus*) and 1-octen-3-one (1.94% in *B. edulis*, 0.55% in *L. deliciosus*, 1.85% in *C. gambosa* and 1.92% in *H. marzuolus)* were present. According to bibliography, the C8 compounds (45.38% in *B. edulis*, 39.14% in *L. deliciosus*, 32.60% in *C. gambosa* and 55.30% in *H. marzuolus*) are the primary volatiles in many edible mushrooms [4-5].

In the present study, aliphatic aldehydes such as 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and 3-methylpentanal were also found from fungal fruiting bodies. The aliphatic aldehydes were, with the C8 components, the main volatile group in the four species, 39.52% in *B. edulis*, 49.78% in *L. deliciosus*, 56.75% in *C. gambosa* and 39.98% in *H. marzuolus*.

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## P-58
## HPLC-UV FINGERPRINTS FOR THE AUTHENTICATION OF CRANBERRY-BASED PRODUCTS BASED ON MULTIVARIATE CALIBRATION APPROACHES

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Food products and pharmaceuticals prepared with American red cranberries (*Vaccinium macrocarpon*) are increasingly used because of the claimed healthy effects on humans, including antioxidant, anti-inflammatory and bacteriostatic activities. Although some of these properties have been attributed to the occurrence of high levels of polyphenols, antibiotic effects are particular of A-type proanthocyanidins (PACs), a kind of flavonoid compounds highly specific of cranberries. Nowadays, some concerns have arisen on the evaluation of authenticity of red cranberry as well as on the detection of possible adulterations with less expensive fruits like grapes or blueberries, which do not contain the A-type PACs.

This work was focused on the development of a strategy appropriate to detect fakes of cranberry extracts that were adulterated with grape-based products. For such a purpose, genuine cranberry and grape samples as well as extract mixtures of the two fruits (containing grape concentrations in the range 2.5 to 50%) were analyzed by reversed-phase HPLC-UV using Kinetex C18 column (100 mm × 4.6 mm i.d., particle size 2.6 µm from Phenomenex). Sample components were separated using an elution gradient based on 0.1% (*v/v*) formic acid aqueous solution and methanol as the components of the mobile phase. The flow rate was 1 mL $min^{-1}$ and the injection volume 10 µL. Chromatograms were recorded at 280, 310, 370 and 520 nm.

Data resulting from pure samples and mixtures, consisting of chromatographic fingerprints at each detection wavelength, were analyzed chemometrically. A preliminary exploratory study by Principal Component Analysis showed that the sample extracts were clearly distributed depending on the amounts of adulteration. Besides, mixture samples containing low percentages of grape could be distinguished from the group of genuine cranberry extracts. Data was further treated by Partial Least Square regression to determine the percentages of grape contamination. Results obtained were highly satisfactory, with overall errors in quantification lower than 5%. As a conclusion, the method proposed here resulted in an excellent approach to carry out the authentication of cranberry-based products relying on polyphenolic fingerprints. Although this presentation faces a particular example, we believe that it could be extended to other similar cases of food authentication.

## P-59
## DETERMINATION OF ENDOCRINE DISRUPTORS RELATED TO THE PACKAGING AND STORAGE OF FOOD IN DAIRY PRODUCTS BY UHPLC-ESI-QqQ(MRM)

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Endocrine disruptors (ECD, *Endocrine Disrupting Chemicals*) are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife[1]. Due to this, it has been necessary to develop rapid and sensitive method for the determination of ECD. This work presents the results of the analysis of **four phthalates**[2] (PAEs: dimethyl phthalate, diethyl phthalate, dibutyl phthalate and butyl benzyl phthalate), **seven parabens**[3] (PBs: methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-propyl paraben, *n*-butyl paraben, *iso*-butyl paraben and benzyl paraben) and **bisphenol A**[4] (BPA) in 42 commercially available milk samples, related to the packaging and storage.

Because of contamination problems, a method based on the simultaneous extraction and purification of the sample, was chosen to reduce the manipulation[5]. Briefly, extraction in glass column was carried out by matrix solid phase dispersion (MSPD) put on a layer of Florisil® (purification). The column was eluted with ethyl acetate and concentrated for its final quantification by ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS). With this method, all the analyte recoveries were higher than 70%.

Different brands (11 commercial trademarks) and different types of containers (metallic bag, metal pail, HDPE, PET, Combibloc®, Tetra Brick®, Tetra Gemina® and Tetra Prisma®) have been investigated to study the effect of the container and the possible contamination during the production and processing of the product. The concentration levels and profiles found (expressed as median in pg/g fresh weight) were different for each type of analyte. PBs showed the highest concentration (1564 – 42879 pg/g f.w.), followed by PAEs (2810 – 9094 pg/g f.w.). Finally, BPA showed the lowest concentration (7 – 13 pg/g f.w.).

Depending on the type of packaging, the highest PAE concentrations were found for **metal pail** and **carton packages** (which incorporate on their inside four layers of polyethylene which protects the content from the exterior moisture). Regarding BPA, the highest concentration was found for **HDPE** containers (high density polyethylene 2 ). Finally, the highest PB concentrations were obtained for those milk samples packed in **PET** bottles (polyethylene terephthalate 1). However, to the best of our knowledge, this fact seems to be more related with the nature of these samples (fresh milk, not UHT milk) than with the container itself.

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## P-60
## ASSESSMENT OF HIGH INTENSITY ULTRASOUND IMPACT ON CITRUS AND APPLE PECTIN STRUCTURE AND PROPERTIES

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Pectins are complex carbohydrates composed of up to seventeen different monosaccharides covalently bonded and distributed in different domains mainly constituted by chain of homogalacturonan (65%), rhamnogalacturonan (20-30%) and rhamnogalacturonan-II (1-8%).
Although pectins are used in pharmaceutical formulations, it is in the food industry where they can find more applications as gelling agents, thickeners, stabilizers, among others [1]. However, an increasing interest toward pectins is arousing due to their bioactivity derived from their role as dietary fiber, although other properties such as antioxidant capacity are also reported [2].
The composition, structure and properties of pectins depend on the sources and conditions of their obtainment, thus, it is possible to find pectins with similar molecular parameters but with different functional properties [3]. Also, the obtained pectins can be transformed into fractions with different structural characteristics and improved bioactivity by enzymatic or chemical hydrolysis. However, emergent technologies such as high intensity ultrasound (US) is an effective, energy-saving and environmentally friendly way for depolymerisation of biomolecules, that is the mechanical degradation during collapse of bubbles (20-100 kHz) by cavitation. Although the application of US for pectin depolymerisation is known [4], hardly information exists about the evaluation of the structure-function relationship of pectins treated by US.
The aim of this work has been the application of different analytical techniques to evaluate the structural changes and the potential modifications in the antioxidant activity suffered by pectin subjected to power US. Aqueous solutions of apple and citrus pectin (0,5%) were treated in an ultrasonic bath (45 kHz) (continuous and intermittent) and in a sonicator (20 kHz) provided by two probes of 3 and 12.7 mm at 30, 50 and 70% of amplitude, controlling the temperature between 30-40°C. Chemical modifications were examined by the determination of reducing power (DNS method), Size Exclusion Chromatography (SEC), Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). Regarding to citrus pectin whose initial relative molecular mass (Mr) was 2900 kDa, the results indicated a Mr reduction of 87%, while in the apple pectin (initial Mr 660 kDa) the Mr reduction reached 51%. The main effects were more pronounced in citrus than in apple pectin, due to the higher compound size in the former. Despite the high reduction of Mr, hardly any change was detected in the antioxidant activity of samples, indicating the suitability of power US to the production of modified pectin but preserving the bioactivity of the initial structures.

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## P-61
## DETERMINATION OF CITRININ IN RICE BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND FLUORESCENCE DETECTION

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Citrinin (CIT) is a mycotoxin produced by several species of fungus *Penicillium*, *Aspergillus* and *Monascus*. It is generally formed after harvest and occurs mainly in stored grains (as rice). In addition to economic losses, CIT contamination entails a risk for human and livestock health due to its nephrotoxicity and other chronic effects [1]. Therefore rapid and sensitive analytical methodologies for the accurate quantification of CIT are needed in order to ensure consumer health and to promote international trade.

In this study a rapid, simple and effective method for the determination of CIT in rice by ultra-high performance liquid chromatography coupled to fluorescence detection (UHPLC-FLD) was developed and validated. The separation was performed in a Zorbax Eclipse Plus RRHD (50×2.1 mm, 1.8 µm) column; mobile phase consisted on water-acetonitrile containing 2 % formic acid, and was optimized to enhance sensitivity. The gradient elution was adjusted to provide a rapid analysis and enough resolution from CIT and matrix interferences, so that extract clean-up could be avoided, increasing significantly the overall throughput of the method. With a flow rate of 0.5 mL $min^{-1}$, a column oven temperature of 45°C and an injection volume of 5 µL, FLD was performed with excitation/emission wavelengths of 331/500 nm. The total run time was 5 min. Extraction of CIT from rice was achieved with a simple QuEChERS-based extraction/partitioning process with water and acetonitrile and the aid of partitioning salts (4 g $MgSO_4$, 1 g NaCl, 1 g tri-sodium citrate dehydrate and 0.5 g sodium hydrogen citrate sesquihydrate).

The method was fully validated for white rice, using spiked samples, and its applicability for brown and red rice was ensured by means of recovery experiments. Under optimum conditions, recoveries ranged from 72.5 to 92.8 %, with relative standard deviations lower than 7.1 %. Detection and quantification limits were estimated to be 1.5 and 5.0 µg $kg^{-1}$, respectively. The method was applied to the analysis of 21 organic rice samples of different varieties (white, red and brown) and none of them were contaminated with CIT above the detection limit of the method.

