

1st Iberian Meeting on Separation Sciences and Mass Spectrometry

XIX Conference of the Spanish Society of Chromatography and Related Techniques
IX Conference of the Spanish Society of Mass Spectrometry
VI Conference of the Mass Spectrometry Group of the Portuguese Society of
Chemistry

Santiago de Compostela, October 8th-11th 2019

BOOK OF ABSTRACTS 1ST IBERIAN MEETING IN SEPARATION SCIENCES & MASS SPECTROMETRY

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Welcome

Dear Colleagues:

On behalf of the organizing committee, it is our pleasure to welcome you to the "1st Iberian Meeting in Separation Sciences and Mass Spectrometry" and to the city of Santiago de Compostela, a city declared World Heritage by UNESCO thanks to its monumental beauty, and for being the goal of a millenary pilgrimage route: the Camino de Santiago, which since the ninth century transformed this place of the *finis terrae* into a meeting point of the western world.

It is indeed a great place for us to meet for this international conference bringing together in the same event the "IX conference of the Spanish Society of Mass Spectrometry", the "VI conference of the Mass Spectrometry Group of the Portuguese Society of Chemistry", and the "XIX Conference of the Spanish Society of Chromatography and Related Techniques".

Last several decades have witnessed the continued evolution and scientific advances in development in the fields of separation sciences and mass spectrometry. The scientific program of the "1st Iberian Meeting in Separation Sciences and Mass Spectrometry" presents an outstanding spectrum of research papers from multidisciplinary areas to focus on the challenges of developing technologies and methodologies for biomedical, environmental, food control, and so many other applications.

We are proud to bring you a great synergy of expertise from internationally renowned scientists in separation sciences and mass spectrometry through seven plenary lectures. Sessions throughout the meeting are focused on addressing the current and future trends and challenges in scientific research, technology development, and practice applications. The scientific program is targeted to provide tangible benefits and networking opportunities to current researchers, and the most important, it provides a tremendous environment for younger scientists to learn about future prospects and professional development activities.

We would like to acknowledge the companies that have given a so important support to this meeting, presenting their latest advances and products.

In addition to the outstanding scientific program, we hope that you will enjoy the social program that we have prepared so that, practically from day one, we will have the opportunity not only to strengthen scientific ties but also friendship in the best monumental and gastronomical environments. Find also time to explore Santiago de Compostela, which is a great place to explore on your own.

With our best wishes for your participation in the "1st Iberian Meeting in Separation Sciences and Mass Spectrometry", welcome to Santiago de Compostela!

Marta Lores Aguín & Carmen García Jares

Chairwomen



Committees

Conference Chairwomen

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Óscar Yanes (Universitat Rovira i Virgili)



General information

Venue

Facultad de Medicina
Rúa de San Francisco, s/n
15782 Santiago de Compostela

Conference language

*The official language of the Meeting is **English**.*

Oral Presentations

Plenary lectures (PL): 45 min.

Oral communications (O): 20 min.

Young scientists oral communications (OY): 10 min.

Posters flash presentations: 5 min.

Poster presentations

Posters are displayed from Tuesday 8 to Thursday 10.

WIFI

<p>SSID: TRIPLE2019 Login: TRIPLE2019 Password: jZ5HUsuhCLX+</p>

Social program

Tuesday 8

20:30-1:30h. Welcome cocktail and dancing party. San Francisco Hotel Monumento (50 m from the Meeting Venue)

Wednesday 9

19:30-21:30h. Compostela City Walk. Departure from the Facultad de Medicina (Meeting venue)

Thursday 10

21:30-12:30h. Gala dinner. Hostal de los Reyes Católicos. Plaza do Obradoiro (50 m from the Meeting Venue)

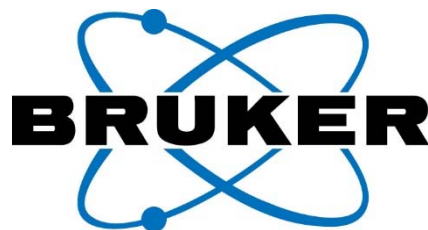
Friday 11

14:00h. Farewell cocktail. Facultad de Medicina (Meeting Venue)



Sponsors

Collaborating companies





Participant companies





Full paper publication

Participants are invited to submit manuscripts based on presentations at the 1st Iberian Meeting in Separation Sciences & Mass Spectrometry (IMSS&MS) to be held in Santiago de Compostela, Spain, 8-11 October 2019 for possible publication in [Journal of Chromatography A](#), with the intention of publishing in an online Special Issue (VSI) that is dedicated to this meeting.

The Special Issue rules out possible delays in publication for contributors to the special issue and will make the conference special issue more complete and accessible than it has ever been. Please see below its advantageous characteristics:

- All submissions will go through normal peer review process per journal standard;
- Accepted articles will be published individually in regular journal volumes at [Science Direct](#) as soon as they are accepted;
- They will also be simultaneously added to the online Special Issue hosted at Science Direct, which is gradually built up as individual articles are published;
- Articles grouped together in an online Special Issue will retain their original citation details.

Submission instructions:

- Submission links: <http://ees.elsevier.com/chroma/>
- Click on the “Submit Paper” option from the top menu;
- Enter your user name and password (first-time users will need to register);
- Please select article type name “**VSI: IMSS&MS 2019**” during submission process;
- Follow the remaining step-by-step instructions to submit your paper;
- Submission deadline: **31st January 2020**

When preparing your manuscript(s), please carefully follow the [Guide for Authors](#) of the journal. In the cover letter please mention that your manuscript is intended for the IMSS&MS 2019 Special Issue.

All manuscripts will be subjected to the regular selection process for the journal, including the strict peer review procedure; therefore acceptance for presentation at the meetings is not a guarantee for publication in the journal.

Thanks in advance for your contribution!

Elsevier Team



Invited Speakers



Prof. Dr. Adrian Covaci

University of Antwerp, Belgium

adrian.covaci@uantwerpen.be

PL1 *Trends in mass spectrometry for human biomonitoring and exposomics.*



Prof. Dr. Rosario Pereiro

University of Oviedo, Spain

mrpereiro@uniovi.es

PL2 *Laser ablation ICP-MS for simultaneous quantitative imaging of elements and biomolecules in biological studies: achievements and trends.*



Prof. Dr. Valerie Gabelica

National Institute of Health and Medical Research (Inserm), France

valerie.gabelica@inserm.fr

PL3 *Ion mobility mass spectrometry: principles of separation and applications in the DNA structural characterization.*



Prof. Dr. Jane Thomas-Oates

University of York, United Kingdom

jane.thomas-oates@york.ac.uk

PL4 *Alternative chromatographic and mass spectrometric approaches to metabolomic challenges.*



Prof. Dr. Frantisek Svec

University Hradec Kralove, Czech Republic

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PL5 *Porous polymer monoliths: Versatile materials for numerous applications.*



Prof. Dr. Miguel Herrero

Institute of Food Science Research (CIAL-CSIC), Spain

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PL6 *Application of comprehensive two-dimensional liquid chromatography to characterize very complex food-related samples.*



Prof. Dr. Cristina Barrocas Dias

University of Évora, Portugal

cmbd@uevora.pt

PL7 *Unveiling the secrets of the past using hyphenated chromatographic techniques.*

Program summary

<i>Tuesday 8</i>	<i>Wednesday 9</i>	<i>Thursday 10</i>	<i>Friday 11</i>
8:15 Registration			
8:40 Opening Session			
9:00-9:45 PL1	9:30-10:15 PL3	9:00-9:45 PL5	9:30-10:15 PL7
9:45-10:45 Oral Session 1	10:15-10:55 Oral Session 3	9:45 -10:45 Oral Session 6	10:15-10:55 Young Scientists Oral Session 8
10:45-11:30 Coffee Break & Poster Exhibition	10:55-11:40 Coffee Break & Poster Exhibition	10:45-11:30 Coffee Break & Poster Exhibition	10:55-11:25 Coffee Break
11:30-12:15 PL2	11:40-12:25 PL4	11:30-12:15 PL6	11:25-12:05 Oral Session 9
12:15-13:15 Oral Session 2	12:25-13:25 Oral Session 4	12:15-13:15 Oral Session 7	12:05-12:25 Flash Poster Session 4
13:15-13:25 Flash Poster Session 1	13:25-13:40 Flash Poster Session 2	13:15-13:35 Flash Poster Session 3	12:25-13:05 Oral Session 10
13:25-13:55 Young Scientists Oral Session 1	13:40-14:00 Young Scientists Oral Session 4	13:35-13:55 Young Scientists Oral Session 6	13:05-13:30 Closing Lecture
14:00-15:00 Lunch	14:00-15:00 Lunch	14:00-15:00 Lunch	13:30-14:00 Closing Session & Awards
15:00-15:30 Technical Seminar 1 & Poster Exhibition	15:00-15:30 Technical Seminar 3 & Poster Exhibition	15:00-15:30 Technical Seminar 4 & Poster Exhibition	14:00 Farewell cocktail
15:30-16:00 Young Scientists Oral Session 2	15:30-16:30 Oral Session 5	15:30-16:30 Oral Session 8	
16:00-16:30 Coffee Break	16:30-17:00 Coffee Break & Poster Exhibition	16:30-17:00 Coffee Break & Poster Exhibition	
16:30--17:00 Young Scientists Oral Session 3	17:00-17:30 Young Scientists Oral Session 5	17:00-17:20 Young Scientists Oral Session 7	
17:00-17:30 Technical Seminar 2 & Poster Exhibition		17:20-17:45 EU-FT-ICR Project	
17:30-19:30 SEEM Meeting & Poster Exhibition	17:30-19:30 SECyTA Meeting & Poster Exhibition		
20:30-1:30 Welcome cocktail & Dancing party	19:30-21:30 Compostela City Walk	21:30-00:30 Gala Dinner	



Scientific and Social Program

Tuesday 8	
8:15	REGISTRATION
8:40	OPENING SESSION: Authorities, Chairs of Scientific Societies, Chairwomen
9:00 Plenary Lecture PL1	<p style="text-align: center;"><i>Trends in mass spectrometry for human biomonitoring and exposomics.</i> Adrian Covaci, University of Antwerp, Belgium</p> <p><u>Session chairs:</u> <i>Francisco Javier Santos, Universidad de Barcelona</i> <i>Marta Lores, Universidade de Santiago de Compostela</i></p>
9:45-10:45 Oral Session 1	<p><i>Omics & Other applications</i></p> <p><u>Session chairs:</u> <i>Francisco Javier Santos, Universidad de Barcelona</i> <i>Montserrat Carrascal, IIBB-CSIC, Barcelona</i></p>
9:45	<p>O1 <i>Evaluation of the anticancer potential golden berry calyx under a foodomics perspective.</i> Gerardo Álvarez-Rivera. CIAL-CSIC, Madrid.</p>
10:05	<p>O2 <i>Glycoproteomic profile of extracellular vesicles in cancer.</i> Hugo Osório. University of Porto.</p>
10:25	<p>O3 <i>Extreme make-up and cosmetic applicators: are they safe?</i> María Celeiro. University of Santiago de Compostela.</p>
10:45-11:30	<div style="display: flex; justify-content: space-between;"> <div style="background-color: #FFD700; padding: 5px;">Coffee Break</div> <div style="background-color: #90EE90; padding: 5px;">Poster Exhibition</div> </div>
11:30 Plenary Lecture PL2	<p style="text-align: center;"><i>Laser ablation ICP-MS for simultaneous quantitative imaging of elements and biomolecules in biological studies: achievements and trends.</i> Rosario Pereiro. University of Oviedo, Spain</p> <p><u>Session chairs:</u> <i>Pablo Rodríguez, Universidad de Oviedo</i> <i>Carmen García Jares, Universidade de Santiago de Compostela</i></p>
12:15-13:15 Oral Session 2	<p><i>Omics</i></p> <p><u>Session chairs:</u> <i>Pablo Rodríguez, Universidad de Oviedo</i> <i>Hugo Osório, University of Porto</i></p>
12:15	<p>O4 <i>Untargeted metabolomics of full scan MS hyphenated data – mining for minor peaks with finnee, risks and benefits.</i> Guillaume L. Erny. University of Porto.</p>



12:35	O5 Profiling the skin volatilome as powerful tool to the neurodegenerative diseases diagnosis. Jorge Pereira. University of Madeira.	
12: 55	O6 A metabolomics-based workflow for adductomics studies. Alexandra M.M. Antunes. IST Lisbon.	
13:15-13:25 Flash Poster Session 1	Flash Poster Session 1	
	<u>Session chairs:</u> <i>Belén Gómara Moreno, IQOG–CSIC, Madrid</i> <i>Juan Francisco García Reyes, Universidad de Jaén</i>	
13:15	P1 Advanced membrane assisted solvent extraction (MASE) for isolating new psychoactive substances from urine before HPLC-MS/MS determination. Cristian Suárez-Oubiña. University of Santiago de Compostela.	
13:20	P2 Analytical challenges associated to essential oils and their individual components in cosmetics. Laura Rubio. University of Santiago de Compostela.	
13:25-13:55 Young Scientists Oral Session 1	Clinical & Toxicological applications	
	<u>Session chairs:</u> <i>Belén Gómara Moreno, IQOG–CSIC, Madrid</i> <i>Juan Francisco García Reyes, Universidad de Jaén</i>	
13:25	OY1 Synthesis and characterization of isotopically labelled ¹⁵N-3-monoiodotyrosine and ¹³C-diiodotyrosine for their use as internal standards in urine analysis by LC-ESI-MS/MS. Jesús Nicolás Carcelén. University of Oviedo.	
13:35	OY2 Sustainable extraction and characterization of bioactive peptides and polyphenols from brewer's spent grain: evaluation of synergic effects. Estefanía González García. University of Alcalá.	
13:45	OY3 Volatolomics approach for the identification of pup pheromones promoting maternal care in mice. Leticia Lacalle-Bergeron. Universtiy Jaume I.	
14:00-15:00	Lunch	
15:00-15:30	Technical Seminar 1 LECO	Poster Exhibition
15:30-16:00 Young Scientists Oral Session 2	Clinical & Toxicological applications	
	<u>Session chairs:</u> <i>Núria Fontanals Torroja, University of Rovira i Virgili</i> <i>José A. González Pérez, IRNAS-CSIC, Sevilla</i>	
15:30	OY4 Determination of syntethic cathinones in meconium by LC-MS/MS. Ángela López-Rabuñal. University of Santiago de Compostela.	



15:40	OY5 State-of-the-art of analytical methods for aflatoxin human biomonitoring. Beatriz Arce-López. University of Navarra.	
15:50	OY6 Analytical considerations for the LC-MS/MS determination of endogenous steroids. Alex Gomez-Gomez. Fundación IMIM.	
16:00-16:30	Coffee Break	
16:30-17:00 Young Scientists Oral Session 3	<p style="text-align: center;">Clinical & Toxicological applications</p> <p>Session chairs: <i>Núria Fontanals Torroja, University of Rovira i Virgili</i> <i>José A. González Pérez, IRNAS-CSIC, Sevilla</i></p>	
16:30	OY7 A LC-MS/MS method for determination of antipsychotic drugs in nails. María Cobo Golpe. University of Santiago de Compostela.	
16:40	OY8 Multiclass method for the determination of endocrine disrupting chemicals in human nails using alkaline digestion prior to UPLC-MS. Laura Martín Pozo. University of Granada.	
16:50	OY9 Determination of synthetic cathinones in oral fluid by LC-MS-MS. Sergi Pascual. University Rovira i Virgili.	
17:00-17:30	Technical Seminar 2 IZASA-SHIMADZU	Poster Exhibition
17:00-19:30	SEEM Meeting	
20:30-1:30	Welcome cocktail & Dancing party	



Wednesday 9

9:30 Plenary Lecture PL3	<p><i>Ion mobility mass spectrometry: principles of separation and applications in the DNA structural characterization.</i> Valérie Gabelica, Inserm, France</p> <p><u>Session chairs:</u> <i>Encarnación Moyano, Universidad de Barcelona</i> <i>Juan Vicente Sancho Llopis, Universitat Jaume I</i></p>	
10:15-10:55 Oral Session 3	<p><i>Clinical & Toxicological applications</i></p> <p><u>Session chairs:</u> <i>Encarnación Moyano, Universidad de Barcelona</i> <i>Juan Vicente Sancho Llopis, Universitat Jaume I</i></p>	
10:15	<p>O7 <i>Mass spectrometry contribution to metabolic studies: new insights into montelukast metabolism.</i> Gonçalo C. Justino. IST, Lisbon.</p>	
10:35	<p>O8 <i>Combination of multiple heart-cutting two dimensional LC and isotope dilution ESI-MS/MS for the accurate quantification of clinical biomarkers.</i> Pablo Rodríguez-González. University of Oviedo.</p>	
10:55-11:40	Coffee Break	Poster Exhibition
11:40 Plenary Lecture PL4	<p><i>Alternative chromatographic and mass spectrometric approaches to metabolomic challenges.</i> Jane Thomas-Oates, University of York, United Kingdom</p> <p><u>Session chairs:</u> <i>Maria Helena Florêncio, Universidade de Lisboa</i> <i>Marta Lores, Universidade de Santiago de Compostela</i></p>	
12:25-13:25 Oral Session 4	<p><i>Clinical & Toxicological applications</i></p> <p><u>Session chairs:</u> <i>Juan Vicente Sancho Llopis, Universitat Jaume I</i> <i>Sandra Pérez, IDAEA-CSIC, Barcelona</i></p>	
12:25	<p>O9 <i>Quantification of related synthetic cathinones in rat brain by UHPLC-MS/MS. Relationship between structure and blood-brain barrier permeability.</i> David Fabregat-Safont. University Jaume I.</p>	
12:45	<p>O10 <i>Identification of growth hormone-releasing hormones in urine doping controls by immunoaffinity purification and LC-MS.</i> Érida Alechaga. Catalanian Anti-doping Laboratory.</p>	
13:05	<p>O11 <i>HPLC-ESI-MS/MS as a valuable tool to unravel the metabolism of new psychoactive substances.</i> Noélia Duarte. University of Lisbon.</p>	