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## P-62
## SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION AS CHEAP AND EASY SAMPLE TREATMENT FOR MONITORING 5-NITROIMIDAZOLE RESIDUES IN MILK SAMPLES BY UHPLC

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In the recent years the interest on Green Analytical Chemistry has involved a great development of sample treatment procedures. In this sense, salting-out assisted liquid-liquid extraction (SALLE) has proved to be an effective sample treatment, showing as main advantages the simplicity in operation, low cost, reduction the extraction time and use of non-halogenated or aromatic solvents compared to the conventional liquid-liquid extraction. In this work, an exhaustive study of parameters involved in a SALLE procedure has been carried out for the extraction of eight 5-nitroimidazole (5-NDZ) antibiotics from milk samples, previous to their analysis by ultra-high performance liquid chromatography (UHPLC) with UV detection.

From the optimization study, ethyl acetate (EtAc) and sodium sulphate ($Na_2SO_4$) were established as extraction solvent and salting-out agent, respectively. The influence on the extraction efficiency of different variables involved in the SALLE, such as salt amount, EtAc volume and agitation and centrifugation times, was evaluated through a screening experimental design. Finally, optimum values of these parameters were subsequently obtained through a surface response Doehlert design. The final SALLE procedure consisted on the following steps: 4 mL of milk sample were centrifuged for 10 min at 7500 rpm and most fat was removed. Afterwards, 10 mL of EtAc were added to the supernatant and the mixture was centrifuged for 5 min at 7500 rpm. Then, 1.0 g of $Na_2SO_4$ was added to the sample followed by centrifuging for 10 min at 7500 rpm. Finally, 6.3 mL of the organic supernatant were recovered. This volume was dried under nitrogen current at 40°C and it was reconstituted in 200 µL of acetonitrile (MeCN):water 6:94 (v/v) containing formic acid 0.1% (v/v). This extract was filtered and analysed by UHPLC, using a $C_{18}$ Zorbax Eclipse Plus column (50 mm x 2.1 mm, 1.8 µm) in gradient mode, using 0.1% (v/v) formic acid aqueous solution and acetonitrile containing 0.1% formic acid (v/v) as mobile phase solvents. Separation was performed in 8 min under a mobile phase flow rate of 0.45 mL/min. Column was thermostatically controlled at 45 °C using an injection volume of 20 µL. Analytical signals were monitored at 320 nm. Matrix-matched calibration curves showed satisfactory linearity ($R^2 \geq 0.996$). Detection limits ranging from 2 to 4 µg/L were obtained. Precision studies resulted in relative standard deviations (RSDs) lower than 10% and recoveries over 71.1% were obtained for all studied compounds in milk samples.

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## P-63
## DETERMINATION OF POLAR PESTICIDES IN OLIVE OIL AND OLIVES BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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The presence of polar pesticides in virgin olive oil is unlikely given their different physicochemical properties and, thus the relative preference of polar pesticides towards the aqueous-phase during virgin olive oil production instead of the oil phase. With the aim to estimate the behaviour of these pesticides, and the extent of their transfer to olive oil during olive oil production (processing factor) [1], two methodologies have been developed for the determination of these polar and challenging compounds in both virgin olive oil and olive matrices. The proposed methodology is based on liquid partitioning with methanol, adapted from QuEPPe-Method method for polar pesticides [2], followed by LC-MS determination using hydrophilic interaction liquid chromatography (HILIC) -with a 1.8 µm particle size HILIC column- coupled to either a triple quadrupole instrument operated in multiple reaction monitoring mode (HILIC-MS/MS) or electrospray time-of-flight mass spectrometry (HILIC-TOFMS). The selected polar pesticides included in the study were: amitrol, cyromazine, diquat, paraquat, mepiquat, trimethylsulfonium (trimesium, glyphosate counterion) and fosetyl aluminium. Both recovery rates and matrix effects were studied in both olive oil and olives matrices with the two instrumental methods used. The results in terms of extraction efficiency were satisfactory in most cases except for some particular pesticide/commodity combinations, such as the extraction of diquat and paraquat from olives, which yielded very poor recovery rates. Matrix effects were minor in the case of olive oil (ca. 20 % suppression average), while in olive matrix the suppression was more notorious (30-50 % suppression average). The studied approaches were found to be useful for the determination of the pesticides studied in olive oil and olives with limits of quantitation in the low µg $Kg^{-1}$ range in most cases.

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## P-64
## PRESENCE OF PHLORIDZIN IN ALBARIÑO Vitis vinifera L. MUSTS.

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In the course of an ongoing research on the phenolic composition of cv. Albariño musts using high performance liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS), a chromatographic peak with retention time and spectral data similar to those of Phloridzin, was found. Phloridzin belongs to the phenolic group of the dihydrochalcones, with a chemical structure that consists of two aromatic rings connected by a $C_3$ chain and with a β-D-glucopyranose moiety attached at position 2' (dihydronaringenin-2'-*O*-β-D-glucoside or phloretin-2'-*O*-β-D-glucoside). Phloridzin is the major phenolic compound found in apple where is present in high concentrations in many tissues (bark, leaves, roots, fruits,…) and it has been found in small quantities in other different plant species [1].
The extraction of phenolic compounds was carried out according to the procedure described previously [2] and Phloridzin was characterized by spectrophotometric and chromatographic methods by comparison with standard. Further evidence for the structure of Phloridzin was by analyzing the products of the acid hydrolysis and by LC-MS/MS. Aglycone was identified as dihydronaringenin by comparison with an authentic standard and sugar as glucose according to Markham [3].
As far as we know, this compound is reported here for the first time as a *Vitis vinifera* L. grape constituent. Moreover, this is the first dihydrochalcone identified in this genus. Presence of this phenolic compound in Albariño cultivar is of significant interest because of the ability of Phloridzin to block sodium-linked glucose transport and block renal re-absorption of glucose in the kidney [4]. Moreover, numerous authors link the presence of phloridzin to resistance against apple pathogens [5].

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## P-65
## POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS IN COFFEE BEANS BY UHPLC-FLU

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During the years, various studies about Polycyclic Aromatic Hydrocarbons (PAHs) have attracted attention among some experts from scientific committees because of their toxicological effects (carcinogenic and their genotoxic potential), such as reproductive and development toxicity. It is believe that they make a substantial contribution to the overall burden of cancer in humans. In order to assess the health risks to consumers associated with exposure to PAHs in foodstuffs, UE has been established controls for some PAHs in food by EFSA (2006/1881/CE). There is a food category for coffee beans and coffee brew in the European Legislation for limits of PAHs known as "coffee, tea, etc", which establish the limit of 1 µg/kg of Benzo(a)Pyrene and 1 µg/kg for the sum of Benzo(a)Pyrene, Benzo(a) Anthracene, Benzo(b)Fluoranthene and Chrysene in this group.

A procedure has been developed in order to quantify PAHs in coffee beans. Sample preparation is based on an *Accelerated Solvent Extraction* of coffee beans, using hexane as a solvent. The extract is concentrated before a basic saponification step followed by a liquid-liquid extraction with hexane. Then the extract is concentrated and it is purified using a cartridge *SPE-PAHs* (SupeIMip™ SPE PAHs)[1]. After it, the extract is concentrated and reconstituted with acetonitrile to be finally analysed by UHPLC-FLU using a ZORBAX Eclipse PAH (3.0x50 mm, 1.8 µm). This procedure has been set to determine PAHs which are included in the European Legislation (Benzo(a)Pyrene, Benzo(a)Anthracene, Benzo(b)Fluoranthene and Chrysene). We have added four additional PAHs such as Benzo(k)Fluoranthene, DiBenzo(a,h)Anthracene, Benzo(g,h,i)Perylene and Indeno(1,2,3-c,d)Pyrene.

Moreover, quality parameters have been evaluated using sample additions: selectivity, linearity, accuracy, precision and the LOD and LOQ.

About twenty samples of green and toasted coffee beans of Arabic and Robusta coffee have been analysed. The results obtained have been compared taken into account the coffee bean type of the sample.

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## P-66
## VOLATILE FRACTION CHARACTERIZATION IN TREPAT WINE VARIETY BY SPME-HRGC/MS

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Many factors contribute to characterize a wine. Aroma (volatile fraction) is the main organoleptic characteristic and key to satisfy consumers. Actually, the wine variety, ripeness, yeast activity, aging, geographic zone and vinification process affects on aromatic profile.

*Trepat* is a grape variety, typical from *Conca de Barberà* (north of Tarragona). This type grape variety is used to produce rose wine and rose sparkling wine giving a light-coloured. However, since 2004, there are red wines of *Trepat with* ruby cherry color and red fruits, liquorice, black pepper and bay leaf aroma.

In our laboratory, we have been studying the volatile fraction of different *Trepat* red wines. First of all, a solid phase microextraction technique (SPME) has been used to concentrated wine volatile compounds. Then, these retained compounds have been desorbed inside the GC injection port and they have been analyzed by gas chromatography coupled to a mass spectrometry detector (HRGC-MS) with electronic impact ionization working in SCAN mode. No previous studies has been found applying SPME-HRGC/MS for the *Trepat* grape variety.

Different types of fibers have been used to concentrate the volatile fraction of *trepat* wine: (a) Polyacrylate (85 µm), (b) polydimethylsiloxane (100 µm), (c) polydimethylsiloxane/divinylbenzene (65 µm), (d) carboxen/polydimethylsiloxane (75 µm) and (e) polydimethylsiloxane (7 µm).

In order to characterize the *Trepat* aroma have been analyzed 18 different types of wine (*Trepat (from Conca de Barberà)*, *Pinot Noir*, *Ull de llebre*, *Cabernet Sauvignon*, *Syrah* and *Merlot)* and their results have been analyzed using multivariate statistical methods.

## P-67
## POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS IN COMMERCIAL AND RAW MILK BY UHPLC-FLU

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Studies show that certain Polycyclic Aromatic Hydrocarbons (PAHs) are genotoxic, causing several diseases and heritable genetic damage in humans. PAHs exposure occurs on a regular basis for most people but the major contribution can be associated with diet.