<p>13:25-13:40 Flash Poster Session 2</p>	<p>Flash Poster Session 2</p> <p><u>Session chairs:</u> <i>Juan Vicente Sancho Llopis, Universitat Jaume I</i> <i>Sandra Pérez, IDAEA-CSIC, Barcelona</i></p>	
<p>13:25</p>	<p>P3 Comparison of two chemometric approaches for the identification by LC-QTOF based untargeted metabolomics of potential plasma biomarkers in pediatric chronic kidney disease. Sandra Benito. University of the Basque Country.</p>	
<p>13:30</p>	<p>P4 Time-series proteomic study of diabetic nephropathy in human proximal tubular HK-2 cells by UHPLC-ORBITRAP MS/MS. María Castro-Puyana. University of Alcalá.</p>	
<p>13:35</p>	<p>P5 Annotation of oxidized phospholipids in metabolomics guided cancer study Ángeles López-López University CEU San Pablo.</p>	
<p>13:40-14:00 Young Scientists Oral Session 4</p>	<p>Food analysis</p> <p><u>Session chairs:</u> <i>Begoña Jiménez Luque, IQOG-CSIC, Madrid</i> <i>Jordi Díaz Ferrero, IQS-Universidad Ramón Llull</i></p>	
<p>13:40</p>	<p>OY10 Simple and effective method for the determination of neonicotinoid residues in honeys and cereals by capillary LC. Laura Carbonell Rozas. University of Granada.</p>	
<p>13:50</p>	<p>OY11 GC-MS characterisation of novel pectic-oligosaccharides derived from artichoke pectin using machine learning and competitive fragmentation modelling (CFM-ID). Carlos Sabater. CIAL-CSIC-UAM.</p>	
<p>14:00-15:00</p>	<p>Lunch</p>	
<p>15:00-15:30</p>	<p>Technical Seminar 3 AGILENT</p>	<p>Poster Exhibition</p>
<p>15:30-16:30 Oral Session 5</p>	<p>Food analysis</p> <p><u>Session chairs:</u> <i>Antonia Carro, University of Santiago de Compostela</i> <i>Maria da Conceição Oliveira, IST, Lisboa</i></p>	
<p>15:30</p>	<p>O12 Preliminary identification of specific markers to guarantee the authenticity of Galicia's honey by a non-target HRMS approach. Thierry Dagnac. AGACAL.</p>	
<p>15:50</p>	<p>O13 Chemical profile of coffee varieties by FT-ICR-MS. Ana Marques. University of Lisbon.</p>	
<p>16:10</p>	<p>O14 LC-MS/MS confirmation of pinnatoxins and high levels of esterified OA group toxins in commercial mollusks from the Atlantic coast of Spain. Paz Otero. University of Santiago de Compostela.</p>	



16:30-17:00	Coffee Break	Poster Exhibition
17:00-17:30 Young Scientists Oral Session 5	Food analysis	
	<p><u>Session chairs:</u> <i>Begoña Jiménez Luque, IQOG-CSIC, Madrid</i> <i>Jordi Díaz Ferrero, IQS-Universidad Ramón Llull</i></p>	
17:00	<p>OY12 <i>New procedure for the selective isolation of mercaptans using copper extraction. Application to the determination of three ultratrace odorants in wine by dual GC-MS/MS.</i> Elena Bueno Aventín. University of Zaragoza.</p>	
17:10	<p>OY13 <i>Detection and quantitation of almond adulterations by UHPLC-HRMS polyphenolic profiles.</i> Guillem Campmajó. University of Barcelona</p>	
17:20	<p>OY14 <i>Determination of the food exposure to bisphenol A and its analogs with obesogenic activity in the school population.</i> Yolanda Gálvez Ontiveros. University of Granada.</p>	
17:30-19:30	SECyTA Meeting	Poster Exhibition
19:30-21:30	Compostela City Walk	



Thursday 10

9:00 Plenary Lecture PL5	<p><i>Porous polymer monoliths: Versatile materials for numerous applications.</i> Frantisek Svec, University Hradec Kralove, Czech Republic</p> <p><u>Session chairs:</u> <i>Ana M^a García Campaña, University of Granada</i> <i>Carmen García Jares, Universidade de Santiago de Compostela</i></p>	
9:45-10:45 Oral Session 6	<p><i>Environmental analysis</i></p> <p><u>Session chairs:</u> <i>Joan Grimalt, IDAEA-CSIC, Barcelona</i> <i>Cristina Barrocas Dias, University of Évora, Portugal</i></p>	
9:45	<p>O15 <i>Evaluating plant uptake of pharmaceuticals and their metabolites from water reuse with UPLC-QTOF-MS.</i> Nicola Montemurro. IDAEA-CSIC.</p>	
10:05	<p>O16 <i>Passive sampling: calibration, detection and quantification of POPs in high-mountain lakes from the Aigüestortes National Park.</i> Raimon M. Prats. IDAEA-CSIC.</p>	
10:25	<p>O17 <i>Fate and occurrence of micro and nanoplastics in the Ebro Delta.</i> Marta Llorca. CSIC.</p>	
10:45-11:30	Coffee Break	Poster Exhibition
11:30 Plenary Lecture PL6	<p><i>Application of comprehensive two-dimensional liquid chromatography to characterize very complex food-related samples.</i> Miguel Herrero, CIAL-CSIC, Spain</p> <p><u>Session chairs:</u> <i>Francisco Javier Santos, Universidad de Barcelona</i> <i>Marta Lores, Universidade de Santiago de Compostela</i></p>	
12:15-13:15 Oral Session 7	<p><i>Environmental analysis</i></p> <p><u>Session chairs:</u> <i>Esteban Abad, IDAEA-CSIC, Barcelona</i> <i>Rosa Antonia Lorenzo, Universidade de Santiago de Compostela</i></p>	
12:15	<p>O18 <i>SPE-UPLC-HRMS suspect screening method for the identification of pharmaceuticals and their transformation products in surface water after photolysis.</i> Sandra Pérez. IDAEA-CSIC.</p>	
12:35	<p>O19 <i>Direct compound specific isotope analysis ($\delta^2\text{H}$, $\delta^{13}\text{C}$) of biomass components using analytical pyrolysis (Py-CSIA).</i> Layla M. San Emeterio. IRNAS-CSIC.</p>	



12:55	O20 Occurrence of pharmaceuticals and toxic elements in well water samples collected in Galicia. Carolina Nebot. University of Santiago de Compostela.	
13:15-13:35 Flash Poster Session 3	Flash Poster Session 3	
	<u>Session chairs:</u> <i>Esteban Abad, IDAEA-CSIC, Barcelona</i> <i>Rosa Antonia Lorenzo, Universidade de Santiago de Compostela</i>	
13:15	P6 Visible light photocatalytic degradation of trazodone using different modified titanate nanowires. Rodrigo Osawa. University of Lisbon.	
13:20	P7 Analysis of palytoxin and its analogues (ovatoxins) produced by <i>Ostreopsis cf. ovata</i> microalgae. Noemí-Inmaculada Medina Pérez. University of Barcelona.	
13:25	P8 Microplastics adsorption capacity and transport of cocontaminants in seawater. Albert Vega. IDAEA-CSIC.	
13:30	P9 Chiral analysis of amphetamine-related drugs in wastewater. Andrea Estévez Danta. University of Santiago de Compostela.	
13:35-13:55 Young Scientists Oral Session 6	Environmental analysis	
	<u>Session chairs:</u> <i>Belén Gómara Moreno, IQOG-CSIC, Madrid</i> <i>Sandra Pérez, IDAEA-CSIC, Barcelona</i>	
13:35	OY15 Fabric phase sorptive extraction for the determination of fungicides in environmental waters by GC-MS/MS. Lúa Vázquez. University of Santiago de Compostela.	
13:45	OY16 A high-sensitivity method for analysis of a mixture of relevant anthropogenic emerging organic contaminants in waters from remote areas. Ester López-García. IDAEA-CSIC.	
14:00-15:00	Lunch	
15:00-15:30	Technical Seminar 4 BRUKER	Poster Exhibition
15:30-16:30 Oral Session 8	Instrumental I	
	<u>Session chairs:</u> <i>Sandra Pérez, IDAEA-CSIC, Barcelona</i> <i>Antonio Moreda, Universidade de Santiago de Compostela</i>	
15:30	O21 High sensitivity applications in High Resolution MS QTOF. Javier López. Bruker	



15:50	O22 Determination of pyrrolizidine alkaloids in plant material using SFC-MS/MS. Diego Esteban-Fernández. Izasa Scientific.	
16:10	O23 Quantitation and Non-Target Detection of Pesticides in Spinach Extract with Pegasus BT 4D. Improvement to Targeted & Untargeted Pesticide Residue Analysis: Fast and Flexible Analyte Finding For GC-MS and GCxGC-MS Julio Lluch. LECO Sep Science.	
16:30-17:00	Coffee Break	Poster Exhibition
17:00-17:20 Young Scientists Oral Session 7	Food analysis	
	Session chairs: <i>Begoña Jiménez Luque, IQOG-CSIC, Madrid</i> <i>Jordi Díaz Ferrero, IQS-Universidad Ramón Llull</i>	
17:00	OY17 UHPLC-API-MS/MS for the determination of azo-dyes in red spices. Ane Arrizabalaga Larrañaga. University of Barcelona.	
17:10	OY18 GC-MS analysis of six lipid peroxidation products with DNPH derivatization using a new GDME-DLLME procedure. Jorge Antonio Custodio-Mendoza. University of Santiago de Compostela.	
17:20-17:45	EU-FT-ICR project C. Cordeiro. University of Lisbon.	
21:30-00:30	Gala Dinner	



Friday 11	
9:30 Plenary Lecture PL7	<i>Unveiling the secrets of the past using hyphenated chromatographic techniques.</i> Cristina Barrocas Dias, University of Évora, Portugal Session chairs: <i>María Helena Florêncio, Universidade de Lisboa</i> <i>Encarnación Moyano, Universidad de Barcelona</i>
10:15-10:55 Young Scientists Oral Session 8	<i>Environmental analysis</i> Session chairs: <i>Francisco Javier Santos, Universidad de Barcelona</i> <i>Juan Vicente Sancho Llopis, Universitat Jaume I</i>
10:15	OY19 <i>Atmospheric pressure photoionization for GC-HRMS analysis of PCDD/Fs and dioxin-like PCBs in food and environmental samples.</i> Juan Francisco Ayala Cabrera. University of Barcelona.
10:25	OY20 <i>Potential of micro-liquid chromatography for the determination of psychoactive substances in wastewater.</i> Alberto Celma. Research Institute for Pesticides and Water, University Jaume I.
10:35	OY21 <i>High throughput analysis of medium to highly polar pesticides in surface and ground water from agriculture-impacted areas of Catalonia using on-line SPE-LC-MS/MS.</i> Maria Vittoria Barbieri. IDAEA-CSIC.
10:45	OY22 <i>Enantiomeric determination of cathinones in environmental water sample.</i> Yandi Fu. University Rovira i Virgili.
10:55-11:25	Coffee Break
11:25-12:05 Oral Session 9	<i>Other applications</i> Session chairs: <i>María Llompart, Universidade de Santiago de Compostela</i> <i>Rosario Pereiro, Universidad de Oviedo, Spain</i>
11:25	O24 <i>Miniaturized SPE follow by GC-MS for the determination of UV filters in water.</i> Piyaluk Nurerk. Prince of Songkla University.
11:45	O25 <i>Evolution and developments of proton transfer reaction-mass spectrometry for security applications.</i> Ramón González Méndez. Coventry University / University of Birmingham.



12:05-12:25 Flash Poster Session 4	Flash poster session 4
	<p><u>Session chairs:</u> <i>María Llopart, Universidade de Santiago de Compostela</i> <i>Rosario Pereiro, Universidad de Oviedo, Spain</i></p>
12:05	<p>P10 Capsule phase microextraction with mixed-mode properties to selectively determine acidic or basic compounds from environmental water. Joan Carles Nadal. University Rovira i Virgili.</p>
12:10	<p>P11 Application of multivariate analysis to identify peptides responsible for the hypocholesterolemic capacity of protein hydrolysates released from olive seeds. Isabel M. Prados. University of Alcalá.</p>
12:15	<p>P12 Evaluation of vermicompost biofertilization on the aroma and polyphenolic composition of Albariño musts and wines. Perla Ferrer. University of Santiago de Compostela.</p>
12:20	<p>P13 Isotopic pattern as filtering rule for the screening of authorized green colorants in foods. María Roca. Instituto de la Grasa (CSIC), Sevilla.</p>
12:25-13:05 Oral Session 10	Instrumental II
	<p><u>Session chairs:</u> <i>Juan Francisco García Reyes, Universidad de Jaén</i> <i>Marta Lores, Universidade de Santiago de Compostela</i></p>
12:25	<p>O26 Development and Application of a Cyclic ion mobility mass spectrometer. Emma Marsden-Edwards. Waters Corporation.</p>
12:45	<p>O27 Does your dog have anxiety after a rough day at the mountain: analysis of CDB extracts for dogs treat. Jose Rivero. Agilent Technologies.</p>
13:05	<p>Closing Lecture “Año Internacional de la Tabla Periódica de los Elementos Químicos”: ¿Cómo se mide el tiempo con la tabla periódica? Joan Grimalt, IDAEA–CSIC, Barcelona</p>
13:30	Closing session & Awards
14:00	Farewell cocktail



List of contributions

PLENARY LECTURES

PL1. Trends in mass spectrometry for human biomonitoring and exposomics. Adrian Covaci, University of Antwerp, Belgium

PL2. Laser ablation ICP-MS for simultaneous quantitative imaging of elements and biomolecules in biological studies: achievements and trends. Rosario Pereiro. University of Oviedo, Spain

PL3. Ion mobility spectrometry: principles of separation and applications in structural characterization. Valerie Gabelica, Inserm, France

PL4. Alternative chromatographic and mass spectrometric approaches to metabolomic challenges.

Jane Thomas-Oates, University of York, United Kingdom

PL5. Porous polymer monoliths: A versatile tool in chromatography. Frantisek Svec, University Hradec Kralove, Czech Republic

PL6. Application of comprehensive two-dimensional liquid chromatography to characterize very complex food-related samples. Miguel Herrero, CIAL-CSIC, Spain

PL7. Unveiling the secrets of the past using hyphenated chromatographic techniques. Cristina Barrocas Dias, University of Évora, Portugal.

ORAL COMMUNICATIONS

O1. Evaluation of the anticancer potential goldenberry calyx under a foodomics perspective. Gerardo Álvarez-Rivera, Diego Ballesteros-Vivas, Alberto Valdés, Fabián Parada-Alfonso, Elena Ibañez, Alejandro Cifuentes.

O2. Glycoproteomic profile of extracellular vesicles in cancer. Hugo Osório, Daniela Freitas, Ana Magalhaes, Celso Reis.

O3. Extreme make-up and cosmetic applicators: are they safe? María Celeiro, Carmen García-Jares, Marta Loes.

O4. Untargeted metabolomics of full scan MS hyphenated data – mining for minor peaks with finnee, risks and benefits. Guillaume L. Erny, Arminda Alves.

O5. Profiling the skin volatilome as powerful tool to the neurodegenerative diseases diagnosis. Jorge Pereira, Beatriz Andrade, José Câmara.

O6. A Metabolomics-based Workflow for Adductomics Studies. João Nunes, Catarina Charneira, Sofia Gouveia-Fernandes, Jacinta Serpa, Judit Morello, Alexandra M.M. Antunes.

O7. Mass spectrometry contribution to metabolic studies: new insights into montelukast metabolism. Cátia F. Marques, Gonçalo C. Justino, M. Matilde Marques.

O8. Combination of multiple heart-cutting two dimensional liquid chromatography and isotope dilution ESI-MS/MS for the accurate quantification of clinical biomarkers. Pablo Rodríguez-González, Amanda Suárez Fernández, Adriana González Gago, Héctor González Iglesias, Adela Cortés Giménez-Coral, Belén Prieto García, Carlos Manuel López López, Maria Funes Menéndez, J. Ignacio García Alonso.

O9. Quantification of related synthetic cathinones in rat brain by UHPLC-MS/MS. Relationship between structure and blood-brain barrier permeability. David Fabregat-Safont, Manuela Barneo-Muñoz, Ferran Martínez-García, Juan Vicente Sancho, Félix Hernández, María Ibañez.



- O10. Identification of growth hormone-releasing hormones in urine doping controls by immunoaffinity purification and Liquid Chromatography Mass Spectrometry.** Élida Alechaga, Laura Pont, Alejandro Terrero, Núria Monfort, Rosa Ventura.
- O11. HPLC-ESI-MS/MS as a valuable tool to unravel the metabolism of new psychoactive substances.** Joana Carrola, Cristina Sampayo, M. Jesus Perry, Pedro Florindo, Rui Moreira, Álvaro Lopes, M. Rosário Bronze, Noélia Duarte.
- O12. Preliminary identification of specific markers to guarantee the authenticity of Galicia's honey by a non-target HRMS approach.** Thierry Dagnac, Lúa Vázquez, Meruyert Sergazina, María Celeiro, María Llompart.
- O13. Chemical profile of coffee varieties by FT-ICR-MS.** Mariana Pereira, Carlos Cordeiro, Ana Marques.
- O14. LC-MS/MS confirmation of the emerging pinnatoxins and high levels of esterified OA group toxins in commercial mollusks from the Atlantic coast of Spain.** Paz Otero, Luis Botana.
- O15. Evaluating plant uptake of pharmaceuticals and their metabolites from water reuse with UPLC-QTOF-MS.** Nicola Montemurro, Sandra Pérez.
- O16. Passive sampling: calibration, detection and quantification of persistent organic pollutants in high-mountain lakes from the Aigüestortes national park.** Raimon M. Prats, Barend L. van Drooge, Pilar Fernández, Joan O. Grimalt.
- O17. Fate and occurrence of micro and nanoplastics in the Ebro Delta.** Marinella Farré, Marta Llorca, Gabriella Schirinzi, Manuela Ábalos, Esteban Abad.
- O18. SPE-UPLC-HRMS suspect screening method for the identification of pharmaceuticals and their transformation products in surface water after photolysis.** Enelton Fagnani, Nicola Montemurro, Sandra Pérez.
- O19. Direct compound specific isotope analysis ($\delta^2\text{H}$, $\delta^{13}\text{C}$) of biomass components using analytical pyrolysis (Py-CSIA).** Layla M. San Emeterio, Nicasio T. Jiménez-Morillo, José M. de la Rosa, Francisco J. González-Vila, José-A González-Pérez.
- O20. Occurrence of pharmaceuticals and toxic elements in well water samples collected in Galicia.** Carolina Nebot, Elena Falqué, Benita Pérez, María Veiga, Carlos Franco.
- O21. High sensitivity applications in High Resolution MS QTOF.** Javier López.
- O22. Determination of pyrrolizidine alkaloids in plant material using SFC-MS/MS.** Diego Esteban-Fernández, Raymond Wong, Anja Grüning, Gesa J. Schad, Jan Stenzler.
- O23. Quantitation and non-target detection of pesticides in spinach extract with Pegasus BT 4D. Improvement to targeted & untargeted pesticide residue analysis: Fast and flexible analyte finding for GC-MS and GCxGC-MS.** Julio Lluch.
- O24. Miniaturized solid-phase extraction follow by Gas Chromatography-tandem Mass Spectrometry for the determination of UV filters in water.** Piyaluk Nurerk, Pajaree Donkhampa, Opas Bunkoed, Thierry Dagnac, Maria Llompart.
- O25. Evolution and developments of proton transfer reaction-mass spectrometry for security applications.** Ramón González Méndez.
- O26. Development and application of a cyclic ion mobility mass spectrometer.** Dale A. Cooper-Shepherd, Martin Palmer, Jakub Ujma, Kevin Giles, Emma Marsden-Edwards, James I. Langridge.
- O27. Does your dog have anxiety after a rough day at the mountain: analysis of CDB extracts for dogs treat.** Jose Juan Rivero.