In the EU, regulation 2006/1881/EC (modified by regulation 584/2011/EC) has established maximum levels of some PAHs in food. There is no classified group including limits of PAHs for commercial and raw milk although one of the food product categories is the “Infant formulae and follow-on formulae, including infant milk and follow-on milk”. In this case, a limit of 1 µg/kg of Benzo(a)Pyrene [B(a)P] and 1 µg/kg of the sum of B(a)P, Benzo(a)Anthracene [B(a)A], Benzo(b)Fluoranthene [B(b)F] and Chrysene [Chr] have been set.

In our laboratory, a whole procedure has been developed to quantify PAHs in commercial and raw milk. In this procedure, sample preparation consists of a basic saponification step followed by a liquid-liquid extraction with hexane. After it, sample is concentrated and reconstituted with acetonitrile to be finally analysed by UHPLC-FLU. The procedure has been set in order to determine the four PAHs included in the European Legislation (B(a)P, B(a)A, B(b)F and Chr). Additionally, four PAHs have been included: Benzo(k)Fluoranthene [B(k)F], DiBenzo(a,h)Anthracene [DiB(a,h)A], Benzo(g,h,i)Perylene [B(ghi)P] and Indeno(1,2,3-c,d)Pyrene [IP]. Furthermore, the chromatographic method is suitable to separate the 16 EPA-PAHs included in the European Legislation in less than 5 minutes.

The procedure has been validated in a concentration range between 0.01 and 10 ng/mL with commercial cow milk. Selectivity, linearity, accuracy and precision have been evaluated. In addition, the LOD and LOQ have been established.

About 70 samples of raw milk from different locations of Catalonia have been analysed. The results obtained have been compared considering the sampling location and the possibility to find PAH contamination sources near the sampling area.

## P-68
## PURIFICATION BY ION-EXCHANGE CHROMATOGRAPHY OF PREBIOTIC OLIGOSACCHARIDES DERIVED FROM LACTOSE-ISOMERIZED WHEY PERMEATE

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Whey permeate (WP) is a by-product obtained by ultrafiltration of cheese whey, lactose and salts being the main components. Today, one of the most promising uses of WP is the synthesis of prebiotic oligosaccharides for their potential use as functional ingredients. On the other hand, lactose is a reducing carbohydrate susceptible to be isomerized to lactulose. Among the different catalysts tested the highest yields (70-80%) are obtained using aluminates or borates [1, 2]. However, the main drawback of the use of these two catalysts is that they must be removed before used for human consumption. Therefore, the aim of this study was to develop a feasible method to purify isomerized whey permeate (IWP), treated with sodium borate as catalyst and selective ion-exchange resins to remove boron. Then, purified IWP was used to synthesize oligosaccharides derived from lactulose (OsLu) and from lactose (galacto-oligosaccharides, GOS).

WP (lactose 200 mg/mL) isomerization was performed with sodium borate (molar ratio boron/lactose 1:1) at pH 12, 70 ºC for 10-180 min. Boron was retained from the IWP using the combination of a strong acid (IR-120) and a weak base (IRA-743) anion-exchange resins. The elimination of boron was followed by HPLC-SEC, VIS-spectrophotometry (Azomethine H method) and ICP-MS analysis. Finally, transgalactosylation reactions were performed using β-galactosidase from *Bacillus circulans* with WP and IWP with different molar ratios of lactose:lactulose as substrate. The analysis of carbohydrates was carried out by GC-FID [3].

According to the results obtained, formation of lactulose with yields >71% was achieved after 20 and 30 min of reaction. Treatment with resins IR-120 and IRA-743, gave rise to purified IWP (99.98% boron elimination), without loss of lactulose. In the transglycosylation reactions maximum formation (75-80% total carbohydrates) of prebiotic compounds (lactulose, GOS and OsLu) was obtained after 3 h with purified IWP (ratio lactose:lactulose 1:2.4).

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## P-69
## EFFECTS OF HIGH INTENSITY ULTRASOUND ON DEPOLYMERISATION AND BIOACTIVITY OF TANNINS FROM GRAPE SEEDS VITIS VINIFERA L.

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Nowadays, the interest of consumers in healthy food is increasing due to the increase of certain diseases related to unhealthier habits. Therefore, it is required that food not just provides nutritious constituents but also with bioactive properties to help in the prevention of certain pathologies and improving health. Procyanidins, also known as condensed tannins, are flavonoid polymers found in a widespread variety of plants. Moreover, they can be linked to other compounds such as proteins and/or polysaccharides [1]. Tannins have been used in enology, pharmacology, adhesives and foams and are traditionally used in the tanning process of leather [2]. Furthermore, tannins are important because of their antioxidant potential which can contribute to the prevention of many pathologies as inflammatory and cardiovascular diseases [3].On the other hand, there is a renewed interest about emergent technologies that maintain as far as possible, the quality and bioactivity of food, as is the case of high intensity ultrasound (US), a technology that by itself or in combination with others can be employed for extraction and preservation of foods [4]. The US technology is based on acoustic cavitation spreading by compression and rarefaction waves which include the formation of bubbles that can give rise to depolymerisation of macromolecules [5]. Although the use of US for depolymerisation purposes is well-known, no studies have been reported on the application to tannins.
The aims of the present work are: i) to assess the effect of high intensity US on the depolymerisation of tannins from grape seeds *Vitis vinifera* L. by means of different analytical techniques and ii) to relate this structural change to their antioxidant properties. Assays with solutions of tannins (0.01, 0.1, 1%) were performed with US at 45 and 20 kHz using bath and probe systems, respectively. Structural modifications were evaluated by NP-HPLC and an increase in the amount of procyanidins polymers was detected in treated samples as compared to controls, indicating a possible rupture of complexes that they form with other molecules. In agreement with this, an increase in the antioxidant activity was observed in samples treated by US, which varied depending on the concentration and the system employed. The results here obtained could indicate that US would be an efficient tool to modify the structures and to improve the bioactivity of procyanidins.

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## P-70
## SCREENING AND QUANTIFICATION OF PHARMACEUTICALS AND THERAPEUTICAL AGENTS IN REAUTHORIZED PROCESSED ANIMAL BY-PRODUCTS

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Limited access to fish meal and fish oil for rapidly growing aquaculture has led to the development of aquafeeds that rely less on marine resources [1]. This, in conjunction with the recent reauthorization of the use of non-ruminant processed animal by-products (PAPs) by the European Commission, has stimulated the utilization of PAPs in alternative aquafeeds. However, the use of those new components can introduce new non-reported undesirables in food products [2].

This remarks the necessity of designing wide scope and robust analytical techniques to face this novel food safety challenge. The capability to examine the presence of a broad spectrum of analytes is required within this field. Hybrid quadrupole time-of-flight mass analyzer (QTOF MS) is among the most powerful analytical tools for wide-scope screening. The high sensitivity in full-spectrum acquisition mode together with accurate-mass measurements offers an extraordinary potential for qualitative screening purposes [3]. Once the compounds of interest are identified in the samples by QTOF MS, the use of liquid chromatography coupled tandem MS with triple quadrupole analyzer (QqQ) allows the reliable quantification of these target analytes at very low concentrations thanks to its excellent sensitivity, selectivity and robustness.

In this work, the applicability of this analytical strategy (first screening by HRMS followed by quantification of target analytes by LC-MS/MS) has been tested to assure possible emergent risk exposition of fish farming industry to a wide group of pharmaceuticals. To this aim, around 1000 pharmaceuticals and metabolites, including previous non-reported undesirables in salmon and also several dyes, have been considered in the screening. A total of 19 non-ruminant category 3 PAPs provided by the European Fat Processors and Renderers Association (EFPRA) were studied (poultry blood meal, poultry meal, feather meal, pork blood meal, pork meal and pork greaves). Quality Controls were injected for every matrix at two concentration levels to assure reliability of the analytical strategy.

After application of the screening to these PAPs, several undesirable compounds were identified, such as acetaminophen, enrofloxacin, flumequine, monensin, oxyphenylbutazone, salicylic acid, trimethoprim, tylosin A, and the dye leucocrystal violet (metabolite of the crystal violet dye). These compounds were afterwards confirmed and quantified by the use of the LC-MS/MS.

The strategy applied in this work is among the most powerful approaches for investigation of organic contaminants/residues in food and feed samples, and has allowed getting relevant information of these ingredients in the aquaculture field.

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## P-71
## ANALYTICAL METHODOLOGY FOR THE CONTROL IN EGGS OF RESIDUES OF THE ALLIUM DERIVATIVE PROPYL PROPANE THIOSULFONATE USED AS NATURAL ADDITIVE IN FEED

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A method for the determination of the allium derivative propyl propane thiosulfonate (PTSO) in eggs by UHPLC-MS/MS has been developed. PTSO is a natural ingredient derived from *Allium spp*. like onion, shallot or chives, which is added in laying hens feed as substitutive of antibiotics-based growth promoters. The major purpose of using PTSO is to maintain or reestablish a favorable relationship between friendly and pathogenic microorganisms that constitute the flora of intestinal of animals, so that it is essential to ensure that effective amounts of this compound reach the animals tissues. PTSO has a beneficial effect and it does not cause any harm to the host. In this sense, sensitive and selective analytical methods to control PTSO in animal tissues or products are needed.

In the proposed work, sample treatment consisted on protein precipitation by the addition of acidified acetonitrile following by a clean-up step based on a novel solid phase extraction (SPE) method employing mixed-cation exchange (MCX) cartridges. PTSO reacts instantaneously with cysteine and glutathione, giving s-propyl mercaptocysteine (CSSP) and s-propyl mercaptogluathione (GSSP). Therefore, PTSO was quantified as sum of CSSP and GSSP by UHPLC-MS/MS. The chromatographic method for the determination of CSSP, GSSP and PTSO was carried out with a C18 column (Zorbax Eclipse Plus RRHD 50 x 2.1mm, 1.8 µm) using a mobile phase consisting of 0.05 % aqueous FA solution (solvent A), and MeOH (solvent B) at a flow rate of 0.4 ml $min^{-1}$ in gradient mode. The temperature of the column was set at 25°C and the injection volume was 5 µL (full loop). The MS was working with ESI+ under MRM conditions.

Matrix-matched calibration curves were established for two different types of eggs (free-farming and battery cage farming). The method was characterized obtaining limits of quantification of 0.2 mg $kg^{-1}$. The precision was evaluated in terms of repeatability and intermediate precision and RSDs were lower than 10% in all cases.

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## P-72
## CHANGES IN THE VOLATILE ORGANIC COMPOUNDS PROFILE OF TWO EDIBLE MUSHROOMS AFTER FREEZE-DRYING TREATMENT

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Mushrooms are edible fungi of commercial importance for their nutritional value and appreciated flavor and aroma [1]. Fresh mushrooms are very perishable products with a limited shelf life whose sensory properties are rapidly lost together with their economic value; thus, post-harvest treatments are mandatory to extend their shelf life [2]. Freeze-drying treatment is one of the important preservation methods employed for storage of mushrooms. Although the main functional compounds like β-glucans and polyphenols content are conserved after lyophilization, the composition of volatile compounds changes owing to partial loss of the high volatility existing compounds and formation of new ones as a result of various chemical and enzymatic reactions.

The influence of lyophilization on the volatile compounds profile of two cultivated edible mushrooms, *Cantharellus cibarius* (a) and *Pleurotus ostreatus* (b), was evaluated by static head-space analysis (HS) combined with gas chromatography-mass spectrometry (GC-MS). The analysis allowed the identification of 38 volatile compounds in total. As results showed, after a freeze-drying treatment the total content of volatile compounds increased from 28 in fresh samples to 34 in the dry ones. According with bibliography [3], the key substances responsible for mushroom flavor in fresh mushrooms are mainly alcohols, ketones and aldehydes with eight carbon atoms: 1-octen-3-ol, 1-octen-3-one, 3-octanone, octanal and 2-octenal. These compounds decreased their concentration after lyophilization, in contrast to the aldehydes 2-methylpropanal, 3-methylbutanal and 2-methylbutanal that increase considerably. We also observed that the content of alcohols in freeze-dried mushrooms considerably decreased while the number and concentration of substituted pyrazines increased, i. e. 2,5-dimethylpyrazine, ethylpyrazine, 2-ethyl-5-methyl pyrazine and 3-Ethyl-2,5-dimethyl pyrazine, which are typical products of the Maillard Reaction [4]. As for the only sulfur compound identified in *C. cibarius*, dimethyl sulfide, it seems to disappear after lyophilization probably due to its instability. The aldehyde 3-methylpentanal was identified only in fresh samples, and the heterocyclic compound 2-pentylfuran decreases considerably in dry mushrooms.

Thus, we found that the aroma of both fresh and lyophilized mushrooms was formed by volatile organic compounds of different classes. Unsaturated alcohols and ketones with eight carbon atoms, hexanal, 3-methylpentanal and 2-pentylfuran were the main volatile compounds identified in fresh mushrooms, while the volatile compound profile of freeze-dry mushroom was formed mainly by the aldehydes 2-methylpropanal, 3-methylbutanal and 2-methylbutanal.

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## P-73
## DETERMINATION OF SULFONAMIDE RESIDUES IN CHICKEN MUSCLE AND EGGS BY LIQUID CHROMATOGRAPHY WITH PHOTOINDUCED FLUORESCENCE DETECTION USING QuEChERS AS SAMPLE TREATMENT

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Sulfonamides (SAs) are a group of synthetic antimicrobials that are frequently employed for clinical and veterinary purposes, in order to prevent the growth of bacteria and treat the infections from certain microorganisms and protozoa. To safeguard human health, the European Union (EU) has established a maximum residue limit (MRL) of 100 μg $kg^{-1}$ for the total amount of SAs in foods from animal origin such as muscle [1]. Using sulfonamides in animals producing eggs for human consumption is not allowed, and therefore the "zero tolerance" principle applies for these matrices.

In this work, a simple and effective method for the determination of eight SAs (sulfadiazine, sulfapiridine, sulfamerazine, sulfamethazine, sulfachloropiridazine, sulfamethoxazole, sulfadoxine, sulfadimethoxin) in chicken muscle and eggs by liquid chromatography with fluorescence detection has been validated. SAs do not present native fluorescence, however their direct determination was achieved by on-line post-column photochemical derivatization by UV irradiation [2]. Sample treatment was based on QuEChERS with several modifications depending on the matrix. Egg extracts were cleaned-up using PSA for the dispersive solid phase extraction step. On the other hand, a new clean-up sorbent, SupelTMQuE Z-Sep+, has been successfully applied in chicken muscle extract, and has proved to be effective for interference removal from this matrix. Under optimum conditions, recoveries from 65.9 to 88.1%, relative standard deviations lower than 10% (except for sulfachloropiridazine), and limits of quantification from 14 to 85 μg $kg^{-1}$ were achieved. Thus, the method complies with current European requirements.

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## P-74
## DEVELOPMENT AND VALIDATION OF A METHODOLOGY BASED ON PURGE AND TRAP EXTRACTION AND GAS CHROMATOGRAPHY DETERMINATION FOR VOLATILES IN MUSKMELON AND WATERMELON SAMPLES

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Nowadays, there has been increasing concern among consumers regarding fruit and vegetable organoleptic quality. Considering that muskmelons and watermelons are important horticultural crops around the world with an increasing demand, the determination of the organoleptic quality characteristics is becoming highly important. The high variability of the aromatic characteristics of muskmelon [1,2] and watermelon [3] makes this an attribute to be considered as a quality trait with increasing importance. Until now, around 300 volatile compounds have been identified in muskmelon and watermelon samples. A dynamic headspace purge and trap (DHS-P&T) methodology for the determination and quantification of 61 volatile compounds responsible for muskmelon and watermelon aroma has been developed and validated. The methodology is based on the application of purge and trap extraction followed by gas chromatography coupled to (ion trap) mass spectrometry detection. For this purpose two different P&T sorbent cartridges have been evaluated. The influence of different extraction factors (sample weight, extraction time, and purge flow) over extraction efficiency has been studied and optimized using response surface methodology. Precision, expressed as repeatability, has been evaluated by analysing six replicates of real samples, showing relative standard deviations between 3 and 27%. Linearity has been studied in the range of 10 to 6130 ng $mL^{-1}$ depending on the compound response, showing coefficients of correlation between 0.995 and 0.999. Detection limits ranged between 0.1 and 274 ng $g^{-1}$. The methodology developed is well suited for analysis of large number of muskmelon and watermelon samples in plant breeding programs.

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## P-75
## GC-FID DETERMINATION OF MONOSACCHARIDES FROM APPLE AND CITRUS PECTIN HYDROLYZATES PRODUCED BY THREE COMMERCIAL ENZYME PREPARATIONS

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Pectin is one of the most structurally complex families of polysaccharides in nature. This heterogeneous polymer is mainly composed of a linear chain of α-1, 4-D-galacturonic acid called homogalacturonan (HG) which is the backbone where are ramified chains rhamnogalacturonan I (RGI), (alternating sequences GalAc and α-(1, 2) linked L-rhamnosyl residues) and rhamnogalacturonan II (RGI) a most complex chain [1]. The properties and applications of pectin are affected by several factors related to pectin structure, including composition, presence and distribution of side chains, degree of methyl-esterification and acetylation, molar mass, and the charge distribution along their backbone [2]. Different chromatographic methods can be used to analyze monosaccharides such as HPAEC-PAD [1] and GC (as their alditols and aldonic acids) [3] or trimethylsilyl oximes (TMSO) [4]. Regardless of the methods used to determine the monosaccharide composition, pectins needs to be hydrolyzed in order to release its monomeric units. The aim of this work was to determine monosaccharide composition (neutral sugars and galacturonic acid) by GC-FID in commercial apple and citrus hydrolyzates obtained using three commercial enzymatic preparations.

Solutions from apple and citrus pectins (10 mg/mL) were prepared in acetate buffer 0.05M pH 4.5. For enzymatic hydrolysis, three commercial enzymatic extracts, Viscozyme, Mannozyme and Gluconex (50 U/mL) were used. Reactions were performed at 50 ºC during 24 h. Sampling was at 0, 1, 3 and 24 h. Monosaccharides and galacturonic acid in reaction mixtures were analyzed as their trimethylsilyl oximes by GG-FID using a capillary column DB-5HT (15m x 0.32 x 0.10µm). GC profiles of carbohydrates from apple and citrus pectin enzymatic hydrolyzates showed the presence of arabinose, rhamnose, galactose, mannose, glucose and galacturonic acid. Enzymatic hydrolysis after 24 h of apple pectin was almost complete using Viscozyme or Mannozyme while only a minor release of monosaccharide was observed in Gluconex hydrolyzates. In citrus pectins, the highest content of neutral sugars was detected in Viscozyme hydrolyzates followed by Mannozyme and Gluconex being of 731, 652 and 189 mg/g of pectin, respectively, measured after 24h of hydrolysis. These differences could be attributed to the low release of mannose and galactose. On the contrary, galacturonic acid was found at high levels in apple pectins reaching 726, 682 and 555 mg/g of pectins when, respectively, Viscozyme, Manozyme and Gluconex were used. These results highlight the influence of the commercial enzyme characteristics on the determination of monosaccharides from apple and citrus pectin.

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## P-76
## OCCURRENCE OF POLYCHLORINATED BIPHENYLS (PCBs) IN HONEY SAMPLES FROM DIFFERENT GEOGRAPHIC REGIONS.