YOUNG SCIENTISTS ORAL COMMUNICATIONS

- OY1. *Synthesis and characterization of isotopically labelled ¹⁵N-3-monoiodotyrosine and ¹³C-3,5-diiodotyrosine for their use as internal standards in urine analysis by LC-ESI-MS/MS.*** Jesús Nicolás Carcelén^{1*}, Pablo Rodríguez González¹, Alfredo Ballesteros Gimeno², Juan Manuel Marchante Gayón¹, José Ángel Cocho de Juan³, J. Ignacio García Alonso.¹
- OY2. *Sustainable extraction and characterization of bioactive peptides and polyphenols from brewer's spent grain: evaluation of synergic effects.*** Estefanía González García, María Luisa Marina, María Concepción García.
- OY3. *Volatolomics approach for the identification of pup pheromones promoting maternal care in mice.*** Leticia Lacalle-Bergeron, Carlos Sales-Martínez, Tania Portolés-Nicolau, Juan V Sancho-Llopis, Joaquim Beltrán-Arandes, Rafael Goterris-Cerisuelo, Cinta Navarro-Moreno, Fernando Martínez-García.
- OY4. *Determination of synthetic cathinones in meconium by LC-MS/MS.*** Ángela López-Rabuñal, Elena Lendoiro, Marta Concheiro, Manuel López-Rivadulla, Angelines Cruz, Ana de Castro.
- OY5. *State-of-the-art of analytical methods for aflatoxin human biomonitoring.*** Beatriz Arce-López, Elena González-Peñas, Ariane Vettorazzi, Elena Lizarraga.
- OY6. *Analytical considerations for the LC-MS/MS determination of endogenous steroids.*** Alex Gomez-Gomez, Olha Khymenets, Oscar J Pozo.
- OY7. *A LC-MS/MS method for determination of antipsychotic drugs in nails.*** María Cobo Golpe, Ana de-Castro-Ríos, Angelines Cruz, Manuel López-Rivadulla, Elena Lendoiro.
- OY8. *Multiclass method for the determination of endocrine disrupting chemicals in human nails using alkaline digestion prior to Ultrahigh Performance Liquid Chromatography tandem Mass Spectrometry.*** Laura Martín-Pozo, Samuel Cantarero-Malagón, Alberto Navalón, Alberto Zafra-Gómez.
- OY9. *Determination of synthetic cathinones in oral fluid by Liquid Chromatography-tandem Mass Spectrometry.*** Sergi Pascual, Núria Fontanals, Francesc Borrull, Carme Aguilar, Marta Calull.
- OY10. *Simple and effective method for the determination of neonicotinoid residues in honeys and cereals by capillary liquid chromatography.*** Laura Carbonell Rozas, Alex Rizo-Zapata, Francisco J. Lara, Monsalud del Olmo-Iruela, Ana M. García-Campaña.
- OY11. *GC-MS characterisation of novel pectic-oligosaccharides derived from artichoke pectin using machine learning and competitive fragmentation modelling (CFM-ID).*** Carlos Sabater, Antonia Montilla, Nieves Corzo.
- OY12. *New procedure for the selective isolation of mercaptans using copper extraction. Application to the determination of three ultratrace odorants in wine by dual Gas Chromatography-tandem Mass Spectrometry.*** Elena Bueno Aventín, Ignacio Ontañón, Vicente Ferreira.
- OY13. *Detection and quantitation of almond adulterations by UHPLC-HRMS polyphenolic profiles.*** Guillem Campmajó, Javier Saurina, Oscar Núñez.
- OY14. *Determination of the food exposure to bisphenol A and its analogs with obesogenic activity in the school population.*** Yolanda Gálvez Ontiveros, Laura Martín-Pozo, Victoria Martín-Laguna, Samuel Cantarero-Malagón, Margarita Aguilera, Celia Monteagudo, Lourdes Rodrigo, Ana Rivas, Alberto Zafra-Gómez.
- OY15. *Fabric phase sorptive extraction for the determination of fungicides in environmental waters by GC-MS/MS.*** Lúa Vázquez, María Celeiro, Piyaluk Nurerk, Abuzar Kabir, Thierry Dagnac, Maria Llompert.
- OY16. *A high-sensitivity method for analysis of a mixture of relevant anthropogenic emerging organic contaminants in waters from remote areas.*** Ester López-García, Cristina Postigo, Luis Moreno-Merino, Miren López de Alda.



- OY17. UHPLC-API-MS/MS for the determination of azo-dyes in red spices.** Ane Arrizabalaga-Larrañaga, S. Epigmenio, F.J. Santos, E. Moyano.
- OY18. GC-MS analysis of six lipid peroxidation products with DNPH derivatization using a new GDME-DLLME procedure.** Jorge Antonio Custodio-Mendoza, J. Aja-Macaya, I. Valente, R. Ramos, J. A. Rodriguez, A. M. Carro, R. A. Lorenzo.
- OY19. Atmospheric pressure photoionization for GC-HRMS analysis of PCDD/Fs and dioxin-like PCBs in food and environmental samples.** Juan Francisco Ayala Cabrera, M. Ábalos, E. Abad, E. Moyano, F. J. Santos.
- OY20. Potential of micro-liquid chromatography for the determination of psychoactive substances in wastewater.** Alberto Celma, Juan V. Sancho, Noelia Salgueiro-González, Sara Castiglioni, Ettore Zuccato, Félix Hernández, Lubertus Bijlsma.
- OY21. High throughput analysis of medium to highly polar pesticides in surface and ground water from agriculture-impacted areas of Catalonia using on-line SPE-LC-MS/MS.** Maria Vittoria Barbieri, Cristina Postigo, Vinyet Sola, Miren López de Alda.
- OY22. Enantiomeric determination of cathinones in environmental water samples.** Yandi Fu, Francesc Borrull, Núria Fontanals, Rosa Maria Marcé.

POSTERS

SELECTED FOR FLASH PRESENTATION

- P1. Advanced membrane assisted solvent extraction (MASE) for isolating new psychoactive substances from urine before HPLC-MS/MS determination.** Cristian Suárez-Oubiña, Ana María Bermejo, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro.
- P2. Analytical challenges associated to essential oils and their individual components in cosmetics.** Laura Rubio, Marta Lores, Carmen García-Jares.
- P3. Comparison of two chemometric approaches for the identification by LC-QTOF based untargeted metabolomics of potential plasma biomarkers in pediatric chronic kidney disease.** Sandra Benito, Nora Unceta, Alicia Sánchez, Alberto Gómez, M^a Aránzazu Goicolea, Ramón J. Barrio.
- P4. Time-series proteomic study of diabetic nephropathy in human proximal tubular HK-2 cells by UHPLC-ORBITRAP MS/MS.** Alberto Valdés, Coral García-Pastor, María Castro-Puyana, Francisco J. Lucio-Cazaña, María Luisa Marina.
- P5. Annotation of oxidized phospholipids in metabolomics guided cancer study.** Ángeles López-López, Joanna Godzien, Vanesa Alonso-Herranz, Anna La Salvia, Genoveva Gómez-Esteves, Beatriz Soldevilla, Rocío García-Carbonero, Ángeles López-González, Coral Barbas.
- P6. Visible light photocatalytic degradation of trazodone using different modified titanate nanowires.** Rodrigo Osawa, Beatriz Barrocas, Olinda Monteiro, M. Conceição Oliveira, M. Helena Florêncio.
- P7. Analysis of palytoxin and its analogues (ovatoxins) produced by *Ostreopsis cf. ovata* microalgae.** Noemí-Inmaculada Medina Pérez, E. Berdalet, E. Moyano.
- P8. Microplastics adsorption capacity and transport of cocontaminants in seawater.** Albert Vega, Marta Llorca, Gabriella Schirinzi, Manuela Ábalos, Esteban Abad, Marinella Farré.
- P9. Chiral analysis of amphetamine-related drugs in wastewater.** Andrea Estévez-Danta, Vanessa Guttman, Rosa Montes, Iria González-Mariño, Rosario Rodil, Ailette Prieto, Manuel Miró, Rafael Cela, José Benito Quintana.



P10. **Capsule phase microextraction with mixed-mode properties to selectively determine acidic or basic compounds from environmental water.** Joan Carles Nadal, Francesc Borrull, Kenneth G. Furton, Abuzar Kabir, Rosa Maria Marcé, Núria Fontanals.

P11. **Application of multivariate analysis to identify peptides responsible for the hypocholesterolemic capacity of protein hydrolysates released from olive seeds.** Isabel M. Prados, Merichel Plaza, María Luisa Marina, María Concepción García.

P12. **Evaluation of vermicompost biofertilization on the aroma and polyphenolic composition of Albariño musts and wines.** Perla Ferrer, María Celeiro, Hugo Martínez-Cordeiro, Tamara Vázquez, Carmen García-Jares, Marta Lores, Jorge Domínguez.

P13. **Isotopic pattern as filtering rule for the screening of authorized green colorants in foods.** María Roca, Isabel Viera, Antonio Pérez.

FUNDAMENTALS ON CHROMATOGRAPHY AND ELECTRO-DRIVEN SEPARATION TECHNIQUES

P14. **Dual retention behavior of benzalkonium chloride in core-shell column with biphenyl groups.** María Carmen Prieto-Blanco, Angela Planas-Franco, Soledad Muniategui-Lorenzo, María José González-Castro.

P15. **Statistical comparison of the retention mechanism of polychlorinated biphenyls in different gas chromatography stationary phases.** J. Escobar-Arnanz, J. Sanz, Belén Gómara, L. Ramos

MASS SPECTROMETRY FUNDAMENTALS, STATE-OF-THE ART, APPLICATIONS AND INNOVATIONS

P16. **Applicability of atmospheric pressure photoionization for the determination of dechlorane plus and analogues by GC-HRMS.** Juan Francisco Ayala-Cabrera, E. Ayvazyan-Arutyunyan, E. Moyano, F. J. Santos.

MS HYPHENATED TECHNIQUES, APPLICATIONS AND NEW DEVELOPMENTS

P17. **Combining in vitro bioanalysis and High Resolution Mass Spectrometry for a refined non-target screening workflow using surface waters from mediterranean littoral (Spain) as a case study.** Alberto Celma, Geeta Mandava, Agneta Oskarsson, Frank Menger, Foon Yin Lai, Oksana Golovko, Juan V. Sancho, Félix Hernández, Lutz Ahrens, Johan Lundqvist, Lubertus Bijlsma.

P18. **$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope signature of *Spondyliosoma cantharus otoliths*.** Ana Isabel Janeiro, Ana Neves, Miguel Gaspar, Ana Rita Vieira, Vera Sequeira, Jorge M. R. S. Relvas, Leonel Serrano Gordo.

P19. **Hormones and their metabolites as prehistoric shepherds' activities and milk storage biomarkers.** Alicia Sanchez, Blanca Navarro, Ane Gorostizu, Asier Vallejo, Josep María Vergés, Juan Antonio Quiros, Nora Unceta, Maria Aranzazu Goicolea, Ramón J. Barrio.

P20. **Determination of melatonin and its metabolites by different quantification approaches using liquid chromatography and tandem mass spectrometry (LC-MS/MS).** Adriana González Gago, Amanda Suarez Fernández, Javier García Calleja, Juan Carlos Mayo, Rosa María Sáinz, Pablo Rodríguez González, José Ignacio García Alonso.



P21. **Development of a MS/MS spectral library of phenolic compounds using electrospray ionization and triple-quadrupole mass spectrometer.** Noélia Duarte, Andreia Bento da Silva, Joana Carrola, Beatriz T. Martins, João Mota, M. Rita Ventura, M. Rosário Bronze.

P22. **Building a collision cross section (CCS) database for environmental organic micropollutants screening under travelling wave ion mobility spectrometry coupled to high resolution mass spectrometry.** Alberto Celma, Lubertus Bijlsma, Félix Hernández, Juan V. Sancho.

P23. **GC meets VION IMS-QTOF. Facilitating ion mobility measurements for GC-amenable compounds in food and environmental analysis.** Tania Portolés, David Fabregat, Juan V. Sancho, Félix Hernández.

NEW ADVANCES AND DEVELOPMENTS IN CHROMATOGRAPHY AND RELATED TECHNIQUES

P24. **Optimization and validation of a method based on capillary electrophoresis coupled to laser induced fluorescence detection (CE-LIF) for chiral analysis and absolute quantitation of 3 amino acids: L and D-ASP, L and D-GLU and L and D-SER. Application to osteocyte and osteoblast cell lines.** M^a Paz Lorenzo, Luis Valiente, Antonia García.

P25. **Pyrolysis compound-specific nitrogen isotope analysis ($\delta^{15}\text{N}$ PY-CSIA): novel analytical approach for archaeological studies.** Nicasio T. Jiménez-Morillo, José M. De la Rosa, Ana Z. Miller, Francisco J. González-Vila, Cristina Dias, Anne-France Maurer, José A. González-Pérez.

P26. **Optimization of microwave assisted extraction of bioactive carbohydrates from alfalfa.** Andrea Martín-Ortiz, Alejandra Daniela Solarte-Sarasty, Maite Rada-Mendoza, Ana Isabel Ruiz-Matute, María Luz Sanz.

SAMPLE PREPARATION, HANDLING AND TRACE ANALYSIS

P27. **Effect of the purification steps of glycoproteins from biological fluids on their recovery.** Angel Puerta, Sergio Lopez-Duque, Raquel Saez-Brox, Laura Gomez Ruiz, Jose Carlos Diez-Masa, Mercedes de Frutos.

P28. **Development of a rapid method to confirm contamination by mineral oils through SPE-GC-MS.** Janira Jaén, Celia Domeño, Chiara Gajotti, Cristina Nerín.

P29. **Application of dispersive liquid liquid micro extraction (DLLME) for determination of aflatoxins B1, B2, G1 and G2 in fish.** G.D. Thilini Madurangika Jayasinghe, Raquel Domínguez-González, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro.

P30. **Application of molecularly imprinted polymer micro solid phase extraction (MIMSPE) for determination of aflatoxins B1, B2, G1, G2 and M1 in fish.** G.D. Thilini Madurangika Jayasinghe, Raquel Domínguez-González, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro.

P31. **Identification of peptides in the olive seed responsible for in vitro and in vivo hypolipidemic capacity.** Isabel M. Prados, José María Orellana, María Luisa Marina, María Concepción García.

P32. **Molecularly imprinted polymers for solid-phase extraction of aryloxyphenoxypropionate herbicides from water samples.** Sagrario Torres-Cartas, Susana Meseguer-Lloret, Carmen Gómez-Benito, Mónica Catalá-Icardo, Natalia Prima Margiotta, José Manuel Herrero-Martínez, Ernesto Francisco Simó-Alfonso.



- P33. **Development and application of a molecularly imprinted polymer for the extraction of phenoxy herbicides from water samples.** Susana Meseguer-Lloret, [Sagrario Torres-Cartas](#), Carmen Gómez-Benito, Mónica Catalá-Icardo, Roberto Beltrán Martí, Ernesto Francisco Simó-Alfonso, José Manuel Herrero-Martínez.
- P34. **Development of stir bar sorptive extraction directly on polytetrafluoroethylene magnets modified with monoliths.** [Carmen Gómez-Benito](#), Mónica Catalá-Icardo, Adrián Torres-Prades, Sagrario Torres-Cartas, Susana Meseguer-Lloret, Ernesto Francisco Simó-Alfonso, José Manuel Herrero-Martínez.
- P35. **Potential of cationic carbosilane dendrimers for sustainable protein sample preparation.** Estefanía González-García, Javier Sánchez-Nieves, Francisco Javier de la Mata, M. Luisa Marina, [M. Concepción García](#).
- P36. **Immobilization of proteolytic enzymes on silica supports functionalized with carbosilane and pamam dendrimers and evaluation of their potential in successive hydrolysis.** Ester Hernández-Corroto, María Sánchez-Milla, Javier Sánchez-Nieves, Francisco Javier de la Mata, M. Luisa Marina, [M. Concepción García](#).
- P37. **Miniaturized Solid-Phase Microextraction followed by GC-MS for the identification of compounds with organoleptic characteristics in honey.** Lúa Vázquez, [Meruyert Sergazina](#), María Celeiro, Thierry Dagnac, María Llompart.
- P38. **Ultrasound assisted extraction for the determination of toxic substances in surfaces made of recycled rubber by GC-MS/MS.** [Daniel Armada](#), María Celeiro, Thierry Dagnac, María Llompart.
- P39. **Development and application of polymeric sorbents with Zwitterionic character.** [Joan Carles Nadal](#), Francesc Borrull, Peter A. G. Cormack, Rosa Maria Marcé, Núria Fontanals.

OMICS

- P40. **Mass Spectrometric Immunoassay of proteoforms of Apolipoprotein A and C in individuals with obstructive sleep apnea.** Ana Margarida Pereira, Amelia Feliciano, Olgica Trenchevska, Fatima Vaz, Dobrin Nedelkov, Randal Nelson, Paula Pinto, Cristina Barbara, Marilia Antunes, Deborah Penque, [Vukosava Milic Torres](#).
- P41. **Carbon isotopic fractionation of urinary metabolites in transgenic mice during the development of prostate cancer.** [Laura Covadonga Rodas Sánchez](#), Pablo Rodríguez-Gonzalez, Pedro Gonzalez-Menéndez, Juan Carlos Mayo Barrallo, Rosa M^a Sainz Menéndez, J. Ignacio García Alonso.
- P42. **Biosynthesis and characterization of isotopically labelled DNA and RNA methylated nucleosides for the quantification of global DNA and RNA methylation by isotope dilution LC-ESI-MS/MS.** [Jesús Nicolás Carcelén](#), Juan Manuel Marchante Gayón, Luis Valledor, Pablo Rodríguez González, M^a Jesús Cañal, J. Ignacio García Alonso.
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- P76. **Basin-scale monitoring of emerging contaminants in South American coastal lagoon using Liquid Chromatography High Resolution Mass Spectrometry.** Luciana Griffero, Jaime Alcántara-Durán, Cecilia Alonso, Lorena Rodríguez-Gallego, David Moreno-González, Juan F. García-Reyes, Antonio Molina-Díaz, Andrés Pérez Parada.
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- P81. **A simple methodology for the analysis of lindane in indoor air.** María Celeiro, María Fabelo, M. Carmen Monterroso, Carmen García-Jares.
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- P85. **Propagation of pesticides from regenerated water to the crops by irrigation.** Maria Jesus Martínez Bueno, M. García Valverde, F.J. Díaz-Galiano, M.M. Gómez-Ramos, A. Goday, A.R. Fernández-Alba.
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- P95. **Determination of nine cyanotoxins in saline water samples by using salting-out assisted Liquid-Liquid extraction and UHPLC with UV and MS/MS detection.** Monsalud del Olmo-Iruela, Maryam Hemmati, Carmen Tejada-Casado, Francisco J. Lara, Ana M. García-Campaña, M. Carmen Contreras-Morales.
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- P97. **Determination of organophosphate flame retardants in fish samples using quechers extraction followed by GC-MS/MS.** Óscar Castro, Rosa Maria Marcé, Eva Pocurull, Francesc Borrull.
- P98. **Development of a simple and fast HPLC-MS/MS method for α - and β -ODAP quantification in Lathyrus Sativus (grass pea) samples.** Andreia Bento-Silva, Letice Gonçalves, Filipe Pereira, Elsa Mecha, Noélia Duarte, Maria Carlota Vaz Patto, Maria do Rosário Bronze.
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- P102. **Isolation of proteins from spent coffee ground. Identification of peptides in the protein hydrolysates by RP-HPLC-ESI-Q-TOF.** Alberto Valdés, María Castro-Puyana, María Luisa Marina.
- P103. **Development and optimization of a method for determination of organochlorine pesticides in mussel based on miniaturized Matrix Solid-Phase Dispersion combined with Gas Chromatography-tandem Mass Spectrometry.** Nieves Carro, A. Lopez, J. Cobas, I. García, M. Ignacio, A. Mouteira.
- P104. **A novel method to determine fipronil and fipronil sulfone in eggs by capillary electrophoresis and salting-out assisted liquid-liquid extraction.** M. Mar Aparicio-Muriana, Ivona Lhotská, Francisco J. Lara and Ana M. García-Campaña.