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Honey is a natural product produced by *Apis mellifera* bees from the nectar or secretions of plants, and has been consumed by many people around the world as a natural food, in medical therapies, and as food supplements [1]. Polychlorinated biphenyls (PCBs) are a family of toxic and persistent organic pollutants that are present in food an environmental samples at different concentrations levels. For the general population, dietary intake is the main route of PCB exposure, contributing with more than 90% to daily exposure [2]. Some PCB congeners are toxic to humans, and the European Commission has recently established maximum permissible levels of dioxin-like PCBs (DL-PCBs) in foods [3].
Twenty PCB congeners (# 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 180, 189, and 194), including non-*ortho*, mono-*ortho*, and the most abundant PCBs were determined in 35 commercially available honeys collected between 2010 and 2012 in Brazil (n=16), Spain (n=10), Portugal (n=4), Slovenia (n=4), and Morocco (n=3). The analytical procedure followed was previously validated by the working group and briefly consists on liquid-liquid extraction followed by a clean-up step carried out using a multilayer column filled with neutral, acid, and basic modified silica. The final instrumental determination was carried out by GC-QqQ(MS/MS), using the isotope dilution technique as quantification method [4].
The results reveal the presence of low amounts of all PCB congeners (between <LOD to 878 pg/g fresh weight, f.w.) in all samples analyzed. The highest total PCB concentrations were found in Brazilian honeys (median of 1423, range of 513-3267 pg/g f.w.), followed by Portuguese (median of 1214, range of 1073-2210 pg/g f.w.), Spanish (median of 1200, range of 458-2439 pg/g f.w.), Moroccan (median of 1022, range of 955-1496 pg/g f.w.), and Slovenian (median of 558, range of 505-626 pg/g f.w.) honey samples. The WHO-TEQ concentrations are far below the maximum permissible levels for low-fatty foods in the UE legislation [3]. The principal component analysis (PCA) shows differences and similarities between PCB honey profiles from the five countries and those with Aroclors 1242, 1248, 1254, and 1260.

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Acknowledgments: Financial support was obtained from the Spanish MINECO (project AGL2012-37201), CM (Spain) and European funding from FEDER programme (project S2013/ABI-3028, AVANSECAL).

## P-77
## ANTIOXIDANT AND ANTIHYPERTENSIVE ACTIVITIES OF PEPTIDES EXTRACTED DURING THE MATURATION OF A FERMENTED SAUSAGE MANUFACTURED FROM CAMEL MEAT

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Fermentation and drying are the oldest methods to extend food shelf life. Fermentation of meat products depends on endogenous microflora. Nowadays, the addition of starter cultures during meat fermentation guarantees to obtain a standard quality and desirable technological properties in the fermented meat product. Lactic acid bacteria and coagulase negative staphylococci present proteolytic and lipolytic capacities. Formed peptides, in addition to contribute to the development of characteristic flavors in fermented products, may exert important bioactive functions such as antioxidant and antihypertensive activity.

Many studies have examined the possibilities of reformulating fermented sausages using different meat types [1,2]. The potential of camel meat has received increasing attention since it contains less fat and cholesterol and higher polyunsaturated fatty acids than other meats. The aim of this work was to evaluate the presence of antioxidant and angiotensin converting enzyme (ACE) inhibiting peptides in dried-fermented sausages prepared with camel meat and different starter cultures of staphylococci and lactic acid bacteria.

Camel sausages were prepared by mixing camel lean meat (70%, w/w), camel fat (22%, w/w), salt (4 %, w/w), glucose (1 %, w/w), $KNO_3$ (0.01%, w/w), and spices (black pepper and paprika). The mixture was divided into four batches: batch A was inoculated with isolated strains of Staph. xylosus and Lb. plantarum (107 UFC/g), batch B was inoculated with isolated starters of Staph. xylosus and Lb. pentosus (107 UFC/g), batch C was inoculated with a commercial starter powder containing a strain of Staph. carnosus and Lb. sakei (107 UFC/g), and batch D was not inoculated and used as control. Sausages were subjected to fermentation for 5 days at 23 °C and 85-90% relative humidity (RH) followed by drying at 14 °C and 75-80% RH for 23 days. Samples were taken on the 0, 7, 14, 21, and 28 days of ripening.

Protein and peptides from dried-fermented sausages were extracted according to a previous method [3]. Extracted proteins and peptides were fractionated by ultrafiltration using 5 and 3 kDa molecular weight cut-off filters. Antioxidant and ACE-inhibitory capacities of whole extracts and fractions (> 5 kDa, 5-3 kDa, and < 3 kDa) were evaluated. All samples (control and inoculated) showed antioxidant and ACE-inhibitory capacities. These properties were mainly due to the peptides below 3 kDa. Antioxidant and ACE-inhibitory capacities were affected by the ripening time and the starter culture used.

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## P-78
## PURIFICATION AND IDENTIFICATION OF NATURAL ANGIOTENSIN CONVERTING ENZYME INHIBITORY PEPTIDES FROM PEACH AND OLIVE SEED BYPRODUCTS

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Hypertension is a major risk factor linked to cardiovascular diseases. Hypertension affects about quarter of world's population and has become a public-health challenge worldwide. Despite diet and lifestyle could prevent and improve the risk of hypertension, synthetic drugs are often necessary for its treatment. The renin-angiotensin system is the main regulator of blood pressure in human organism. In this system, the angiotensin converting enzyme (ACE) converts the decapeptide angiotensin I into the vasoconstrictor peptide angiotensin II and, at the same time, deactivates the vasodilator bradykinin [1]. Although synthetic drugs exhibit high capability to inhibit ACE, they can cause various side effects. Natural ACE inhibitor peptides are effective blood pressure reducers and, unlike synthetic drugs, they do not exert side effects.

Processing of fruits generates large amounts of wastes constituted by leaves, seeds, stems, and skins. Previous researches demonstrated that stones from fruits such as plum, peach, apricot, cherry, and olive presented high protein contents and were evaluated for their content in ACE-inhibitory peptides [1, 2]. Among these fruit stones, peach and olive seeds were selected since they showed peptide fractions with high ACE-inhibitory capacity. The aim of this work was the isolation, identification, and characterization of ACE-inhibitory peptides from peach and olive seeds.

Peach and olive seeds proteins were extracted and hydrolysed with Thermolysin using previously optimized methods [2, 3]. Peptide hydrolysates were fractionated by ultrafiltration observing the highest ACE-inhibitory activity in fractions below 3 kDa. Peptides in these fractions were further purified by semipreparative reverse-phase HPLC collecting 8 fractions. The peptide concentration, percentage of ACE-inhibition, and $IC_{50}$ were measured. Peptides in most active fractions were identified by HPLC-ESI-Q-ToF using RP-HPLC and HILIC modes and *de novo* sequenced using PEAKS software. Finally, most active peptides were synthetically obtained and characterized.

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## P-79
## ORGANOLEPTIC CHARACTERIZATION OF VIRGIN OLIVE OIL BY HS-GC-MS

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In this communication we present the development of qualitative and quantitative, rapid and highly sensitive methodology for the analysis of volatile and semi-volatile organic components present in virgin olive oils for the organoleptic assessment thereof, as a complementary method to the official method (named panel test method).

The analysis is performed on a HS-GC-MS (Perkin-Elmer, Clarus TurboMatrix 40 Trap-600T), working with electron impact ionization and SIFI mode for ion detecting (FULLSCAN and SIM at the same time).. Extraction of volatile and semi-volatile components from olive oils, is conducted by the technique of static headspace from 2 g of sample, at 120 ° C for 15 min and with sample vial shaking in order to facilitate the analytes extraction. 1-Fluorobenzene is added to all samples as internal standard and used for the relative quantification of analytes (chemical markers). Chromatographic separation is carried out on a 5% phenylsilicone GC column (60 m length, 0.32mm i.d. and 1 um film thickness). Under these conditions, we selected 39 compounds as marked compounds.

Different prediction models of the three olive oils categories, Extra-Virgin-Lampante by discriminant analysis and partial least squares regression (PLS-DA) gave optimal prediction results (>85%).

The method developed for the qualitative and quantitative characterization of volatile components, as well as the identification of chemicals with positive/negative attributes in samples of virgin olive oil with different organoleptic qualities. The development of the method is being carried out with more than 700 samples of olive oils previously characterized by accredited tasting panels.

## P-80
## THE QUANTITATION OF MYCOTOXINS IN BABY FOOD USING A SIMPLE EXTRACTION AND LC-MS/MS WITH FAST POLARITY SWITCHING AND MRM SCHEDULING

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Regulations for mycotoxin contamination for some of the major classes have been set in different countries. In the European Union the mycotoxin limits were harmonized in the regulation for contaminants in foodstuffs [1,2,3] and amended by regulations in September 2007 [4]. Traditionally mycotoxin analyses have been carried out using multiplemethods, each method just suitable for one single mycotoxin or a group of chemically similar compounds e.g. aflatoxins [5]. This has been due to the wide range of polarities and physical properties of these compounds. These single mycotoxin methods include two new analytical methods for measuring aflatoxin B1 (AFB1) and ZON in baby food which were adopted as European benchmark methods in July 2010 [6]. Both methods are based on an immuno-affinity column cleanup of the sample followed by HPLC with fluorescence detection. However, it is possible that many different classes of mycotoxins could be present in the same sample of food or feed [7,8,9] and not just AFB1 and ZON.
In this work we show the ability to analyze AFB1 and ZON at comparable detection levels, to the benchmark methods, as well as implementing these two mycotoxins into an LC-MS/MS screening method. For these measurements the AB SCIEX Triple Quad™ 5500 system was used. In one single LC-MS/MS run of 13 minutes 17 compounds were detected; 12 of them in the positive ionization mode and 5 of them in the negative ionization mode. The crude extracts of different foods were diluted and injected without any extensive sample clean up or concentration steps. Detection limits of AFB1 and ZON were found to be comparable to the required values set by EN standards6 and reproducibility was found to be better than 20% without the use of any internal standards. The method itself incorporates fast polarity switching using the *Scheduled* MRM™ algorithm, unlike previous work10, and expands on the previous number of toxins detected.