- P105. **Authentication of *Garcinia cambogia* supplements by GC-MS.** Adal Mena-García, Angie J. Belleizac, Diana M. Chito, Maite P. Rada-Mendoza, Ana C. Soria, María L. Sanz, Ana I. Ruiz-Matute.
- P106. **Olive ripeness as a tool to modulate phenolic composition of oils and in vitro inhibition of α -glucosidase.** María Figueiredo-González, Patricia Reboredo-Rodríguez, Laura Castro Sobrino, Lucía Olmo-García, Alegría Carrasco-Pancorbo, Jesús Simal-Gándara, Carmen González-Barreiro, Beatriz Cancho-Grande.
- P107. **Extraction of chlorogenic acid from blueberry pomace using novel extraction technologies.** Ante Lončarić, María Celeiro, Antun Jozinović, Drago Šubarić, Jurislav Babić, Marta Lores.
- P108. **Alterations in Mediterranean mussel (*Mytilus galloprovincialis*) composition exposed to cyanotoxins as revealed by analytical pyrolysis.** Leticia Diez-Quijada, Flavio L. de Oliveira, Ángeles Jos, Ana M. Cameán, Ramón Aparicio-Ruiz, Victor Vasconcelos, Alexandre Campos, Francisco J. González-Vila, José A. González-Pérez.
- P109. **Multi-mycotoxin determination in unifeed by (D)SPE-QuEChERS-HPLC-HRMS.** Rocío Facorro, María Llompart, Thierry Dagnac.
- P110. **Development of a HPLCS-DAD-MS method for the quality evaluation of guarana (*Paullinia cupana*) supplements.** Andrea Martín-Ortiz, Belén Gonzalo-Martínez, Ana Cristina Soria, María Luz Sanz, Ana Isabel Ruiz-Matute.

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- P112. **Body-decorating products: current analytical perspective of permanent and temporary tattoos ingredients.** Laura Rubio, Eugenia Guerra, Carmen Garcia-Jares, Marta Lores.

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- P113. **Non-targeted UHPLC-HRMS (ORBITRAP) fingerprinting in the authentication of cranberry natural products and pharmaceuticals.** Sergio Barbosa, Javier Saurina, Lluís Puignou, Oscar Núñez.
- P114. **Determination of bioactive compounds in serial extracts of chia leaf (*Salvia Hispanica* L.) by UHPLC-HRMS.** M. Carolina Zúñiga-López, Guillem Campmajó, Javier Saurina, Oscar Núñez.
- P115. **Phytochemical characterization of *Ramalina Striatula* extracts obtained by green compressed fluid technologies in a biorefinery approach.** Mónica Bueno, Carlos Areche, Gerardo Álvarez-Rivera, Elena Ibáñez, Alejandro Cifuentes.
- P116. **Analysis and quantification of *Sambucus Nigra* extracts for potential skin application.** Ana Henriques Mota, Noélia Duarte, Maria Rosário Bronze, Andreia Bento Silva, Elias Fattal, António José Almeida, Catarina Pinto Reis.
- P117. **Extraction of bioactive compounds from pomegranate seeds using High Intensity Focussed Ultrasounds and Pressurized Liquids.** Miriam Guzmán-Lorite, Estefanía González-García, María Luisa Marina, María Concepción García.



P118. **Analytical platform for the analysis of variability introduced by 10 *S. Cerevisiae* strains in tempranillo wines.** Marie Denat, Dolores Perez, Amparo Querol, Vicente Ferreira.

P119. **Natural pigments-based tattoos: analytical approaches for their safety control.** Laura Rubio, Carmen Garcia-Jares, Marta Lores.

P120. **Chromatographic insights for tracking bioactive compounds in woody by-products.** María Celeiro, J.Pablo Lamas, Rosa Arcas, Marta Lores.

P121. **Effect of experimental parameters in the matrix solid-phase dispersion extraction on the individual polyphenols from apple peel.** Ante Lončarić, Perla Ferrer, Katarina Matanović, Tihomir Kovač, Marta Lores.

OTHER APPLICATIONS

P122. **Evolution of aroma and polyphenols of Mencía wine during winemaking.** Perla Ferrer, María Celeiro, Hugo Martínez-Cordeiro, Tamara Vázquez, Carmen Garcia-Jares, Marta Lores, Jorge Domínguez.

P123. **Driving proteomic nanoLC-MS to new crossroads, are we heading towards extra sensitive or increased throughput applications?** Gilles Jaouen, R. van Ling, C. Mitterer, G. van Raemdonck, J. Op De Beeck.

P124. **Maximize the output of routine proteome analyses by using micro pillar array column technology.** Geert Van Raemdonck, Jeff Op de Beeck, Kurt Van Mol, Bo Claerebout, Natalie Van Landuyt, Wim De Malsche, Gert Desmet, Paul Jacobs, Gilles Jaouen.

LAST MINUTE POSTERS

P125. **Translating analytical pyrolysis fingerprints of soil organic matter to climatic variables.** Marco A. Jiménez-González, Ana M. Álvarez, Pilar Carral, José A. González-Pérez, José M. De la Rosa, Nicasio T. Jiménez-Morillo, Francisco J. González-Vila, Gonzalo Almendros.

P126. **Assessment of a new derivatization reagent for the analysis of glyphosate and aminomethylphosphonic acid.** Juan M Sánchez, Clàudia Fontàs.

P127. **A HPLC-FLD method for the simultaneous quantification of mycotoxins in animal feed using solid phase combined with dispersive liquid-liquid extraction.** Borja Muñoz Solano, Elena González-Peñas.

P128. **LC-MS analysis of ISRIB in plasma and brain samples.** Argitxu Esquivel, Elías Marlin, Obdulia Rabal, Tomas Aragón, Montserrat Arrasate, Antonio Pineda-Lucena.

P129. **Lipid analysis of beef meat: can MALDI-TOF mass spectrometry be used to define differential profiles for bovine breeds fed under distinct fact content diet?** Newton V. Verbisck, A.A. Perestrelo, A.L.J. Ferraz, L.C. De Paula, M.N. Bonin, S.R. Medeiros, M. Mele, R.G. Almeida, G.L.D. Feijó.

P130. **Extraction of mycotoxins from edible insects using natural deep eutectic solvents: A green alternative to conventional methods.** Fernando Pradanas-González, Gerardo Álvarez-Rivera, Elena Benito-Peña, Miguel Herrero, Fernando Navarro-Villoslada, María Cruz Moreno-Bondi.

P131. **Photo-transformation products from halogenated pharmaceuticals.** Enelton Fagnani, Nicola Montemurro, Sandra Pérez.

PLENARY LECTURES



PL1. TRENDS IN MASS SPECTROMETRY FOR HUMAN BIOMONITORING AND EXPOSOMICS

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Human biomonitoring methods are well established for the exposure assessment of many chemicals. Such methods have resulted in establishing measures for regulations, following time trends of concentrations in human populations and in establishing reference values and ranges for a selected group of 250–300 known persistent and non-persistent chemicals.

Yet, as more and more chemicals are added to the market, there is an increasing need to estimate the human exposure to these emerging contaminants. Recent efforts and advances in mass spectrometry have seen the unprecedented rise of screening techniques with the aim to identify emerging contaminants and/or their metabolites present in humans. Such analytical approaches based on high-resolution mass spectrometry (HRMS) are: 1) target screening; 2) suspect screening and 3) non-target (or untargeted) screening. Using these advanced tools, we can capitalize even more on the identification of lifestyle-specific exposure profiles, i.e. compounds that may differ in relation to specific behavioural patterns. Furthermore, the use of HRMS screening techniques allows the coupling of human biomonitoring with the exposome approach. An exposomic approach (exposomics) theoretically includes all exposures of potential health significance, whether they are derived from exogenous sources (e.g., pollutants, diet, drugs) or endogenous sources (e.g., hormones, human and microbial metabolites). Since levels of chemicals in biological samples reflect a wide range of exposures (biomarkers of exposure), but also consequences of exposures (biomarkers of effect), exposomic biomonitoring offers an efficient means for characterizing the overall individual exposure profiles. Incorporating the exposome paradigm into traditional biomonitoring approaches offers a means to improve exposure assessment in many ways. With only a few hundred chemicals routinely measurable through targeted methods and with limitations for short-lived compounds, exposomic approaches are critical to understanding the daily exposure to thousands of chemicals and the consequences of exposure in exposome-wide association studies (EWAS). The processing of rich sets of data from untargeted analyses offers a path for discovering health-impairing exposures that have thus far escaped scrutiny, a largely unrecognized benefit of exposomics. This should give guidance towards more accurate prevention measures that protect against exposure to (emerging) environmental contaminants and their substitutes in new materials and products.



PL2. LASER ABLATION ICP-MS FOR SIMULTANEOUS QUANTITATIVE IMAGING OF ELEMENTS AND BIOMOLECULES IN BIOLOGICAL STUDIES: ACHIEVEMENTS AND TRENDS

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The combination of laser ablation (LA) with inductively coupled plasma – mass spectrometry (ICP-MS) is currently considered as a powerful tool for element and isotopic direct analysis of solids, offering limits of detection in the order of ng per gram and a spatial resolution in the μm range. In addition, LA-ICP-MS allows studies with isotopically-enriched tracers such as the investigation of supplementation in single-cells [1]. New strategies for quantitative elemental mapping of biological tissues and cells with LA-ICP-MS are being developed in recent years. On the other hand, another growing research area with LA-ICP-MS is related to obtaining molecular information as well. For example, mapping of specific proteins in biological tissues and cells can be performed by the combination of metal-labelled immunoprobes with LA-ICP-MS [2]. The use of several atoms of a given metal/isotope per label (e.g. polymeric labels containing several metal chelates and metallic nanoparticles) provides high sensitivity. However, although LA-ICP-MS is in principle a quantitative technique, critical requirements should be met for absolute quantification of protein distribution. Progresses based on the use of metal-labelled antibodies for LA-ICP-MS mapping of specific proteins will be reviewed. Also, critical requirements to obtain absolute quantitative mapping of specific proteins by LA-ICP-MS will be highlighted. Illustrative examples with the last advances achieved, for example related to imaging of human brain sections and ocular tissues, will be presented.

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PL3. ION MOBILITY SPECTROMETRY: PRINCIPLES OF SEPARATION AND APPLICATIONS IN DNA STRUCTURAL CHARACTERIZATION

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Ion mobility spectrometry separates ions based on their drift velocity in a cell filled with gas under the influence of an electric field. In addition to separation, one of the possible applications of ion mobility spectrometry is structural assignment based on the collision cross section (CCS). This physical quantity derived from the ion mobility measurement can also be calculated on a structural model. The structural assignment is based on the matching between experimental and calculated CCS values [1]. The talk will review some fundamental aspects of ion mobility spectrometry and collision cross sections [2], with first a focus on the metrology aspects of ion mobility mass spectrometry measurements [3], and new results about the gas-phase structures of artificial foldamers and nucleic acid higher-order structures and how these relate to the solution structures [4, 5].

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PL4. ALTERNATIVE CHROMATOGRAPHIC AND MASS SPECTROMETRIC APPROACHES TO METABOLOMIC CHALLENGES

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Metabolomic analyses frequently involve analysis of largely water-soluble analytes, and so my group has for over a decade made use of chromatographic stationary phases that are designed to retain polar, as well as those focussed on retaining non-polar analytes. Porous graphitic carbon (PGC) and hydrophilic interaction liquid chromatography (HILIC) materials are polar-analyte-retaining stationary phases that in our hands have proven especially effective. In parallel, we are involved in improving sample extraction protocols for metabolomic analyses, and I will review some of our recent innovations in handling and extracting plant material. The talk will be illustrated with some of the broad applications which have driven our metabolomics development work.

Acknowledgement

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PL5. POROUS POLYMER MONOLITHS: VERSATILE MATERIALS FOR NUMEROUS APPLICATIONS

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The modern monolithic columns emerged about 30 years ago. Their well-known advantages include ease of the preparation, robustness, high permeability to flow, mass transport via convection, and a vast variety of chemistries. The early polymer-based monoliths were used almost entirely for the rapid liquid chromatography separations of proteins and other large molecules. A number of new chemistries and functionalization methods were meanwhile developed to produce monolithic columns for the separations in various chromatographic modes including gas chromatography, electrochromatography, and microfluidics. In addition to typical chromatographic applications, new uses were recently described thus confirming versatility of the monoliths. For example, reversible functionalization via attachment of gold nanoparticles to thiols provides materials for highly sensitive surface enhanced Raman spectroscopy (SERS). Thin monolithic layers are gaining more attention as well since they enable efficient separations of proteins using very simple means followed by an easy detection using mass spectrometry or SERS. This presentation will focus on three applications, liquid chromatography, SERS, and thin layer chromatography.

This work was supported by the STARSS project (Reg. No. Z.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.



PL6. APPLICATION OF COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY TO CHARACTERIZE VERY COMPLEX FOOD-RELATED SAMPLES

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The chemical characterization of food and food-related products is of utmost importance in many research fields, including the study of the food and health relationship. However, foods are really complex matrices, making this characterization very challenging. Nevertheless, it is necessary to precisely know the food constituents that would be responsible for the health beneficial effects.

Although liquid chromatography (LC) coupled to mass spectrometry (MS) is a very robust and useful tool to chemically characterize food bioactive components, there are lots of samples that are simply too complex to be analyzed by conventional one-dimensional approaches. In this situations, the use of comprehensive two-dimensional liquid chromatography (LC×LC) is able to provide with the additional separation power required. LC×LC is based on the use of two independent and complementary (thus, orthogonal) separation mechanisms through which the whole sample is analyzed. By using this approach, resolving power is greatly enhanced as, theoretically, the peak capacity attainable in each dimension can be combined.

Moreover, the combination of LC×LC with MS increases the capabilities of this analytical tool to characterize samples composed by a great variety of closely related unknown compounds. In this work, different applications devoted to the separation and elucidation of the secondary metabolite pattern of several food-related complex matrices are described, including different couplings and modifications at the modulator level in order to solve some important problems and limitations in LC×LC practice.



PL7. UNVEILING THE SECRETS OF THE PAST USING HYPHENATED CHROMATOGRAPHIC TECHNIQUES

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Cultural heritage artefacts usually yield very complex samples for analysis. In the making of the artefacts, an array of materials are used alongside ancient and sometimes unknown recipes and manufacture technologies. Moreover, their chemical analysis is generally further complicated by the chemical changes induced by the ageing of the materials.

The questions that need to be answered vary depending on the object to be studied, but are usually related either with the history and the manufacture of the object (where and how it was produced, or how was it used throughout times, crucial for art history and archaeological studies), or about the decay processes of their constituent materials (crucial in terms of their conservation).

The characterization of the organic materials occurring, for example, as residues in archeological ceramics, or as adhesives, bindings and lake pigments on paintings, relies heavily on the separation capabilities of the chromatographic techniques (both gas chromatography, GC, and liquid chromatography, HPLC), and the structural information at a molecular level, provided by the mass spectrometry (MS).

In this presentation, three case studies will be presented to show the wide range of questions and analytical methodologies need to address them. The identification of the previous contents of archaeological ceramics is usually done using GC-MS, and this will be exemplified in the identification of the illuminant used in Roman oil lamps from two archaeological sites from the south of Portugal ⁽¹⁾. The study of paleodiets is done throughout the analysis of the stable isotopic ratios for carbon and nitrogen of collagen extracted from osteological remains. These analysis are done by isotopic ratio mass spectrometry coupled with elemental analyser (EA-IRMS), and this will be exemplified with the study of the paleodiet of the Late Antiquity population of Monte da Cegonha (Vidigueira, Beja) ⁽²⁾. Finally, the application of mass spectrometry in the study of art objects will be briefly exemplified with the study of bindings and lake pigments in mural paintings which are done by Pyrolysis-GC-MS and LC-MS, respectively ⁽³⁾.

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ORAL COMMUNICATIONS



01. EVALUATION OF THE ANTICANCER POTENTIAL OF GOLDENBERRY CALYX UNDER A FOODOMICS PERSPECTIVE

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The prevention of human diseases by means of the proper diet control and the intake of functional food or nutraceutical products is becoming an emerging trend in medicine, food and bioscience fields. In recent years, Foodomics has become a powerful multi-omic platform to investigate the potential health benefits of some dietary agents of interest that can reverse, suppress or prevent cancer progression [1]. In this regard, the calyx of goldenberry fruit is an interesting agri-food waste which represents a powerful source of bioactive compounds of great interest from the pharmacological point of view [2,3].

In the framework of a sustainable strategy for goldenberry calyx valorisation, an integrated Foodomics approach is proposed in this work to investigate the bioactive potential of this unexplored food by-product. Thus, a pressurized-liquid extraction (PLE) procedure was optimized to obtain an enriched extract in high-added value compounds, followed by a comprehensive phytochemical characterization by LC and GC coupled to q-TOF-MS(/MS). A broad variety of interesting phytoconstituents such as withanolides (C₂₈-isoprenoids), phenolic acids, flavonoids, sucrose esters, terpenoids, phytosterols, and phytol derivatives (e.g., vitamin E) were identified in the enriched PLE extracts, whose bioactive potential was tested against HT-29 colon cancer cells. After 48 h of treatment, the viability of HT-29 cells was notably reduced without affecting normal human colon fibroblast cells. Metabolomics and transcriptomics data integration revealed alteration of cellular redox homeostasis, inactivation of aminoacyl tRNA charging pathway, dysfunction on carnitine shuttle and beta-oxidation of fatty acids, and pyrimidine ribonucleotide interconversion impairment. The results reported herein represent a valuable contribution to the sustainable valorization of goldenberry calyx and to better understand the molecular mechanisms underlying its bioactivity against human colon cancer, demonstrating the huge potential of Foodomics to carry out this type of studies.

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02. GLYCOPROTEOMIC PROFILE OF EXTRACELLULAR VESICLES IN CANCER

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Glycans are major cellular components. Changes in glycosylation are known to play critical roles in cancer biology¹. Extracellular Vesicles (EVs) are mostly produced by cancer cells and have been demonstrated to impact cancer spread namely by defining the pre-metastatic niches. Exosomes are nanosized extracellular membrane vesicles. We have isolated EVs using different state-of-the-art methodologies namely differential ultracentrifugation (UC), total exosome isolation (TEI), OptiPrepTM density gradient (ODG) and size exclusion chromatography (SEC) and assessed their impact in the subpopulation of EVs obtained². Glycoproteomics mass-spectrometry approaches were performed using a genetically engineered gastric cancer cell line displaying simplified truncated homogeneous O-glycosylation, whereas the wild type cell line presents a heterogeneous glycosylation profile, due to O-glycosylation extension. UC, ODG and SEC methods isolated EVs with similar protein and glycan contents, allowing the detection of the tumour-associated glycan antigen STn. The TEI technique enriched the most different EV population and presented the higher content of non-EV co-purified proteins.

In other study³, in addition to other omics approaches, we performed a glycomic analysis of melanoma, breast and pancreatic cancer cell lines submitted to asymmetric-flow field-flow fractionation (AF4). The glycomics study of the resulting exosome subpopulations demonstrated the prevalence of complex N-glycans in all particle subsets with relatively high levels of sialylation. Furthermore, our study revealed differences in N-glycan composition and structures among exomeres, Exo-S, and Exo-L.

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03. EXTREME MAKE-UP AND COSMETIC APPLICATORS: ARE THEY SAFE?

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The so-called 'Extreme Cosmetics', such as long-lasting lipsticks and eye masks, eyelash lengtheners, waterproof makeup, eye pencils with tattoo effect... are designed to magnify their functions and/or their duration over time. All cosmetic products available in the European market must comply with the EC Regulation No 1223/2009, nevertheless, 'Extreme Cosmetics' may contain ingredients other than those of a classic cosmetic, with the same function or that enhance some of its characteristics, e.g.: anti-wrinkle cream with snake venom. Since most of the analytical methodology developed for the regulatory control of consumer products has been optimized using common or classic cosmetics, the main objective of this work is to assess whether such methodology is useful for the 'Extreme Cosmetics'.

Fast and simple extraction procedures based on Ultrasound Assisted Extraction (UAE) have been optimized for the simultaneous analysis of 70 organic compounds of several families of ingredients which presence in cosmetics needs to be controlled, either because they are restricted or even banned [1]. The evaluated compounds include allergenic fragrances, synthetic musks, plasticizers, and preservatives. Moreover, for cosmetics with plastic applicators, a *Supported-UAE (Sup-UAE)* method was also opportunely optimized to evaluate if a partial transfer of plasticizers to the cosmetics—and thereby to the consumers—could happen. Analysis was performed by gas chromatography-mass spectrometry (GC-MS). The method was successfully validated in terms of linearity, accuracy and precision, with mean recoveries values about 100% and RSD values lower than 5% for UAE and 10% for *Sup-UAE*.

Finally, the proposed methodologies UAE/*Sup-UAE*-GC-MS were applied to a survey of 50 commercial products, demonstrating their analytical suitability. Results revealed that partial transfer of phthalates from the applicators to the cosmetic formulations does indeed happen. Additionally, a labelling study was performed to check if the consumer is correctly and fully informed.

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04. UNTARGETED METABOLOMICS OF FULL SCAN MS HYPHENATED DATA – MINING FOR MINOR PEAKS WITH FINNEE, RISKS AND BENEFITS

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Separation techniques hyphenated with high-resolution mass spectrometry is often the analytical method of choice in untargeted metabolomics studies. While many strategies have been developed, differential metabolomics analysis with full scan data allows extracting the most information from a given set of experiments. In differential metabolomics, datasets resulting in a single experiment are mined for chromatographic like features that are summarized in a peak list. Multiples peak lists are then aligned between all experiments and differentiating features can be identified using chemometrics tools such as PCA, clustering or PLS-DA. The resulting features should then be identified using tandem mass spectrometry. Differential analysis heavily relies on computerized tools; different approaches have been developed, many of them freely available. Finnee is a Matlab toolbox [1] that is being developed in the LEPABE with the original approach of correcting for baseline drift and quantifying and correction for background noise before transforming the MS scans from profile to centroid [2]. This approach allows to avoid intensity thresholding and allows to mine for features near the limit of quantification. Thus, as it will be shown with different examples, features whose intensities spanned multiples order of magnitudes can be reliability extracted. As it will be presented here, the benefits of increased information should be balanced with an increase in computing time, increased potential error during the alignment step as well as the chemometrics analysis.

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05. PROFILING THE SKIN VOLATILOME AS POWERFUL TOOL TO THE NEURODEGENERATIVE DISEASES DIAGNOSIS

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Human metabolism integrates a myriad of endogenous and exogenous inputs to keep homeostasis. Whenever this complex equilibrium is disrupted, different deleterious events can be triggered, leading to disease. In this context, we are exploring the use of volatile biosignatures of human biofluids as snapshots of the human metabolism under health and diseases. Such signatures will allow a potential discrimination between healthy and disease conditions using a non-invasive diagnosis. While this approach is being explored in several oncologic and respiratory diseases, there are growing evidences that it can be also applied to other conditions, particularly neurodegenerative diseases (NDDs). Currently, Parkinson and Alzheimer are among the most prevalent forms of dementia and their increasing trends, effects and costs for the patients and society are very difficult to invert. The main reasons for this scenario lie in the fact that humans are living longer and so more susceptible to accumulation of deleterious effects in their bodies, particularly in their neurons. Furthermore, as NDDs progress slowly and their ethiology is not yet fully characterised, their diagnosis relies essentially on the clinical symptoms. This strategy, however, is very ineffective because neurodegeneration begins long before the patient experiences any symptoms. There is, therefore, an urgent need of reliable tools for the early diagnosis of NDDs, able to support the anticipation of the treatment and mitigate the negative effects of neurodegeneration. In this project, we are developing a methodology for the non-invasive sampling and analysis of the skin volatilome as a tool for the NDDs study and diagnosis, particularly in Parkinson and Alzheimer diseases.

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06. A METABOLOMICS-BASED WORKFLOW FOR ADDUCTOMICS STUDIES

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Identification of protein covalent modifications (covalent adducts) is a challenging task mainly due to the lack of data processing approaches for Adductomics studies. Despite the huge technological advances in mass spectrometry (MS) instrumentation and bioinformatics tools for proteomics studies, enabling the identification of several thousands of proteins in a single injection analysis, these methodologies have very limited success on the identification of low abundant covalent protein adducts.¹ Herein we present a novel strategy to identify protein covalent modifications inspired in metabolomics workflows that consists on LC-MS data pre-processing using the open source software MZmine followed by statistical analysis. The usefulness of this strategy was evaluated using experimental LC-MS data of histones isolated from HepG2 and THLE2 cells exposed to the chemical carcinogen glycidamide.

Our approach (**Figure 1**) was able to identify more adducts than the commonly used methodology in adductomics studies, which rely on producing comprehensive MS/MS data, using data dependent analysis (DDA) acquisition mode, and then use protein database search engines (*eg.* Mascot, MaxQuant) for the identification of adducted peptides. This new untargeted Adductomics workflow consists on a first full scan analysis of samples followed by our metabolomics-inspired data processing strategy that will yield a list of *m/z* values, corresponding to potential adducts. This peak list will be subsequently used for targeted MS/MS analysis of potential adducted-peptides. The selection step is expected to result in higher quality MS/MS spectra of low level adducted-peptides, when compared with DDA and data independent analysis (DIA) approaches, thereby enhancing the chances of identifying low abundant adducted peptides in biological samples. This will exponentially increase the number and accuracy of findings for all fields of Adductomics application, encompassing epigenetic and toxicological studies.

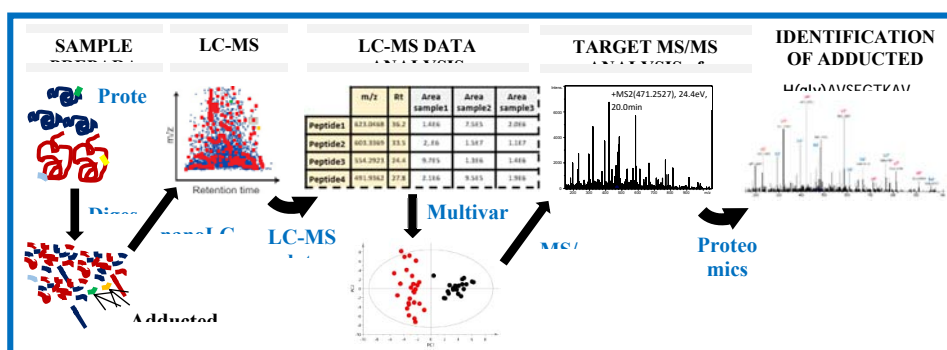


Figure 1: Workflow of our metabolomics-based approach to identify protein covalent modifications.

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07. MASS SPECTROMETRY CONTRIBUTION TO METABOLIC STUDIES: NEW INSIGHTS INTO MONTELUKAST METABOLISM

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Montelukast (MTK) is a leukotriene antagonist used for asthma management in children and adults. Recent evidence points towards new therapeutic indications as controller of neuroinflammation, with application in neurodegenerative disorders or as chemopreventive and cancer therapy adjuvant.[1-3] MTK metabolism is, however, poorly understood, and only 5 phase I and 2 phase II MTK metabolites have been identified.[4]

The goal of this work was to evaluate the *in vitro* metabolism of MTK in human and mice sub-cellular fractions. Metabolites were analyzed by HPLC coupled to HRMS/MS.

In addition to the known MTK metabolites, we identified novel phase I metabolites that resulted from hydroxylation, S-oxidation, N-oxidation and oxidative dealkylation. Analysis of the fragmentation pathways allowed the unequivocal identification of the metabolites. In the presence of the adequate co-factors, we also identified new MTK-derived phase II metabolites, including glucuronide, glutathione, and cysteine conjugates. A new decarboxylation metabolite, specific of brain tissue, has also been observed.

The identified metabolites allowed the creation of a database comprising all identified metabolites that will be used in metabolomics analysis. A data-independent analysis will be applied to patient-derived samples and animal studies, further completing the metabolite database beyond phase I and II metabolites, including conjugation and detoxification products and even adducts with small peptides and other relevant biomolecules.

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08. COMBINATION OF MULTIPLE HEART-CUTTING TWO DIMENSIONAL LIQUID CHROMATOGRAPHY AND ISOTOPE DILUTION ESI-MS/MS FOR THE ACCURATE QUANTIFICATION OF CLINICAL BIOMARKERS

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Quantification of trace amounts of clinical biomarkers in human samples by UHPLC-ESI-MS/MS is still challenging. A relevant problem with the use of ESI source is the signal variabilities due to matrix effects which significantly affect the instrumental sensitivity and the accuracy and precision of the results. The most efficient strategy to overcome matrix effects is standardization through the use of stable isotopically labeled standards. However, even when using adequate labelled analogues coeluting with the analyte after the chromatographic separation, matrix constituents can lead to serious signal suppression and spectral interferences. For these reasons, the application of effective sample purification steps is critical to perform a reliable LC-ESI-MS/MS analyses.

An alternative to the application of time consuming purification steps is the use of multidimensional chromatography. This work presents the combination of multiple heart-cutting (MHC) two dimensional liquid chromatography and isotope dilution mass spectrometry as a powerful strategy to quantify different clinical biomarkers in complex samples. Using the MHC mode the target analytes can be subjected to two different separation mechanisms. Therefore, a purification of the sample is obtained while increasing the chromatographic resolution between analytes and matrix interfering compounds. In addition, this strategy is particularly useful when using mobile phases in the first dimension which are not compatible with the ESI source due to the presence of non-volatile salts or organic modifiers. Examples of applications of this strategy will be presented such as the absolute quantification of protein biomarkers of glaucoma, methylated arginines, creatine and creatinine in human serum and the determination of melatonin and its metabolites in cell cultures.



09. QUANTIFICATION OF RELATED SYNTHETIC CATHINONES IN RAT BRAIN BY UHPLC-MS/MS. RELATIONSHIP BETWEEN STRUCTURE AND BLOOD-BRAIN BARRIER PERMEABILITY

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Synthetic cathinones were the second most seized new psychoactive substance family in Europe during 2018, according to European Drug Monitoring Centre for Drug and Drug Addiction (EMCCDA 2019 Report) [1]. These compounds are used as stimulants, being involved in some overdose death cases. Recently, some synthetic cathinones, such as the 3,4-methylenedioxypropylvalerone (MDPV, and known in the street as “cannibal drug”) or the α -pyrrolidinopentiphenone (α -PVP or “flakka”), had an important social alarm. Both compounds presented high potency even at low doses. The reason of the potency of synthetic cathinones in real consumers has not been studied in depth.

For this purpose, analytical methodology for the determination of 14 synthetic cathinones in rat brain samples has been developed and validated. The aim of this work was to determine the permeability of synthetic cathinones through the blood-brain barrier. These 14 cathinones are related compounds, with some slight changes in the moieties present in their structure. The idea was to test which are the most permeable cathinones depending on their moieties and thus, try to relate this high permeability with the cathinone potency.

The analytical methodology has been validated in Sprague-Dawley female rat brain at 1 ng/g, after extraction with acidified acetonitrile followed by 10-fold dilution and determination by ultra-high performance liquid chromatography coupled to low-resolution tandem mass spectrometry (UHPLC-MS/MS). Sufficient chromatographic separation required, due to the similarities between the 14 cathinones, including two pairs of positional isomers. This validated methodology was then applied to the determination of these cathinones in the brain of rats individually dosed with each compound. The levels of the cathinones found in brain samples was then compared with the reported effects of some drug users that have consumed these cathinones, trying to establish a relationship between structure and potency for these compounds.

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O10. IDENTIFICATION OF GROWTH HORMONE-RELEASING HORMONES IN URINE DOPING CONTROLS BY IMMUNOAFFINITY PURIFICATION AND LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

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The use of growth hormone-releasing hormone (GHRH) and its analogues sermorelin, tesamorelin and CJC-1295 is prohibited in sports according to the regulations of the World Anti-Doping Agency (WADA) [1]. These target peptides are found at very low concentrations in urine (at pg/mL level), reason why hyphenated purification and enrichment steps are required prior to mass spectrometric detection [2]. Compared to other enrichment strategies, immunoaffinity purification based on magnetic beads offers improved extraction efficiency, selectivity and reproducibility, optimum antibody orientation and minimal non-specific interaction.

In this work, we have evaluated the suitability of magnetic beads with different protein affinities and binding capacities (MagnaBind™, LOABeads™ SerAsca and LOABeads™ AffiActive) prior analysis of human urine samples by liquid chromatography coupled to Quadrupole-Orbitrap mass spectrometry. Critical aspects of the extraction protocol with regards to analyte stability will be discussed. After optimization of the immunopurification protocol (sample pretreatment, relative amounts of reagents and elution conditions) with the magnetic beads that provided better recoveries, i.e. LOABeads™ SerAsca, the method was validated in terms of limits of detection, selectivity, matrix effects and intra- and inter-day precisions. Method validation was performed at the minimum required performance levels specified by WADA, hence demonstrating that the immunoaffinity-mass spectrometry-based method can be successfully applied to identify GHRH peptides for doping control purposes.

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O11. HPLC-ESI-MS/MS AS A VALUABLE TOOL TO UNRAVEL THE METABOLISM OF NEW PSYCHOACTIVE SUBSTANCES

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Over the last two decades, new psychoactive substances (NPS), commonly named “legal highs” or “herbal highs”, have increasingly appeared in the illicit market, surpassing controlled substance legislation, ultimately resulting in a drug abuse crisis. Synthetic cathinones (SC), one of the most prevalent NPS's, showed psychostimulatory effects that are similar to methamphetamine and cocaine, being responsible for many intoxications and overdose deaths worldwide [1]. Therefore, a higher knowledge of SC's metabolism and excretion profiles is of major importance. The aim of this study is to identify and quantify Phase I metabolites of two SC's, buphedrone and N-ethylhexedrone, in mice urine, and in mice and human liver microsomes using liquid chromatography coupled to tandem mass spectrometry. HPLC-MS/MS methods were implemented according to each SC and validated. MS full scan mode was used for a preliminary screening aiming at searching for precursor ions corresponding to parent drugs and expected metabolites. Identification and quantification of SC's and metabolites were performed in MRM mode to achieve high selectivity and sensitivity. Results revealed similar *in vivo* and *in vitro* metabolic pathways for both drugs. N-dealkylated metabolites were the most excreted. Other metabolites, such as those resulting from ketone reduction were also identified and quantified. Ultimately, our findings suggested a slower elimination rate for N-ethylhexedrone than for buphedrone, which is in accordance with their lipophilic properties. Further *in vitro* studies are still on going. In conclusion, this work contributes to a better understanding of SC's metabolism through the identification and quantification of metabolites that could be potential new markers to estimate drug consumption.

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O12. PRELIMINARY IDENTIFICATION OF SPECIFIC MARKERS TO GUARANTEE THE AUTHENTICITY OF GALICIA'S HONEY BY A NON-TARGET HRMS APPROACH

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Foodomics, application and integration of advanced "omics" technologies help solving the new challenges facing the safety, quality and traceability of food, including the establishment of more powerful analytical methodologies to guarantee the origin and quality of foods, or the discovery of (bio) markers. Phenolic acids and flavonoids are one of the most important quality parameters of honey since they give it its colour, sensorial properties, organoleptic and antioxidant activity beneficial to health. These phenol derivatives can be used as indicators of floral and botanical origin of honey, especially pollens, nectars, resins and oils. In the present study, honey samples were diluted in water, thus reducing metabolite information loss, and the mixture was homogenized using vortex mixer and an Ultra Sounds. Afterwards, MSPDs with different dispersive agents and SPEs with different sorbents were tested, the latter showing the best results employing a new material based on granulated cork. Liquid Chromatography-High Resolution Mass Spectrometry was used by means of a SCIEX TripleTOF[®] 5600+. As regards non-target screening, a powerful workflow based on a data-independent acquisition (SWATH) was implemented. The exploration of specific markers using this metabolomics "non-target" SWATH approach should allow addressing the discrimination and differentiation of Galicia's honeys with different floral origins, with a particular focus on the content of antioxidants and polyphenols in monofloral honeys. Pinocembrin, isoscapoletin, chrysin, cordycepin, wogonin and shionone were among the most detected compounds.

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O13. CHEMICAL PROFILE OF COFFEE VARIETIES BY FT-ICR-MS

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Coffee is a drink prepared from roasted coffee beans and is one of the most consumed beverages in the world. The beans (i.e. dried coffee seeds) are roasted to varying degrees, depending on the desired flavor. Coffee is darkly coloured, bitter, slightly acidic and has a stimulating effect in humans, primarily due to its caffeine content [1]. Some advantages of coffee are to give more energy, burn fat, improve physical performance and lower the risk of several conditions, such as type 2 diabetes, cancer and Alzheimer's and Parkinson's disease. However, excessive coffee consumption can be bad for our health such as increase of the blood pressure, anxiety, insomnia, tremor and elevate glaucoma risk.