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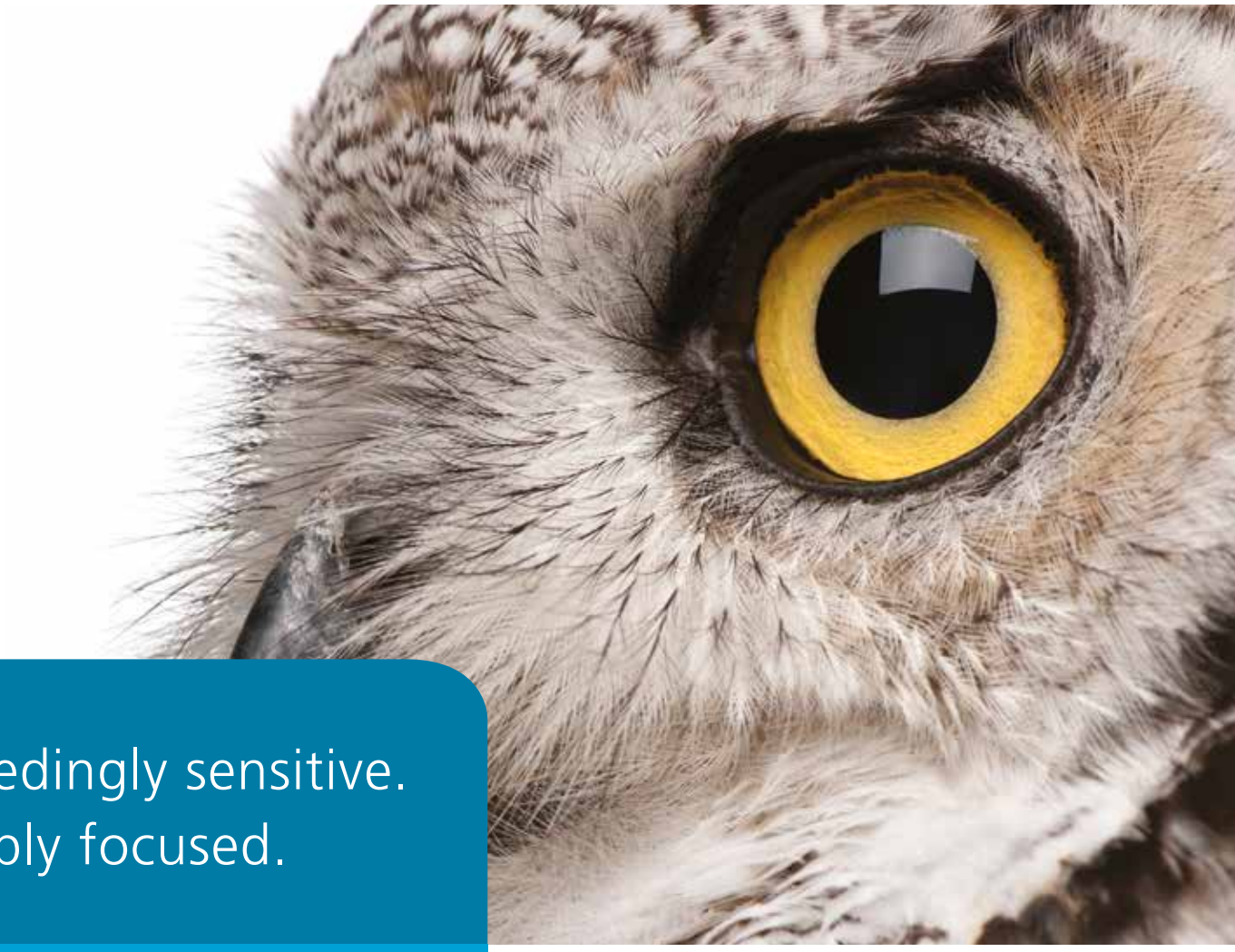

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## P-81
## GENERAL SOLUTION OF THE EXTENDED PLATE MODEL INCLUDING DIFFUSION, SLOW TRANSFER KINETICS, EXTRA-COLUMN EFFECTS AND MULTI-PATH DISPERSION

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The plate model proposed by Martin and Synge has served as a basis for the characterization of columns up-to-date [1,2]. In this approach, the column is divided in a large number of identical theoretical plates (*N*). Mobile phase transference between plates is assumed to be infinitesimally stepwise (or continuous), giving rise to the mixing of the solutions in adjacent plates during the flow. Thus, the plate height is related to the band broadening that occurs in the mixing process due to the microscopic heterogeneities in the mobile phase flow.

According to the original Martin and Synge model, solutes reach the equilibrium instantaneously in each theoretical plate and the separation occurs on the basis of their different affinity for the stationary phase. In this process, without diffusion, solute dispersion of solutes is produced by:

(i) convection or mixing of the mobile phase that reaches a theoretical plate with that existing in that plate, and
(ii) the equilibrium of the solute that is partitioned between the mobile phase and stationary phase.

Therefore, the concept of a theoretical plate is a hypothesis of this model. In a previous work [3], we proposed an extended Martin and Synge model to include slow mass-transfer kinetics (with respect to the flow rate) between the mobile phase and stationary phase. In this work, we go further, proposing a general method to solve the problem of chromatographic elution, assuming besides slow mass transfer, longitudinal diffusion in both phases, extra-column dispersion and multi-path dispersion. An inverse linear relationship was found between the variance and the ratio of the kinetic constants for the mass-transfer between the mobile phase and stationary phase, in the flow direction, and a direct relationship with the ratio of the diffusion constant and the flow rate.

The proposed model was validated with data obtained according to an approach that simulates the solute migration through the theoretical plates. An experimental approach to measure the different contributions to the band broadening using the peak variances and retention times at several flow rates is also proposed.

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## P-82
## ARE IONIC LIQUIDS REALLY ADVANTAGEOUS AGAINST AMINES AS MOBILE PHASE ADDITIVES FOR THE ANALYSIS OF BASIC COMPOUNDS?

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It is not possible to functionalize all silanols on the silica support used in most reversed-phase liquid chromatographic (RPLC) columns, making some of them accessible to the solutes and mobile phase components [1]. Residual silanols are weakly to strongly ionized within the working pH range of typical RPLC columns. This gives rise to a negatively charged stationary phase, which can act as a weak cation exchanger that increases the retention of protonated basic compounds, which are positively charged. Also, the chromatographic peaks of these compounds are broad and tailing, behavior that has been explained by the slow kinetics of the sorption-desorption equilibria of cationic solutes with silanols. The poor peak profiles affect considerably peak resolution in HPLC. The problem is of considerable importance, since a significant fraction of the drugs used in modern therapy and a large number of compounds of biomedical, biological and environmental significance have a basic character, being protonated at the usual working pH in RPLC.

A practical and highly extended practice to suppress the silanol problem is the addition of ionic (cationic or anionic) additives [2]. Recently, the popularity of ionic liquids (ILs) has grown as silanol supressors [3,4]. Added to the mobile phase in RPLC, ILs just function as salts, but keep several kinds of intermolecular interactions, which are useful for chromatographic separations.

In this work, we show that the performance of ILs and amines used as additives is similar. Therefore, the appeal of ILs should be emphasized in other terms. Thus, ILs do not change radically the pH of the mobile phase as found for amines, and seem to be less environmentally harmful than volatile amines. ILs do not appear either to damage the silica-based columns as indicated for amines, although some precautions should be taken, especially when working with ILs associated to hexafluorophosphate. Finally, the main attractive of ILs is their great variety, with the possibility to be task specific for basic compounds and other analytes.

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## P-83
## OPTIMIZATION OF THE INFORMATION IN CHROMATOGRAPHIC FINGERPRINTS OF HERBAL MEDICINES

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Pharmaceutical industries manufacture many products that contain substances extracted from plants that replace traditional herbal treatments. However, the interest in the use of medicinal herbs has not decayed, but even increased. Hence, it is necessary to improve their control. Such analyses are troublesome owing to the number of components, the difficulty in knowing their nature and the absence of standards in the market that allow for their qualitative and quantitative analysis.

Obtaining useful information about the chemical composition of such samples is still challenging. One approach to the analysis of very complex samples of which there are no standards is by obtaining their fingerprints, for which several chromatographic techniques are useful. A chromatographic fingerprint is just a chromatogram that shows multiple peaks of different heights. Samples with similar fingerprints possess similar nature and probably a common origin. Therefore, fingerprints show potential interest to determine the identity, authenticity and consistency between batches of medicinal herbs.

An ideal assessment for fingerprint quality should include the number of visible peaks and their resolution [1–3]. In this work, an HPLC strategy is reported to measure the level of information through the concept of peak prominence as chromatographic objective function, which was developed in our laboratory [4]. The peak prominence is the protruding part of a peak signal in a chromatogram with regard to the valleys that delimit the peak. As advantages, the peak prominence is:

(i) sensitive to signal profile and size
(ii) easily interpretable and normalized
(iii) scores individual peaks, which facilitates the combination of the elementary resolution and peak counting
(iv) does not require standards
(v) measurable from the raw overall signal, being directly applicable to chromatograms of mixtures
(vi) attends to all peaks, corresponding each one to one or more compounds.

In this work, an approach is also developed to optimize the separation conditions, in order to get maximally informative fingerprints, using the peaks present in the sample as internal standards, whose correspondence among experiments is unequivocal.

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## P-84
## OPTIMIZATION OF RESOLUTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PROTEIC AMINO ACIDS CONSIDERING BOTH TIME AND SPECTRAL INFORMATION

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The optimization of the resolution in high-performance liquid chromatography (HPLC) is traditionally performed attending only to the time information. However, even in the optimal conditions, some peak pairs may remain unresolved. Such resolution can still be completed by deconvolution, which can be carried out with more guarantees of success by including spectral information.