Mass spectrometry is an analytical technique that possess high sensitivity, low detection limit, short analysis time and flexibility. Fourier-transform ion cyclotron-resonance mass spectrometry (FT-ICR-MS) provides ultra-high-mass accuracy and a highest mass resolution with a resolving power over 1,000,000 at m/z 400 [2].

In this work, we compared the profile chemical of different coffee varieties by FT-ICR-MS. All the analysis was performed in a Bruker Daltonics solariX XR FT-ICR mass spectrometer equipped with a 7.0 Tesla actively shielded superconducting magnet. MetaboScape software version 4.0 from Bruker Daltonics was used to propose a list of possible compounds and to give some statistical analysis. We identify several compounds presents in coffee and we were able to discriminate between coffee varieties.

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O14. LC-MS/MS CONFIRMATION OF THE EMERGING PINNATOXINS AND HIGH LEVELS OF ESTERIFIED OA GROUP TOXINS IN COMMERCIAL MOLLUSKS FROM THE ATLANTIC COAST OF SPAIN.

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Lipophilic marine toxins, which are produced by harmful microalgae and accumulate in the marine food chain, represents a food safety threat in the shellfish industry. Galicia, which is the main producer of edible bivalve mollusc in the European Union (EU), it was subjected to recurring cases of mussel farm closures in the last decades due to the presence of biotoxins. In this work, we present evidence that okadaic acid (OA) and dinophisistoxin-2 (DTX-2) (especially in the form of esters) constitute the main risk for Galician shellfish consumers. It is established the impact of the analytical error margin when seafood containing toxins at levels below the legal limit (160 µg OA eq/kg) [1] but close to this are commercialized. We also confirm the presence of the emerging pinnatoxins in mussels from Galician markets. In the recent years, evidence has grown for the presence of emerging toxins in European waters, leading to the potential for consumers of contaminated products to be affected by these new risks. Pinnatoxins have been recently detected in seafood from the Mediterranean sea (Catalonia, Italy and Slovenia) [2]. However, to our knowledge, this is the first time that these toxins are recorded in commercial mollusks from the Atlantic Coast of Spain. Identification and quantification of lipophilic marine toxins were performed by a 1290 Infinity ultra-high-performance liquid chromatography system coupled to an Agilent G6460C Triple Quadrupole mass spectrometer equipped with an Agilent Jet Stream ESI source. Analytical method performance provided the optimum sensitivity to unequivocally identify these new emerging toxins by comparing the specific transitions and their ratio in samples and standard. Despite there is no a potential risk through mussel ingestion for the emerging pinnatoxins, the presence of new analogs are issues that must be considered in the shellfish safety monitoring programs through LC–MS/MS methods.

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O15. EVALUATING PLANT UPTAKE OF PHARMACEUTICALS AND THEIR METABOLITES FROM WATER REUSE WITH UPLC-QTOF-MS

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Today there are concerns remaining about the safety of irrigation with treated wastewater from conventional wastewater treatments plants because it contains various contaminants among them pharmaceuticals and their metabolites. Plants have the ability to accumulate pharmaceuticals in their tissues through root uptake. Investigations on pharmaceuticals translocations from soil and water into plant tissues irrigated with wastewater were published recently. With the increasing use of wastewater for irrigation, and thus the potential uptake and translocation of pharmaceuticals and their metabolites in crops, concerns about food safety are growing. Unfortunately, as of today information on the behavior of metabolites in crops is scarce, partly because the lack of simple yet robust methodologies for the analysis of pharmaceuticals and their metabolites in complex matrices such as crops. Recently, an alternative and effective technique for extraction and clean-up has been used for the extraction of pharmaceuticals namely QuEChERS (“quick, easy, cheap, effective, rugged, and safe”). This methodology was first reported in 2003 by Anastassiades and coworkers [1]. QuEChERS has been traditionally applied to analyze pesticides in many food matrices (i.e., fruits, vegetables and cereals) but it is now increasingly accepted in food, environmental and clinical applications. It is a versatile, simple, rapid, inexpensive and multiclass multiresidue method which provides high quality results with a minimal number of steps, with reduced reagent use, and required little glassware [2, 3]. Concerning the detection and quantification of chemically diverse group of pharmaceuticals and their metabolites, high resolution (HR) mass spectrometers such as Orbitrap-mass spectrometry (MS) based instruments and time-of-flight (TOF-MS) systems, are now the most powerful tool for multi-residue determination. LC-HRMS provides robust analysis with high selectivity and sensitivity in even the most complex environmental matrices. Indeed, HR hybrid mass systems, such as TOF spectrometers and quadrupole (quadrupole-TOF or QTOF), have the ability to perform quantitative multi-target analysis detections increasing selectivity with MRM^{HR} or SWATH (Sciex technology). This work proposes the use of QuEChERS in combination with SWATH method for HR-MS for a fast evaluation of the plant uptake of 40 relevant wastewater-derived pollutants, mainly pharmaceuticals and their metabolites. The combination of QuEChERS and LC-HRMS (QTOF-MS X550R) worked satisfactory for the determination of pharmaceuticals and their metabolites in the crops. Neutral pharmaceuticals and metabolites were detected in leaves while acidic compounds were not taken up. Lipophilicity and speciation affected the uptake of pharmaceuticals by plants. However, some basic drugs were detected in higher concentrations in leaves than in soil, probably because they underwent degradation in soil.

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O16. PASSIVE SAMPLING: CALIBRATION, DETECTION AND QUANTIFICATION OF PERSISTENT ORGANIC POLLUTANTS IN HIGH-MOUNTAIN LAKES FROM THE AIGÜESTORTES NATIONAL PARK

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Persistent organic pollutants (POP) are incorporated to high-mountain areas through long-range atmospheric transport and deposition. There is a need for assessment of the impact of these compounds in these ecosystems, since the remoteness of these environments entails difficulties for their adequate monitoring. Passive sampling (PS) techniques have proven to be a suitable approach for the monitoring of such nonpolar and semi-polar organic contaminants in waters and the atmosphere, although their application in remote high-mountain areas is limited. PS devices are easy to deploy and do not require an energy source, since uptake is controlled by diffusive processes. Long-term exposure of PS mediums in the environment decreases limits of quantification (LOQ) of contaminants, which makes them candidate devices for environmental monitoring of POPs at trace levels. However, the assessment of POP concentrations in air and water is an analytical challenge since they must be determined through the estimation of uptake rates using Performance Reference Compounds (PRC).

Active and passive sampling devices, including polyurethane foam (PUF) disks and low-density polyethylene (LDPE) sheets, were deployed in air and water of six remote high-mountain areas in the National Park of Aigüestortes i Estany de Sant Maurici (Pyrenees). The sequestered contaminants were extracted by Soxhlet and solid phase extraction (SPE), depending on the environmental matrix. Separation by high-performance liquid chromatography (HPLC) and silica column chromatography were used as fractionation and clean-up steps. The fractions were analyzed by gas chromatography coupled to electron impact ionization mass spectrometry (GC-EI-MS) and tandem mass spectrometry (GC-EI-MS/MS), achieving in-column LOQs of 0,1–0,5 pg for polychlorinated biphenyls (PCB), organochlorinated pesticides (OCP), polycyclic aromatic hydrocarbons (PAH) and organophosphorous flame retardants (OPFR).

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O17. FATE AND OCCURRENCE OF MICRO AND NANOPLASTICS IN THE EBRO DELTA.

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Microplastic (MPLs) are defined as plastic fragment than less of 5 mm of diameter comprising either manufactured MPLs (primary sources) and fragments or fibbers of plastics derived from the breakdown of plastic products (secondary sources)¹. The MPLs of smaller range of sizes and nanoplastics (NPLs) are of high interest because of their potential impact on living organisms and because the higher surface-active areas that they pose.

In this context, this work presents the development and validation of a method based on ultrasonic assisted extraction by toluene followed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) analysis². The method has been successfully applied for the quantification of polystyrene (PS) MPLs' in environmental waters from one relevant case of study: Ebro Delta. The results denoted the presence of PS between 500 and 3000 Da at concentrations ranging from 32 to 67 ng/L along the Ebro River and up to 586 ng/L in commercial harbour Port de l'Illa (Fangar Bay, Ebro Delta). In addition, the suspect screening of polymer residues in these samples has done using the capabilities of HRMS. The results showed the tentative identification of polyethylene (PE) (1000 – 2000 Da), polypropylene (1500 – 3000 Da), polyisoprene (1000 – 2500 Da) and polybutadiene (1500 – 2000 Da) in samples from the mouth of the river as well as in Fangar Bay and Alfacs Bay.

Finally, the interaction of those plastics detected in Ebro Delta with persistent organic pollutants such as polychlorinated biphenyls (PCBs) and perfluoroalkyl substances (PFASs) has been assessed. The evaluation of sorption isotherms of selected POPs and MPLs has been done working at relevant environmental concentrations and at laboratory scale in controlled microcosms. The results showed that PE and PS can act as carrier materials for those POPs in the environment and, in both cases the model isotherm can be adjusted to a Freundlich model.

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O18. SPE-UPLC-HRMS SUSPECT SCREENING METHOD FOR THE IDENTIFICATION OF PHARMACEUTICALS AND THEIR TRANSFORMATION PRODUCTS IN SURFACE WATER AFTER PHOTOLYSIS.

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The ubiquitous presence of pharmaceutical active compounds (PhACS) and their degradation products in the aquatic environment around the world deserves attention. These contaminants of emerging concern (CECs) are released in domestic and industrial wastewater and not efficiently degraded through the wastewater treatment plants. Because many of them are recalcitrant for microbiological process, these compounds reach the water bodies where abiotic processes as photolysis arises as an important degradation process. As a result, the so-called transformation products (TPs) are formed and present different characteristics in comparison with pattern molecules regarding mobility, bioaccumulation potential and toxicity, and can be even more harmful than their precursors. Identification and quantification of TPs in environmental matrices demand efficient sample preparation and accurate analytical techniques. Solid phase extraction (SPE) is used to clean up the sample and pre concentrate the analytes that in original sample are present in order of ng/L or less; because of environmental matrices contain analytes with widely different physicochemical properties as ionization constant (pKa, pKb) and partition coefficient (log Kow), a multi-sorbent extraction cartridge ensures better recoveries [1], according with it was done in this work. Since no certified standards are available for TPs target analysis, suspect screening approach using ultra-performance liquid chromatography coupled to high resolution mass spectrometry (UPLC-HRMS) is the most indicate methodology for the task [2]. There are two piece of equipment that compete each other in unknown screening of contaminants: quadrupole time of flight (QTOF) and quadrupole Orbitrap; however, the availability of software for acquiring MS/MS data, processing and extracting the information, and comparing with databases have been as crucial as the analytical potentiality in the choice for one of the techniques, as discussed in this work.

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O19. DIRECT COMPOUND SPECIFIC ISOTOPE ANALYSIS ($\delta^2\text{H}$, $\delta^{13}\text{C}$) OF BIOMASS COMPONENTS USING ANALYTICAL PYROLYSIS (PY-CSIA)

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Changes in climatic and environmental conditions can affect both, plant chemical and isotope composition. Nowadays, many studies use bulk isotope values, which represent a weighed mean average of the different plant compounds. An isotopic characterization of individual biogeochemical compounds is desirable in order to differentiate the isotopic composition of the main plant components. However, biomass is composed mainly of high MW biopolymers i.e. polysaccharides (celluloses), polypeptides (lignin), polypeptides (proteins), polyesters (waxes), etc. not amenable to most chromatographic techniques without the use of more or less previous intense extraction and sample preparation.

Here, a particular analytical pyrolysis technique combining Py-GC with a continuous flow isotope ratio mass spectrometer (IRMS) (Py-CSIA) is described and validated. Isotopic values obtained by Py-CSIA of standard *n*-alkanes mixtures (dissolved C₁₆ to C₃₀ series with increasing concentrations along three pentads, Indiana Univ. SIL mix. Type B), fitted well to a straight line ($R^2 > 0.999$). No induced thermal cracking nor deviations from the acclaimed isotope composition (fractionation) was observed up to high pyrolysis temperature (< 400 °C).

Results linking detailed molecular and isotope composition obtained by the direct analysis of various biomass types and biopolymers will be discussed. In general, our results show that Py-CSIA can reveal major differences between biomass components from different biogenic origin with high precision. In addition, despite that biomass pyrolysates from different origins may yield very similar Py-GC/MS patterns, Py-CSIA can make the difference providing additional valuable isotopic information.

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O20. OCCURRENCE OF PHARMACEUTICALS AND TOXIC ELEMENTS IN WELL WATER

SAMPLES COLLECTED IN GALICIA

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Groundwater has always been very important in Galicia not only because it has socioeconomic and ecological functions but also, because in many cases it has mineralization and hydrotherapy qualities. Anthropogenic contamination can occur via wastewater, agricultural and livestock activities. Therefore, as has been shown in Canada, EEUU and other countries emerging contaminants such as pharmaceuticals employed in human and veterinary medicine are susceptible of been present in Galician groundwater. The concerns of these contaminants are based on problems due to long-term accumulation and promotion of continual adverse effects to non-target organism. On the other hand, heavy metals are a group of elements that are toxics to humans and theirs mayor problems is bioaccumulation, therefore maximum level of these elements in drinking water are as lows as $\mu\text{g/L}$ level. Like for pharmaceuticals, the concentration of toxic elements in groundwater can increases due to human activity (industrial contamination, lixiviation of residual water, lixiviation of fertilizer) and also due to the presence deposit nearby the aquifer.

The objective of this research work was to investigate the presence of 22 pharmaceuticals and ten toxic elements in Galician well water samples. Pharmaceuticals were extracted from the water by solid-phase extraction (SPE) and identified and quantified by HPLC-MS/MS and the toxic elements (Cr, Mn Fe, Cu, Zn, As, Se, Cd, Hg, Pb) directly into an ICP-MS.

This study demonstrates that pharmaceuticals, in particular, antimicrobials are present in the Galician subterranean water system and toxic elements such as Mn were above the maximum permitted level for drinking water.

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O21. HIGH SENSITIVITY APPLICATIONS IN HIGH RESOLUTION MS QTOF

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In this work, we will present new data focused on priority pollutant as PCB's and PCDD's in food matrices, focused in fish tissue. The novel approach includes the GC-APCI-HRMS QTOF interface, with parameter optimization and quantification in real samples. The high resolution and selectivity of this technology allows obtaining excellent results even in very complex matrices, being able to differentiate in very complex compound families as PCB's and PCDD's, with a high interest nowadays in food safety and quality control. All the results obtained permit to strictly comply with the actual regulation for food in terms of precision and LOD.



O22. DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN PLANT MATERIAL USING SFC-MS/MS

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Pyrrrolizidine alkaloids (PAs) are potentially carcinogenic plant metabolites. They occur mainly in plants of the Boraginaceae, Asteraceae and Fabaceae families. They contain a pyrrolizidine core and make up a large group of heterocyclic alkaloids mainly derived from the 4 Necin bases platynecine, retronecine, heliotridin and ontonecin. PAs are hepatotoxic if they carry a 1,2-double bond as well as an esterified side chain which is a structural prerequisite for their hepatic activation. Exposure to PAs in food, beverages or phytopharmaceuticals, is a possible long-term concern for human health. Based on available data the Panel on Contaminants in the Food Chain (CONTAM) have proposed a list of PAs to be monitored in foodstuffs.

Since some of the analytes are isomers that can't be distinguished by different mass, they have to be separated chromatographically. LC-MS/MS is the standard method for determination of PAs. However, separation of these compounds often poses a challenge. SFC offers complementary chromatographic selectivity to RP-LC and an advantage for separation of stereoisomers, shown in the development of a separation method for determination of 34 PAs including 5 Lycopsamin and 2 Senecionin isomers.



**O23. QUANTITATION AND NON-TARGET DETECTION OF PESTICIDES IN SPINACH EXTRACT
WITH PEGASUS BT 4D. IMPROVEMENT TO TARGETED & UNTARGETED PESTICIDE RESIDUE
ANALYSIS: FAST AND FLEXIBLE ANALYTE FINDING FOR GC-MS AND GCXGC-MS**

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Accurate detection, identification and quantitation of compounds in high matrix food extracts often proves challenging even to experienced analysts. This work becomes more challenging as limits of detection (LODs) are constantly driven lower by regulatory agencies while they simultaneously increase the number and types of compounds that must be targeted. Selected ion monitoring and MS/MS techniques can help mitigate matrix interferences but may not be selective enough for all compounds in the most challenging matrices. Furthermore, these types of targeted analysis techniques remove the possibility for retrospective non-target analysis of the data, preventing analysts from detecting new or emerging contaminants. In contrast, comprehensive two-dimensional gas chromatography (GC×GC) dramatically improves chromatographic resolution of analytes within a sample often completely separating target compounds from would-be matrix interferences. Additionally, new time of flight mass spectrometers (TOF-MS) allow for full scan collection at SIM level sensitivities obviating the need for quadrupole based systems. In this article, we demonstrate the use of GC×GC TOF-MS as a methodology to combat matrix interferences, quickly target and quantify suspected contaminants while still allowing non-target analyte detection in a single sample injection.



O24. MINIATURIZED SOLID-PHASE EXTRACTION FOLLOW BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF UV FILTERS IN WATER

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UV filters are a class of emerging pollutants that are mainly intended to protect the skin against solar radiation. They enter the environment through aquatic activities or indirectly with domestic discharges. Many of these compounds are lipophilic, therefore they can bioaccumulate and biomagnify through the food chain, and they also cause adverse effects like estrogenic activity on biota and human beings. Consequently, the monitoring methods for UV filters in the environment is of vital importance. In addition, a sample preparation is normally required to isolate and pre-concentrate the target analytes from sample matrices prior to instrumental analysis. In this study, a metal-organic framework material (MIL-101) was developed as an effective miniaturized solid-phase extraction (SPE) adsorbent. The MIL-101 adsorbent was packed into a polypropylene cartridge and connected at the outlet tip with the vacuum manifolds allowing process up to 12-port SPE samples simultaneously. The developed adsorbent was used for the extraction and enrichment of 11 UV filters in water samples. The determination of extracted UV filters was quantified by gas chromatography-tandem mass spectrometry (GC-MS/MS). Several parameters affecting the extraction efficiency of the target analytes, i.e. desorption conditions, sample pH, the addition of salt and sample volume were optimized by statistical analysis. Under the optimal extraction conditions, the SPE-GC-MS/MS method provided good linearity ($R^2 \geq 0.9973$) and the limit of detections were in the range of 1.0 - 11.7 ng L⁻¹. This developed method was successfully applied for the extraction of UV filters in different type of water samples, including lake, river, seawater and swimming pool with satisfactory recovery from 82 to 105 % and a relative standard deviation of less than 10 %.