Current HPLC instruments are able to yield two-way signals, where full spectra are collected as a function of time. With such rich signals readily available, highly specific columns are not so imperative for resolving complex samples at routine level. Hence, the usual strategy of finding the best separation conditions giving prevalence to the chromatographic resolution in the time order seems not too logical nowadays. Even in situations involving two peaks with poor resolution in both data orders, nearly selective wavelength windows may exist, and consequently, the spectrochromatogram can be rich enough in analytical information to retrieve the underlying contributions. As far as the analyte contributions can be correctly retrieved by deconvolution from the spectrochromatogram, the assayed separation condition will be acceptable.

In this work, a two-way chromatographic objective function (COF) that incorporates both time and spectral information using diode-array detection was tested, based on the multivariate selectivity (figure of merit derived from the net analyte signal) [1]. This COF is sensitive to situations where the components that coelute in a mixture show some spectral differences. Therefore, it is useful to locate experimental conditions where the spectrochromatograms can be recovered by deconvolution using the combination of orthogonal projection approach and alternating least squares [2].

In previous work, the proposed approach was applied to the separation of a mixture of 25 phenolic compounds, which remained unresolved in the chromatographic order using linear and multi-linear gradients of acetonitrile-water [3]. A new application is now reported for a mixture of proteic amino acids, which were satisfactorily resolved using a gradient of acetonitrile-water, but still at too high analysis times. By increasing the elution strength, the retention times decreased, but yielding overlapping among the peaks. The approach was used to find experimental conditions where the peaks could be deconvolved in spite of the small spectral differences.

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## P-85
## THE IMPACT OF INSTRUMENT DESIGN CHARACTERISTICS ON REVERSED-PHASE HPLC AND UHPLC METHODS TRANSFER

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Transfer of established reversed phase methods across both HPLC and UHPLC chromatographic instrumentation requires careful consideration of the operating parameters and design of each instrument. Specifically, dwell volume differences across instruments can also lead to changes in retention [1]. With reference to dwell volume, chapter <621> of the USP monograph states [2]"If adjustments are necessary, change in ... the duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed.2" While adjustments for dwell volume can be manually entered into a gradient table, this approach requires calculations and manual changes to the gradient table; both of which can be prone to error. Using a feature in the software to adjust the time between the start of the gradient and the point of injection, the duration of the gradient hold can be adjusted to emulate a system with larger or smaller dwell volume.

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## P-86
## ESTIMATION OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHIC RESPONSE FROM ONE-DIMENSIONAL GAS CHROMATOGRAPHY DATA

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Optimization of a separation in comprehensive two-dimensional gas chromatography (GC×GC) requires the selection of an appropriate set column, and the optimisation of their respective dimensions, temperature programs and flow rates. Until not so long ago, a time consuming trial and error process was the only way to select the columns and chromatographic conditions for the two GC dimensions. During the last few years, several attempts have been proposed to estimate the chromatographic response in GC×GC [1-4]. Although most of these methods are helpful contributions, some of them focussed exclusively on the retention time estimation and do not consider the effect of peak width, also essential for a correct prediction of the chromatographic separation capability. Others are based on theoretical formulas not applicable to the extreme experimental conditions used in GC×GC, or are only valid for specific types of compounds or stationary phases: these models cannot be corrected for different separation problems.

In this work, modelization of retention time and peak width has been carried out from experimental 1D GC data in order to estimate the response in GC×GC separation for different column sets operated under different flows and temperatures.

Several theoretical and experimental models are proposed for estimation of $^{1}$D and $^{2}$D retention time and peak width; in experimental models, 1D GC data are required together with information on column characteristics and operation conditions. Models for hold-up time, retention factor and peak width were first validated in 1D GC from the fit between experimental and calculated data. Their validation in GC×GC was carried out by using *n*-alkanes and a mixture of disaccharides as their trimethylsilyl oxime derivatives. Comparison of experimental and estimated data showed good results for retention time in both first and second dimension. Peak widths presented, however, some errors, caused by the no consideration on estimation of the effect of the modulator or the rather unusual chromatographic conditions used.

The developed programme is very versatile as it can be used for the calculation of the chromatographic response of compounds with different polarity and volatility, analysed under different pressures and temperature ramps, and with different sets of columns.

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## P-87
## SUITABILITY OF REVERSE STATIONARY PHASES WITH DIFFERENT CHEMICAL FUNCTIONALIZATION AND PARTICLE POROSITY: THERMODYNAMIC STUDY AND APPLICATION TO THE ANALYSIS OF LUOTONIN A AND DERIVATIVES

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The chromatographic retention is mainly dependent on the chemical nature of the stationary and the mobile phases. In reverse-phase LC, both retention and selectivity are primarily driven by hydrophobic interactions [1,2]. In the case of phenyl-alkyl- (PH) and perfluorinated-phenyl-alkyl (PFP) stationary phases, additional interactions (such as π–π stacking and steric accommodation onto the stationary phase) are possible in comparison to the well-known C-18 columns. Porosity also exerts an important role on the chromatographic behaviour, and superficially porous particles afford excellent separation features and improve the column's performance [3]. The study of the correlation between retention and temperature is a well-stablished resource making possible to investigate the thermodynamic nature of the interactions between analytes and stationary phase.

Columns packed with three different chemically-modified particles were studied in both fully- and superficially-porous versions at temperatures ranging from 25º C to 60º C. The analytes were a series of 7 compounds with a pentacyclic aromatic backbone (luotonin A and 6 different analogues bearing methyl-, phenyl-, chloro- and bromo- substituents) and the anticancer alkaloid camptothecin. The enthalpy and entropy values were determined for all the 8 compounds by means of the corresponding Van't Hoff plots (Table 1).

*Table 1: Phehyl-hexyl fully- and superficially-porous particles, the enthalpy and entropy values for luotonin A clearly exhibit two different zones related to the temperature.*

| | ***FULLY POROUS PARTICLES*** | | ***SUPERFICIALLY POROUS PARTICLES*** | |
|---|---|---|---|---|
| | ***40-60 ºC*** | ***25-40 ºC*** | ***35-60 ºC*** | ***25-35 ºC*** |
| **Δ*H* (J/mol)** | - 6472.52 | - 3285.29 | - 6481.54 | 1316.40 |
| **Δ*S* (J/mol)** | - 6.65 | 3.48 | - 11.93 | 13.49 |

As expected, the main reason accounting for the differences in the thermodynamic behaviour among the columns was not the porosity of their particles, but the nature of their functionalizations. On C-18 and PFP stationary phases, Van't Hoff plots are linear for all of the compounds, but on the PH stationary phases, two different zones are observed (Table 1) depending on the temperature. This explains the different retentions exhibited by the same compounds on the three stationary phases and depending on the temperature.

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## P-88
## REMOVAL EFFICIENCES IN WASTE WATER TREATMENTS PLANTS FOR EMERGING POLLUTANTS

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Pharmaceuticals and personal care products are extensively used in Human Population and most of these are excreted through urine or feces and come to end up to treatment plants. Recent studies indicates that pharmaceuticals, personal care products or illicit drugs from Waste Water Treatment Plants (WWTP) are a considerable chemical pollution in surface [1, 2]. The purpose of this study is to determine the removal efficiency for two WWT of Pinedo I and II, Valencia (Spain). After obtaining the results of analysis by an Agilent 1260 HPLC in tandem with a 6410 MS/MS triple quad, a simple mathematical operation with the influents and effluents is performed. This operation consists in subtracted from the influent, the effluent, divided by the result of the influent and this multiply by 100. Results are expressed as a percentage with its 95 % confidence interval (CI).

The influent and effluent of the samples were filtered with a 0.50 µm glass fiber filter of 90 mm by Advantec (Minato-ku, Tokyo, Japan). After filtration, 250ml of this water is extracted through a SPE. SPE was performed with Strata-X 33U Polymeric Reversed Phase (200 mg/6 mL) from Phenomenex. These cartridges were conditioned with 6 mL of methanol and 6 mL of distilled water. Extracts were eluted with 6mL of Methanol and evaporated with compressed air. The residue was reconstituted with 1 mL of methanol–water (30:70, v/v).

The removal efficiencies depend on the type of the compound, these rates remain between 23% and 100%. In some cases, removal efficiency is negative since some compounds are accumulated in the sludge and these have more concentration. Tertiary treatment including UV disinfection could efficiently reduce most of the residual pharmaceuticals below their quantification limits.

**Acknowledgments**
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## P-89
## DETERMINATION OF MIXTURES OF THE FOUR MAJOR SURFACTANT FAMILIES IN CLEANERS BY LIQUID CHROMATOGRAPHY WITH COLUMNS IN TANDEM

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The determination and characterization of mixtures of surfactants is important in relation to the quality control of cleaners and other industrial products. Most household and industrial cleaners contain four major surfactant families, namely, linear alkylbenzene sulfates (LAS), alkyl ether sulfates (LES), fatty alcohol ethoxylates (FAE) and oleins (fatty acids of vegetal origin). The determination of mixtures of these families is a complex analytical problem. In this, work we have developed a simple and efficient solution: to use two detectors (UV-Vis and ELSD) and two columns in tandem, combined in such a way that the 1$^{st}$ column mainly provides a separation of anionic (LAS and LES) from non-ionic (FAE) or weakly anionic (oleins) surfactants, whereas the 2$^{nd}$ column increases the separation of the families also affording an excellent separation of the oligomers.

To perform the separation between the families, two stationary phases were tried: pentafluoro-phenyl (F5) and biphenyl (BP). Using an acetonitrile (ACN) gradient (reversed phase conditions), LAS and LES eluted first, followed by FAE and olein. The two columns gave closely similar chromatograms, then, F5 was selected. The introduction of ammonium acetate in the mobile phase increased retention of LAS and LES, with an incipient separation among the oligomers. To lower the pH down to 4.0 with acetic acid further increased retention of these two families.

On the other hand, a C8 column in tandem with the first F5 column was used to increase the separation between the LAS homologues and the successive LES and FAE hydrocarbon series, and to resolve the oligomers within the series. The chromatograms showed the peaks of the LAS homologues overlapped with the LES series, followed by the resolved FAE series and the oleins at longer retention times.

To fully resolve the LAS and LES oligomers, the selectivity provided by the UV-Vis and ELSD detectors connected in series was used. Only LAS was observed on the UV-Vis recording. Subtraction of the UV-Vis from the ELSD chromatogram provided the ELSD peaks of the LES oligomers, free from the LAS interference. A combined gradient in which the ACN concentration increased, and at the same time the ammonium acetate concentration (necessary to resolve the LAS homologues) decreased, and the acetic acid concentration increased (to reduce overlapping between FAE and olein peaks), provided excellent resolution of all the surfactant families and the oligomers within them. The procedure was applied to personal care products and household cleaners.

## P-90
## DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF MYCOTOXINS IN HUMAN PLASMA

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Mycotoxins are toxic secondary metabolites produced by fungal species and can occur naturally in plant products such as cereals, nuts and dried fruit, in their by-products and in food of animal origin if animals have been fed with contaminated products. Through these products, they can reach man and affect human health. Some of them are particularly dangerous for humans. The International Agency for Research on Cancer has classified aflatoxin B1 and naturally-occurring mixtures of aflatoxins as human carcinogens (group 1) and ochratoxin A as a possible human carcinogen (group 2B)[1]. Different studies have been carried out in order to know their presence in food and, taking into account the data obtained, along with toxicity data, the European Commission has set maximum permitted levels of some mycotoxins in different matrices [2]. However, taking into account that food is usually processed before ingestion and that the bioavailability of mycotoxins from food is unknown in many cases, studies regarding their presence, and that of their metabolites, in human biological fluids are needed in order to determine the real human exposure. For that purpose it is necessary to have validated analytical methods. Methods capable of detecting simultaneously several mycotoxins are particularly relevant as human population is usually exposed to multiple mycotoxins because diet is generally varied and the same food might be contaminated by several mycotoxins.

In the present study, an LC-MS/MS (QqQ) method has been developed for simultaneous determination of 21 mycotoxins in human plasma: nivalenol (NIV), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM-1), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), neosolaniol (NEO), diacetoxyscirpenol (DAS), fusarenon X (FUS-X), T-2, HT-2, aflatoxin B1 ($AFB_1$), aflatoxin B2 ($AFB_2$), aflatoxin M1 ($AFM_1$), aflatoxin G1 ($AFG_1$), aflatoxin G2 ($AFG_2$), fumonisin B1 ($FB_1$), citrinin (CIT), zearalenone (ZEA), ochratoxin A (OTA), ochratoxin B (OTB) and sterigmatocystin (STER). Sample treatment is simple and based on the extraction with acidified acetonitrile. Validation of the method has been initiated studying parameters as limits of detection and quantification, linearity and precision (within-day). The limits of quantification (LOQ) were 14, 1.1, 1.4, 3.5, 0.1, 3.5, 0.7, 1.5, 0.02, 0.02, 0.03, 0.02, 0.08, 1.2, 0.3, 0.06, 0.03, 0.06, 9.0, 0.03 and 0.7 ng/mL for NIV, DON, DOM-1, FUS-X, NEO, 3-ADON, 15-ADON, AFG2, AFM1, AFG1, AFB2, AFB1, DAS, CIT, HT-2, OTB, FB1, T-2, ZEA, OTA and STER, respectively. Repeatability (between-day variability) assayed at three levels of concentration was < 15% (20% for the LOQ).

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[2] COMMISSION REGULATION (EC) No 1881/2006

## P-91
## SIMULTANEOUS QUANTIFICATION OF VOLATILE FATTY ACIDS AND LACTIC ACID BY GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Oligosaccharides from goat´s milk have positive effects in human health, through its activity as prebiotics, modulating microbial populations. Volatile fatty acids and lactic acid are produced via fermentation of these oligosaccharides. Quantification and identification of volatile fatty acids and lactic acid is a key step to evaluate the microbial metabolism of these compounds.
Usually, volatile fatty acids (acetic, propionic, butyric, isobutyric, valeric, isovaleric) are detected by GC-FID; lactic acid is quantified in a different way, using spectrophotometric methods (which present reproducibility problems) or HPLC. Our laboratory has developed a method, based on one described by Tabasco et al [1], to analyze all cited compounds by gas chromatography coupled to tandem mass spectrometry, including also formic and succinic acid. We have evaluated different steps of sample extraction and derivatization (solvent, time) and chromatographic and detection parameters (temperature program, collision energies).
Our aim was to evaluate volatile fatty acids and lactic acid production derived from faecal samples from 12 kids (6-30 months old), incubated in anaerobiosis 12 hours at 37°C and inoculated with different oligosaccharides, including control samples (absence of prebiotics). Aliquots were taken at 0, 6 and 24 hours to measure pH decrease and acid production.
The conditions selected for analysis were as follow: extraction was made with diethyl ether (better than hexane or pyiridine) in acidified matrix, adding the internal standard (ethyl butyric acid) before vortex and centrifugation (3000 g, 10 min, 4ºC). 50 µL of the ether layer were derivatizated with 10 µL of MTBSTFA (N-terbutil-dimetil-silil-N-metil-trifluoroacetamida), at 80°C 20 minutes, followed by 48 hours at room temperature to ensure total derivatization of lactic acid . The reaction was finished freezing the derivatizated extracts. Low volume GC inserts were chosen for the final step in order to minimize ether evaporation.
For quantification, a solution of pure standards was made and several amounts of this solution (mixed with water) were extracted and derivatizated as described above, working in different concentration ranges for each compound. One calibration was made for each sample batch, usually freezing all ether extracts before derivatization. For identification, a tandem mass spectra library was made with our fragmentation patterns of standards, after selecting the best collision energies for the chosen mass of each compound.

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## P-92
## FORENSIC ANALYSIS OF BRAND AND IMITATION PERFUME SAMPLES WITH GC, GC×GC, AND HR-TOFMS

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Characterizing perfumes by determining individual components provides important information to differentiate samples. This can maintain quality control, aid process optimization, drive product development through competitive analysis and brand awareness, and screen for fraud. Non-targeted analytical methods, such as gas chromatography with mass spectrometry (GC-MS), are essential as targeted approaches likely do not provide enough analyte coverage. An even greater amount of information about a perfume sample can be gained with two-dimensional gas chromatography (GC×GC) by pairing an additional complementary separation to improve the ability to chromatographically separate coelutions. The addition of a high resolution mass spectrometer allows for confident formula determinations, combining for a powerful analytical method to better understand a sample and confidently discover and identify more analytes.

A brand and two imitation perfume samples were analyzed by GC and GC×GC coupled to TOFMS, and also with GC×GC coupled to high resolution TOFMS (GC×GC-HR-TOFMS). These analytical tools were used to characterize and compare the brand and imitation perfume samples. The samples were diluted in ethanol prior to injection. The GC×GC separations paired a long non-polar column (Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)) with a shorter mid-polarity column (Rxi-17Sil MS, 1.20 m x 0.25 mm x 0.25 µm coating (Restek)). HR-TOFMS detection provided mass spectral information for library searching and formula determinations of molecular ions and fragments for definitive identification via accurate mass measurements.

The brand and imitation perfumes were analyzed with GC-TOFMS, GC×GC-TOFMS, and GC×GC-HR-TOFMS. All platforms provided the ability to characterize the samples and detect specific analyte differences. The brand sample had 16 fragrance ingredients listed on the packaging information that could be considered target analytes for comparison between brands. However, more than 10 times as many peaks were detected in the GC-TOFMS data with this non-targeted approach. Many of these peaks were identified by library matching to the NIST databases and were found to have important odor properties, highlighting the importance of not only focusing on the target analytes. Specific analyte similarities and differences were determined providing greater insight to the samples. For example, numerous esters, aromatic species, terpenes, oxygenated terpenes, and phthalates were measured in these samples and expressed differently. The addition of a second dimension column with GC×GC provided the opportunity to chromatographically separate analytes that were coeluting in the first dimension separation leading to a greater number of detected analytes, some with important odor characteristics. With GC-TOFMS, some coelutions were mathematically deconvoluted, but other cases of near complete coelution exceeded deconvolution's capabilities. For these cases, the second dimension separation provided new information that was not determined through the GC separation and examples are highlighted. The structured nature of the GC×GC separation space that facilitates rapid visual sample comparisons is also demonstrated. The further addition of high resolution TOFMS added another layer of information to the analysis. High resolution MS provided accurate mass information that were used for definitive formula determinations. These analytical technologies together provided a comprehensive picture of these samples and the ability to distinguish differentially expressed analytes and confidently identify them, including some that were challenging to separate with a one dimensional separation.

## P-93
## METABOLITE IDENTIFICATION IN BLOOD PLASMA USING UHPLC-QqTOF-MS/MS

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Ultra-high performance liquid chromatography (UHPLC-QqTOF-MS/MS) offers high separation power and high sensitivity for metabolomic research. The utility of metabolomic screens largely depends on the number of metabolites identified and links to their biological interpretation. Often, the challenging step is in the identification of these metabolites. A homemade XIC manager and a MS/MS Library have been specifically developed to improve the identification of metabolites.

The number of metabolites identified depends on the extraction procedure used. To this end, several methods based on acetonitrile protein precipitation, were compared to establish which of them were able to extract the higher number of metabolites. Only subtle metabolite differences between the different plasma preparations were noticed, which were primarily related to ion suppression or enhancement.

Human blood plasma was used to demonstrate metabolite identification in a complex biological matrix. The identification routines were complemented by mass spectral deconvolution and matching of sample peak spectra to the library spectra via fast, flexible, and high throughput searching.

By implementing identification procedures such as our procedure, will become increasingly important in standardizing the reporting of the metabolomics results.

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