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O25. EVOLUTION AND DEVELOPMENTS OF PROTON TRANSFER REACTION-MASS SPECTROMETRY FOR SECURITY APPLICATIONS

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Proton Transfer Reaction-Mass Spectrometry (PTR-MS) is a broad-based technique that has proved its analytical use for fast trace explosives detection.^{1,2} It has been almost a decade since the first publication for explosives detection came to light.¹ From that moment on, the PTR-MS technique has developed into a more multidimensional technique, overcoming the challenges for achieving fast, selective and sensitive detection of threat agents for security applications. For such a journey to be successful, hardware developments had been necessary. Among these, this talk will revise the developments of a)- new inlet unit based on commercial swabs allowing no (or minimum) memory effects; and new methodologies for improving selectivity, based in enhancing collisional induced dissociation in a controlled way by manipulating the ion-molecule chemistry within the drift tube of a PTR-MS, through b)- the use of a radio frequency ion-funnel (RFIF) DT,³ and c)-rapid switching (less than a microsecond at a frequency of 0.1-10 Hz) of the reduced electric field.⁴ All these improvements translate into allowing identification of a compound of interest with higher specificity in complex chemical environments.

Finally, all these recent developments have been combined and recently applied to the detection of organic additives used in smokeless powders,⁵ expanding thus the range of compounds and applications where PTR-MS can be used as the detection tool.

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O26. DEVELOPMENT AND APPLICATION OF A CYCLIC ION MOBILITY MASS SPECTROMETER

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Introduction

Over the past decade the use of ion-mobility mass-spectrometry (IM-MS) has rapidly expanded with the technique increasingly being used in more routine applications, including structural elucidation. Despite the increased adoption of the technique, until relatively recently improvements in IM resolution have been modest, limiting the separation of compounds with similar mobilities. We will discuss the development and application of a high-resolution cyclic ion mobility enabled mass spectrometer and the application to a variety of structural elucidation studies.

Methods and Results

Studies were performed on a SELECT SERIES Cyclic IMS. The instrument is characterised by the ability to perform high resolution ion mobility separations using a 98cm circular TWAVE device with multi-pass capability. In addition, a multi-functional ion entry/exit array allows multiple rounds of mobility selection and separation (IMSⁿ).

We present a variety of examples that demonstrate the benefits of this novel technology including; the use of high ion mobility resolution to characterise small molecule, oligosaccharide and peptide isomers and to perform high peak capacity separations of mixtures. We will specifically highlight the use of the flexible instrument geometry to perform multi-stage separations incorporating IMSⁿ.

Sequencing of oligosaccharides is challenging because they are made up of isobaric units. High resolution mobility separation of isomeric precursor ions followed by dissociation and further separation of products has allowed identification of anomeric and open ring forms of pentasaccharides. For crude oil analysis, we describe an ion 'enrichment' method in which mobility selection is performed on ions of interest and all others are discarded. Multiple rounds of this experiment are performed to increase selectivity and were coupled with collisional dissociation to provide insights into structural motifs. Finally, we demonstrate how Cyclic IMS enhances the analysis of native protein collision-induced unfolding pathways experiments.

Conclusions

The Cyclic IMS mass spectrometer is a flexible research platform for the in-depth structural elucidation of a range of important samples from oils to biopharmaceuticals. The unique instrument geometry, and the capability to perform high resolution mobility separations and the unique IMSⁿ functionality provide many possibilities for detailed studies



O27. DOES YOUR DOG HAVE ANXIETY AFTER A ROUGH DAY AT THE MOUNTAIN: ANALYSIS OF CBD EXTRACTS FOR DOGS TREAT

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Recent legalization of cannabis, in some US states, has led to the promotion of cannabidiols (CBDs) as a supplement for various ailments. CBDs are usually extracted from the flowers and buds of cannabis or hemp, without the intoxicating or psychoactive chemical tetrahydrocannabinol (THC). Many of the implied benefits: reducing anxiety, anti-inflammatory, anti-depressant and sleep-aid, have been extensively tested only in animal trials. Over 14.4 B\$ was spent on pet supplies/OTC medicine, and pet owners are looking for natural alternatives. Many pet owners are supplementing their pet's diet with products derive from legal hems extractions. Due to the differences in extraction techniques and phenotypical difference between hemp strains, an untargeted chemical analysis approach is needed to know the composition of the oil supplements. The data presented in this work illustrates the analytical capability of an accurate mass high resolution GCQTOF with low energy EI and Chemical ionization functionality (Fig 1) to help with unknown components detection and increased identification confidence to provide detailed information on specific CBD extracts.



Figure 1.- Agilent 7250 GC/QTOF

High resolving power, accurate mass and Low eV provided additional information to a difficult untargeted analysis, on this work we could conclude:

- Feature finding reproducibility provides a reliable differential analysis
- Most of the samples treated were significantly different based of unique components and concentration of specific CBDs
- Low eV provided additional information and confirmation for fragile molecules.
- High resolving power quantification provided excellent results for complex samples.

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YOUNG SCIENTIST ORAL COMMUNICATIONS



OY1. SYNTHESIS AND CHARACTERIZATION OF ISOTOPICALLY LABELLED ¹⁵N-3-MONOIODOTYROSINE AND ¹³C-3,5-DIIODOTYROSINE FOR THEIR USE AS INTERNAL STANDARDS IN URINE ANALYSIS BY LC-ESI-MS/MS

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Iodotyrosine Deiodinase Deficiency (ITDD) is a type of Congenital Hypothyroidism (CH). Inclusion of ITDD in newborn screening programs could prevent growth failure and permanent intellectual disability. Unfortunately, ITDD cannot be easily diagnosed at neonatal stage. The early diagnosis of this endocrine disorder requires the determination of the thyroid hormone metabolites mono- and diiodotyrosine (MIT and DIT, respectively) but this determination is not implemented in a routine clinical basis as it presents serious technical and practical difficulties [1].

LC-ESI-MS/MS is considered one of the gold standard techniques for the identification and quantification of metabolites in clinical samples. [2]. However, a relevant problem with the use of ESI source is the signal variabilities due to matrix effects which affect the instrumental sensitivity and the accuracy and precision. The most efficient strategy to overcome matrix effects is standardization based on the application of IDMS using stable isotopically labeled standards. Unfortunately, isotopically labelled MIT and DIT are not commercially available.

Therefore, we present here a simple approach to synthesize isotopically labelled ¹⁵N-MIT and ¹³C-DIT and their further purification and characterization in terms of stability, purity, isotopic enrichment and concentration by LC-ESI-MS/MS. A double spike isotope dilution methodology will be developed to accurately quantify MIT and DIT in human urine. This approach allows the correction of interconversions and/or degradations during sample preparation. This is the first attempt for the future development of a methodology capable of quantifying these metabolites in blood or urine samples from neonates collected in filter-paper.

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OY2. SUSTAINABLE EXTRACTION AND CHARACTERIZATION OF BIOACTIVE PEPTIDES AND POLYPHENOLS FROM BREWER'S SPENT GRAIN: EVALUATION OF SYNERGIC EFFECTS

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Brewer's spent grain (BSG), a brewing industry by-product resulting after malting of barley, is a protein-rich material mainly used as animal feed [1-2]. However, BSG is a source of value-added compounds that are irretrievably lost. The exploiting of this by-product requires the development of sustainable extraction strategies.

The aim of this work was to develop green strategies using ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) for obtaining bioactive extracts from BSG, to evaluate their bioavailability, and to identify peptides and polyphenols by HPLC-MS/MS. Regarding UAE, ultrasound amplitude and extraction temperature and time were optimized to obtain the highest protein content. In the case of PLE, extraction solvent, temperature, and number and time of static cycles were optimized for obtaining an extract with the highest protein content and/or the highest polyphenols content and antioxidant activity. Extracts obtained under optimal conditions were submitted to simulated gastrointestinal digestion and all hydrolysates were assayed for antioxidant, ACE-inhibitory, and hypocholesterolemic capacities. Comparison of bioactivities of extracts and hydrolysates enabled to evaluate potential synergic effects between bioactive peptides and polyphenols. All hydrolysates were analyzed by HPLC-QTOF-MS/MS to identify peptides and polyphenols responsible for these bioactivities.

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OY3. VOLATOLOMICS APPROACH FOR THE IDENTIFICATION OF PUP PHEROMONES PROMOTING MATERNAL CARE IN MICE

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Untargeted metabolomics aims to classify between different groups of samples based on the metabolomic profile that change in response to external/internal perturbations, with the ultimate goal to identify discriminant markers. When the study is focused on the volatile part of the metabolome it is called Volatolomics [1]. GC coupled with mass spectrometry (MS) has been widely used for the volatolomic studies in the last years. Furthermore, the use of a thermal desorption unit (TDU) or the arrival of Ion Mobility (IM) separator to the GC-MS instruments enhance remarkably the sensitivity, selectivity and elucidation power of this combination. In this work, volatolomics was applied to the study of the volatile compounds released by mice pups at ages 3-24 days. The aim was to find out volatile pup biomarkers that could induce the maternal behaviour in the dam and to investigate how their secretion changes with time. Dams take care of young pups (even alien ones), whereas old pups (15-26 days old) are progressively rejected until weaning [2]. Therefore, identification of compounds of the volatolome of young pups (3-10 days old) that change with age seems a good strategy to investigate the identity of pup pheromones involved in mother-infant communication, e.g. induction of maternal pup care. For this purpose, 66 mice pups of both sexes were used for volatolome extraction at ages 4 to 24 days, using a purge and trap system. A total of 72 extraction were performed from groups of 3-6 pups of the same age (6 extractions per each postnatal day evaluated). Extracts were analysed by GC-TDU-MS and GC-APCI-IM-HRMS. Data were processed with *PARADISE* and *Progenesis Q1*, respectively. Then, *EZinfo* was used for the multivariate statistical analysis to highlight potential pup pheromones, starting by a PCA and followed by two types of supervised analysis (PLS-DA and OPLS-DA), which consider additional information about the groups. The last step was putative pheromone elucidation.

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OY4. DETERMINATION OF SYNTHETIC CATHINONES IN MECONIUM BY LC-MS/MS

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Introduction: New psychoactive substances, including synthetic cathinones, are not controlled by the international law, so they are used to replicate the effects of outlawed drugs. In Spain, up to 0.7% of women on childbearing age admitted the use of these drugs [1], and their consumption during pregnancy has been associated with neonatal complications [2].

Aim: To development and validate a method for the determination of common synthetic cathinones (methyldone, methedrone, mephedrone, 3,4-methylenedioxypropylone [MDPV], 4-fluoromethamphetamine and 4-fluoromethcathinone) in meconium using LC-MS/MS.

Methodology: 2 mL methanol were added to 0.25 g meconium, and sonicated for 30 min. After centrifugation, the sample was extracted with Oasis MCX columns. Chromatographic separation was achieved using an Atlantis T3 column (3 µm, 2.1x50 mm) and a gradient with acetonitrile and 0.1% formic acid. The mass spectrometer was a Quattro Micro™ API ESCI triple quadrupole, operating in electrospray in positive mode (ESI+). The method was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) recommendations [3]. The method was subsequently applied to 28 real meconium specimens that previously tested positive for other drugs of abuse.

Results: Total chromatographic run time was 12 minutes. The method was quantitatively validated for all the compounds, except for methedrone and fluoromethcathinone, which were qualitatively validated. Limits of detection were 0.5-1 ng/g, and limits of quantification 1-2 ng/g. None of the synthetic cathinones were detected in the selected real cases.

Conclusion: A LC-MS/MS method was developed and successfully validated for the determination of synthetic cathinones in meconium. Its application to real samples can contribute to provide data on the prevalence of these drugs among pregnant women.

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OY5. STATE-OF-THE-ART OF ANALYTICAL METHODS FOR AFLATOXIN HUMAN BIOMONITORING

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Aflatoxins (Afs) are highly toxic secondary metabolites produced by fungi of *Aspergillus* species [1,2]. Human exposure to Afs occurs mostly through contaminated food intake, although inhalation of contaminated airborne dust is also a possible route of exposure [3]. Measurement of their biomarkers in human fluids is a useful way of assessing human exposure to these mycotoxins. The aim of this work is to provide an overview of worldwide analytical methodologies employed in the analysis of Afs and/or its metabolites in human biomonitoring studies reported in the last 10 years. This review summarizes the broad range of techniques involving Afs determination in various human biological fluids (urine, plasma, serum and breast milk). Relevant data on limits of detection, sample preparation methodologies and separation and detection techniques that have been applied for the determination and quantification of Afs are included herein. Briefly, HPLC methodology coupled to different detectors, especially MS/MS, has been considered the most useful analytical technique. AFB1, B2, G1, G2 and M1 have been the most analyzed biomarkers for Afs in urine samples. Liquid-liquid extraction (LLE), Immunoaffinity columns or combinations of LLE-Solid phase extraction (SPE) approaches have been used as sample preparation procedures; however, direct injection of urine extracts based on “dilute and shoot” methodology has also been employed. The biomarkers for Afs detection in plasma samples are their adducts, especially Afs-lysine. For Afs-lys detection, most of the articles proposed a digestion with enzymes and a purification step by SPE before being analyzed with HPLC coupled to FLD, MS/MS or HRMS detectors. AFM1 was the most common studied biomarker in breast milk samples.

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OY6. ANALYTICAL CONSIDERATIONS FOR THE LC-MS/MS DETERMINATION OF ENDOGENOUS STEROIDS

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Introduction: The simultaneous determination of a broad panel of steroids (steroidome) provides more accurate information about the hormonal status of a patient than the detection of a single hormone. However, although clearly relevant, the steroidome determination by LC-MS/MS remains an analytical challenge due to several aspects such as (i) the large number of structurally similar compounds, (ii) the need of chromatographically resolve several pairs of isomers separation and (iii) the lack of easily ionizable moieties in steroids. The optimization of LC-MS/MS parameters is the bottleneck for developing reliable method for the steroids determination.

Objective: The main objective of the present work is to provide information about the critical LC-MS/MS parameters for the comprehensive determination of the steroidome.

Methods: The relevance of several parameters in the determination of steroids was evaluated. On the one hand, the effect of stationary phase and mobile phase composition were tested. On the other hand, MS parameters were evaluated in terms of ESI ionization (usefulness of the different species and effect of gas temperature) and MS/MS fragmentation (specificity of the transitions).

Results: Our results show that stationary phase is a key factor in the isomers separation (e.g. convencional BEH C18 stationary phases do not adequately separate androsterone and etiocholanolone and alternative stationary phases must be tested) and in the peak shape of steroids conjugated as sulfates. The mobile phase composition is also crucial in obtaining a correct peak shape of anionic steroids (e.g. sulfates) and in favoring the ionization or poorly ionizable steroids (e.g. the presence of fluorine in mobile phase favors the ionization of estrogens). The ESI importance in the ionization of steroids must not be dismissed. In fact, both the configuration of ESI and the desolvation gas temperature determine the species formed in the ion source ([M+H]⁺, [M+NH₄]⁺, [M+H-H₂O]⁺, etc). Furthermore, the careful selection of specific transitions is required to discern between isobaric steroids that only differ on some specific fragments (e.g. the specific m/z 151 fragment of 5 α -THB not present in 5 β -THB).

Conclusions: Our results show the great importance in the selection of LC-MS/MS conditions in the proper determination of steroids. Unfortunately, the steroids determination by LC-MS/MS is not universal since the selection of optimum parameters for some steroids would hamper the detection of others. Thus, although the steroidome determination of a few numbers of steroids can be relatively easy, the inclusion of a large number of steroids requires the selection of compromise parameters. For this reason, the development of comprehensive methodologies for the evaluation of steroidome must be guided by different aspects such as the matrix and the main hypothesis of the research.



OY7. A LC-MS/MS METHOD FOR DETERMINATION OF ANTIPSYCHOTIC DRUGS IN NAILS

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Introduction: Keratinized matrices, like hair and nails, have a wide window of detection, compared to other biological samples¹. Hair is commonly used for long-term monitoring of drugs of abuse in different fields, but there are still few studies about the usefulness of nails, and its application to toxicological studies is very limited².

Objectives: To develop and validate an analytical method for the detection of 5 antipsychotic drugs (clozapine, haloperidol, levomepromazine, olanzapine and quetiapine) in nails, and to apply it to paired finger- and toenail samples in order to compare concentrations in both samples.

Methodology: Thirty milligrams of nails were washed with dichloromethane, dried and pulverized in a ball mill. After incubation with 1.5mL Water:Acetonitrile (50:50, v:v), samples were purified by solid phase extraction with Oasis MCX cartridges (3cc, 60mg) and analyzed by LC-MS/MS. Paired finger- and toenail samples were obtained from 5 patients under chronic treatment with olanzapine and quetiapine.

Results: The developed method was successfully validated for the following parameters: linearity (from 10 to 10000pg/mg), selectivity (no interferences), limit of detection (2.5pg/mg), limit of quantification (10pg/mg), accuracy (96.7-106.9%), imprecision (<8%), autosampler stability (%difference <13%), matrix effect (-35.6 to 268%), recovery (62.3-109.8%) and process efficiency (47.9-404.6%). Four fingernail and four toenail samples were positive for quetiapine (77.5-1150.2 pg/mg in fingernails and 23.6-1316.2 pg/mg in toenails), and two for olanzapine (40.1-106.9 pg/mg in fingernails and 30.0-553.9 pg/mg in toenails).

Conclusion: The method was successfully developed and validated, and its applicability was demonstrated by the analysis of paired fingernail and toenail samples.

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OY8. MULTICLASS METHOD FOR THE DETERMINATION OF ENDOCRINE DISRUPTING CHEMICALS IN HUMAN NAILS USING ALKALINE DIGESTION PRIOR TO ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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The exposition of humans to new chemicals has increased progressively due to rapid industrial development. These emerging contaminants are usually present in the environment, foods, beverages, and personal care products. Some of these emerging contaminants interfere with the normal functioning of hormones and are widely known as endocrine disrupting chemicals (EDCs) [1,2]. The present work validates a new analytical method to determinate 19 EDCs in human nail samples. In contrast to other common biological samples, nail sampling is non-invasive and since they take several months to grow out, they are well suited for measuring and reflecting the cumulative exposure to harmful substances in the long term [3,4]. The method is based on the digestion of the samples with a basic solution of sodium hydroxide (0.04 M) followed by analysis by UHPLC-ESI-MS/MS in SRM mode. Multivariate optimization strategies were used for the optimization of the parameters that affects the digestion procedure. The compounds were separated in 10 min. The validation was developed using a matrix-matched calibration and a recovery assay with spiked samples. After validation, the method was successfully applied for the analysis of compounds in samples of human nail from volunteers. All samples tested positive for several of the analyzed EDCs.

Acknowledgement

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OY9. DETERMINATION OF SYNTHETIC CATHINONES IN ORAL FLUID BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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The consumption of new psychoactive substances (NPS) is increasing nowadays. An important type of NPS are synthetic cathinones, commonly known as “bath salts” which can be easily obtained in the market [1]. Their analysis has become a great concern and in this sense, different methods have been developed to determine these compounds in biological samples [2]. The main advantages of oral fluid (OF) are the simplicity and non-invasiveness of sample collection. The observed sampling difficult the possible adulteration of the analysis. Moreover, OF allows the drug detection between the first 24 and 48 hours [3].

Even the advantages of OF, there are limited literature focused on the analysis of this kind of biological samples for cathinone determination. To extend the bibliography in this field, the aim of this project was to determine a group of synthetic cathinones by using a commercial device collector (Salivette®) by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The saliva was collected by using a Salivette® device and the extraction parameters were optimised. In this sense, the desorption of the analytes from the device was achieved by centrifugation using only 2 mL of organic solvent. Finally, the extract was evaporated, reconstituted and analysed by LC-MS/MS. Thus, a fast, easy and cheap method is presented for the determination of synthetic cathinones in OF samples.

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OY10. SIMPLE AND EFFECTIVE METHOD FOR THE DETERMINATION OF NEONICOTINOID RESIDUES IN HONEYS AND CEREALS BY CAPILLARY LIQUID CHROMATOGRAPHY

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Neonicotinoids (NNIs) are systemic insecticides easily incorporated into the plant tissues and translocated to all parts of the plant, staying for a long time after application. They represent a risk to wild bees and honeybees, being related with the Colony Collapse Disorder (CCD) syndrome, which is characterized by a rapid loss of adult worker bees. For this reason, in this communication a novel method based on capillary liquid chromatography (CLC) with UV-diode array detection (DAD) has been developed for the determination of seven NNIs (imidacloprid, thiacloprid, clothianidin, thiamethoxam, acetamiprid, nitenpyram and dinotefuran) in honey and cereals. CLC shows several advantages compared to analytical HPLC, such a lower limits of detection, better resolution and lower solvent consumption. In this case, separation was achieved in less than 20 min at 25 °C, using a Zorbax XDB-C18 capillary column (15 mm x 0.5 i.d, 5 µm) with a mobile phase consisting of water/acetonitrile in gradient mode. Two different sample treatments were developed. For honey samples dispersive liquid-liquid microextraction (DLLME) was optimized using acetonitrile and dichloromethane as disperser and extraction solvents, respectively, in presence of MgSO₄ as salting-out agent. On the other hand, solid-liquid extraction (SLE) was used in grain samples employing a mixture of acetonitrile/dichloromethane as extractant. Good linearity was obtained in both cases, with LODs and LOQs lower than 6.6 µg/kg and 22 µg/kg respectively in honey, and lower than 7.5 µg/kg and 25 µg/kg in grain samples, allowing their quantification below the maximum residue limits (MRLs). Recoveries above 80 % for all studied analytes in different honeys (multiflower, orange tree, eucalyptus and rosemary) were achieved, as well as, in different cereals (maize, wheat, oat, barley and rice) showing that both proposed methods, DLLME-CLC-UV and SLE-CLC-UV, are a powerful, simple and green alternatives for the monitoring of NNIs in these matrixes.

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OY11. GC-MS CHARACTERISATION OF NOVEL PECTIC-OLIGOSACCHARIDES DERIVED FROM ARTICHOKE PECTIN USING MACHINE LEARNING AND COMPETITIVE FRAGMENTATION MODELLING (CFM-ID)

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Pectic oligosaccharides (POS) comprise a wide variety of structures containing different monomers including galacturonic acid (GalA), rhamnose (Rha), xylose (Xyl), arabinose (Ara) or galactose (Gal). To characterise these mixtures, several chromatographic techniques might be employed. GC-MS-EI is widely used in the field of metabolomics due to its reproducibility and simplicity. It generates massive amounts of high-dimensional data and standards of many oligosaccharides might not be commercially available. To interpret GC-MS-EI results, *in silico* fragmentation methods such as CFM-ID have been developed [1]. On the other hand, machine learning allows extracting chemically relevant information from complex GC-MS-EI spectra. Therefore, the purpose of this study was to characterise novel POS obtained from hydrolysis of artichoke pectin using different types of enzymes. With this aim, a GC-MS-EI data analysis strategy based on the combination of machine learning and *in silico* fragmentation to interpret these models is presented. First, POS were obtained from pectin using different glycosidases (Pectinex Olio, PO; Cellulase from *Aspergillus niger*, CEL; Pentopan, PEN) and then reaction mixtures were analysed by GC-MS-EI [2]. Spectra of di- and tri-POS were classified by determining structural patterns depending on the enzyme selected. Models used like random forests and artificial neural networks led to high prediction rates, showing accuracies over 95% on classification of new samples [2]. After, chemical structures of most influential m/z ions were calculated using an oligosaccharide *in silico* fragmentation library previously generated using CFM-ID. Some relevant ions in POS obtained with PO (m/z 340, 424 and 443) were originated from Rha- α (1,4)-GalA, GalA- α (1,2)-Rha or Xyl- α (1,3)-GalA (methylated/acetylated or not). Similarly, POS obtained with CEL showed high abundances of m/z 482, derived from GalA- α (1,2)-Rha (acetylated) and Xyl- α (1,3)-GalA (acetylated). In contrast, POS obtained with PEN showed high abundances of m/z 454 which could also be formed from POS containing Ara- α (1,4)-Rha. In conclusion, this methodology allows to tentatively predict some of the most probable POS structures that may be obtained from artichoke pectin using different enzymes.

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OY12. NEW PROCEDURE FOR THE SELECTIVE ISOLATION OF MERCAPTANS USING COPPER EXTRACTION. APPLICATION TO THE DETERMINATION OF THREE ULTRATRACE ODORANTS IN WINE BY DUAL GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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A new procedure for the selective isolation of neutral mercaptans based on copper has been developed. Using such method as base, a method for the quantitative analysis of polyfunctional mercaptans, such as 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (MHA) at levels of nanogram per liter in wine, without derivatization has been further developed.

Methods commonly applied to the selective isolation of mercaptans make use p-hydroxymercuribenzoate (p-HMB) or other toxic mercury agents and in any case, methods are tedious and require processing large volumes of samples and of solvents [1]. The new selective isolation procedure makes use of the ability of some copper salts to be strongly adsorbed in polymeric sorbents. The breakthrough volumes of the salt solutions and their stability on the sorbent were tested by spectrophotometrical determination of eluting copper. Once the bed is formed, aqueous or hydroalcoholic samples containing mercaptans can be further loaded ensuring complete retention of the mercaptans while there are copper nuclei available. Afterwards, neutrals mercaptans can be completely separated from polar and non-polar interferences by washing the cartridge with different organic solvents. The thiol-copper bonds are further cleaved by using aqueous cysteine and mercaptans can be quantitatively recovered in dichloromethane. Different aspects of the method are presented.

The usefulness of the procedure has been demonstrated by applying it to the quantitative analysis of underivatized wine polyfunctional mercaptans by GC-GC-MS at ng/L level.

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OY13. DETECTION AND QUANTITATION OF ALMOND ADULTERATIONS BY UHPLC-HRMS POLYPHENOLIC PROFILES

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Considering the complexity of the food chain in a globalized world, where many players are involved between production and consumption, food manipulation and adulteration practices are raising because of the easiness to conduct fraud that may remain undetected [1]. Nuts, which are food products worldwide consumed with important health benefits, are considered as highly exposed to fraudulent practices since they can be relabeled or replaced with cheaper ones, representing not only an economic deception but also a health risk. Their antioxidant properties can be directly attributed to their high content of polyphenolic compounds, which are a large family of aromatic secondary metabolites of plants [2].

In the present work, target UHPLC-HRMS polyphenolic profiles were proposed as chemical descriptors in order to detect and quantitate almond and almond-based bakery cream adulterations with peanut or hazelnut. Sample treatment consisted of a two-split procedure based on an extraction with acetone:H₂O 70:30 (v/v) followed by a defatting step with hexane. The chromatographic separation was reached with a Kinetex C18 (10 cm x 4.6 mm x 2.6 µm particle size) column in a 35-min run. Moreover, TraceFinderTM 3.3 EFS software was used in order to process data by means of a polyphenolic customized accurate mass database, while PLS_Toolbox 7.8.2 (Eigenvector Research) allowed the partial least squares regression (PLS) study. Satisfactory prediction errors were achieved by the built PLS models, demonstrating the suitability of the proposed method to determine adulterant percentages.

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OY14. DETERMINATION OF THE FOOD EXPOSURE TO BISPHENOL A AND ITS ANALOGS WITH OBESOGENIC ACTIVITY IN THE SCHOOL POPULATION

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Obesity is the main metabolic disease that affects the world population, it is associated with lifestyle and unhealthy dietary habits. However, recent research has shown that a wide variety of chemicals called obesogens (bisphenol A, phthalates and parabens among others) act by altering the endocrine system influencing adipogenesis and obesity. That is why, they begin to take into account other predisposing factors other than dietary habits and lifestyle such as these are obesogens [1]. This study aims determine the dietary exposure to BPA and analogous with obesogenic activity in the school population. The determination of bisphenols in food was carried out using a dispersive solid phase method for extraction of target compounds followed by UPLC-MS/MS analytical detection [2]. BPA was detected in most of the analyzed samples (1 to 409 ng g⁻¹), followed by BPB (1 to 22 ng g⁻¹). BPAF was detected in 12 samples, but could only be quantified in 1 sample (1 ng g⁻¹). BPS was detected in 3 samples (5 to 39 ng g⁻¹). BPF, BPE and BPP were not detected. In recent decades, the consumption of dishes made from packaged and precooked foods has been increased, thus increasing exposure to chemical contaminants such as BPA and its analogues. BPA is still the most abundant bisphenol found in food, but in turn we can see that its analogues are becoming powerful substitutes for it, since they have been detected in a significant number of food products.

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OY15. FABRIC PHASE SORPTIVE EXTRACTION FOR THE DETERMINATION OF FUNGICIDES IN ENVIRONMENTAL WATERS BY GC-MS/MS

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Fungicides are a type of pesticides usually used in viticulture to avoid fungi infection such as grey rot (*Botrytis cinerea*), mildew (*Plasmopara viti-cola*) and oidium (*Uncinula necator*)^{1,2}. The continuous application of fungicides induces their entrance into the environment, especially into the aquatic environment. Indeed, in groundwater and superficial waters, these compounds are considered to pose a risk to the environment as well as to human health³. A method based on FPSE (Fabric Phase Sorptive Extraction) followed by gas chromatography-tandem mass spectrometry (GC-MS/MS) has been developed for the simultaneous determination of seventeen fungicides in water. Some preliminary parameters were evaluated (elution solvent, volume of solvent, type of phase...) and then the method was optimized applying an experimental design. In addition, the method was validated showing good linearity, precision and recoveries. Finally, real water samples were analysed for the quantification of the 17 target. Metalaxyl and folpet were among the most detected compounds due to their usual application in Galicia's viticulture.

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OY16. A HIGH-SENSITIVITY METHOD FOR ANALYSIS OF A MIXTURE OF RELEVANT ANTHROPOGENIC EMERGING ORGANIC CONTAMINANTS IN WATERS FROM REMOTE AREAS

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The aim of this work was to develop a high-sensitivity analytical method to determine anthropogenic organic contaminants of emerging concern in water samples collected in Antarctica. In order to maximize the method sensitivity an on-line solid phase extraction (on-line SPE) approach was selected because this technology allows transferring the total extraction volume into the detection system. Analyte detection was carried out in the selected reaction monitoring mode using liquid chromatography coupled to electrospray tandem mass spectrometry (LC-ESI-MS/MS). The list of selected compounds included pharmaceuticals (acetaminophen, bezafibrate, diclofenac, ibuprofen, hydrochlorothiazide, citalopram, fluoxetine), antibiotics (azithromycin, clarithromycin), stimulants (caffeine, nicotine), UV filters (benzophenone-1, benzophenone-3), one artificial sweetener (acesulfame), and industrial pollutants (the alkylphenolic compounds nonylphenol, and the anticorrosive tolyltriazole). During method optimization different extraction sorbents were evaluated to maximize recovery and several organic gradients and mobile phase compositions were tested to obtain good peak separation and maximize peak signal.

The optimized approach was validated in terms of linearity, recovery, repeatability, sensitivity, and matrix effects. Finally, it was applied to various water samples collected in the Antarctic Peninsula in order to assess the environmental impact caused by humans in that remote region. Levels found were comparable to those reported in previous studies [1].

Acknowledgement This work has been financially supported by the Government of Catalonia (Consolidated Research Group 2017 SGR 1404-Water and Soil Quality Unit).

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OY17. UHPLC-API-MS/MS FOR THE DETERMINATION OF AZO-DYES IN RED SPICES

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A wide range of synthetic dyes is used in food industry to change the food color or to correct it with the aim of increasing the quality or the variety of food products. Unfortunately, dyes are sometimes used to commit fraud, which may represent not only an economical problem but also a human health risk. For instance, the use of azo-dyes as food additive is banned due to human health risk, but they have been detected in spices such as chilli, curcuma and curry.

Generally, LC-UV and LC-MS (electrospray ionization) are the choice techniques for the determination of azo-dyes. Nevertheless, LC-UV offers a low selectivity, while LC-ESI-MS does not provide enough sensitivity for the analysis of these compounds in food products. In this work, the ionization of different azo-dyes using atmospheric pressure chemical ionization (APCI) and photoionization (APPI) are evaluated to develop a sensitive ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for their determination in spice samples that could have been adulterated with the addition of banned azo-dyes.

The proposed method achieves the chromatographic separation in less than 8 min using an Accucore C18 column (100 mm × 2.1 mm id., 2.6 μm particle size), a binary gradient elution (acetonitrile:aqueous buffer, formic acid/ammonium formate, 20 mM, pH 3.75) and a mobile phase flow-rate of 600 μL min⁻¹. All the studied compounds are ionized in positive ion mode yielding the protonated molecule [M+H]⁺ as base peak of the mass spectrum, although Para Red shows better response in negative ion mode by generating the deprotonated molecule [M-H]⁻. Moreover, tandem mass spectrometry is used to obtain structural information for further confirmation of these analytes and to improve selectivity and sensitivity. In addition, a simple and fast solid-liquid extraction is proposed to extract the target compounds from the red spices samples before the UHPLC-MS/MS analysis. The proposed method shows satisfactory quality parameters such as low limits of detection (0.01 – 0.06 mg L⁻¹), good precision (RSD% <10%), high extraction efficiencies (>85%), low matrix effects (<15%) and accurate quantification (relative error % <10%) by using matrix-matched calibration method.

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OY18. GC-MS ANALYSIS OF SIX LIPID PEROXIDATION PRODUCTS WITH DNPH DERIVATIZATION USING A NEW GDME-DLLME PROCEDURE

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Lipid peroxidation is the most aggressive degradation reaction that naturally occurs in oils and oil-based foodstuffs. Because of the exposure to heat, temperature, light, time of exposure, and others, polyunsaturated fatty acids (PUFAS) are submitted to a series of chemical transformation that lead to the formation of secondary peroxidation products such as Malondialdehyde (MDA) and Acrolein (ACRL), that not only modify organoleptic properties but also may cause a health damage due to its toxicological activities due to their capability of DNA adducts formation that lead in mutation [1-3].

Since there is not sufficient analytical data available MDA has been considered as not classifiable as to its carcinogenicity to humans (group 3) by the IARC [4], and only ACRL has a TDI established at the level of 7.5 $\mu\text{g}\cdot\text{kg}^{-1}$ of b. w. per day by the International Program of Chemical Safety (IPCS) in conjunction with members of the United Nations Environment Program (UNEP), the International Labor Organization (ILO) and the World Health Organization (WHO) [5].

Here is present a new analytical method for the GC-MS simultaneous analysis of MDA, ACRL, formaldehyde, acetaldehyde, propanal and pentanal in edible oils using a fast and simple GDME-DLLME procedure with *in-situ* DNPH derivatization.

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OY19. ATMOSPHERIC PRESSURE PHOTOIONIZATION FOR GC-HRMS ANALYSIS OF PCDD/Fs AND DIOXIN-LIKE PCBs IN FOOD AND ENVIRONMENTAL SAMPLES

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Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and *dioxin-like* polychlorinated biphenyls (dl-PCBs) involve a wide group organic pollutants of great concern due to their high toxicity and persistence in the environment. Although in the last decades their emissions have been reduced significantly due to the application of stricter environmental regulations [1], their presence in the environment continues to be the subject of large-scale monitoring programs to guarantee the health of human being and living organisms. Currently, gas chromatography coupled to high-resolution mass spectrometry is the technique of choice for the determination of the target compounds because it provides the required sensitivity and selectivity [2], assuring the quality of the results [3]. Recently, the use of the atmospheric pressure chemical ionization (APCI) in the GC-MS determination of environmental pollutants has shown to be a promising alternative to classical ionization techniques, but information about the real capability of the atmospheric pressure photoionization (APPI) is still limited.

In this work, a GC-APPI-HRMS (Orbitrap) method has been developed for the determination of PCDD/Fs and dl-PCBs in food and environmental samples. For this purpose, the APPI operating conditions and the use of different dopants were evaluated achieving the best results in the negative-ion mode using benzene for PCDD/Fs and diethyl ether for dl-PCBs. The developed method has been validated using certified reference materials and selected samples containing a wide range of concentration levels. The results have been compared with the reference GC-HRMS method demonstrating the good performance of the GC-APPI-HRMS method.

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OY20. POTENTIAL OF MICRO-LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PSYCHOACTIVE SUBSTANCES IN WASTEWATER

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Wastewater-based epidemiology (WBE) can give valuable information on the extent and actual use of new psychoactive substances (NPS) and illicit drugs. Solid-phase extraction followed by UHPLC-MS/MS is normally used for the determination of both illicit drugs and NPS in wastewater. However, recently developed micro liquid chromatographic techniques (μ LC) remained unexplored in this field. The reduced flow rate used in μ LC techniques not only enhances the chromatographic performance because of the higher ionization efficiency, but it also reduces the amount of organic solvents required for the analysis moving μ LC a step forward into a greener analytical chemistry. In this study, a detailed comparison of μ LC and UHPLC, both coupled to tandem mass spectrometry, in terms of sensitivity and reproducibility has been made in the application field of WBE. As expected, a significant increase was observed for sensitivity when comparing mass normalized data (average 14-fold). Also, the overall method performance resulted in an average increase sensitivity factor of 4.5 for μ LC-MS/MS. This is of particular interest for this type of analysis where the presence of NPS and illicit drugs is usually at very low concentrations. However, large deviations in retention time (up to 0.4 min) affected the reproducibility and robustness of the methodology when it was applied to wastewater analysis. Since many of the NPS do not have its isotopically labelled standard, the utilization of retention time as an identification parameter in this study becomes essential. Therefore, further developments need to be accomplished in order to achieve a retention time reproducibility enough to validate the methodology. Although in this work μ LC-MS/MS was strongly influenced by the amount of matrix loaded in the separation device, its enhanced sensitivity and promotion of green chemistry (faster analysis time and less solvent consumption) allow to expect improved future applications, especially when analytes are present at very low concentrations.

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OY21. HIGH THROUGHPUT ANALYSIS OF MEDIUM TO HIGHLY POLAR PESTICIDES IN SURFACE AND GROUNDWATER FROM AGRICULTURE-IMPACTED AREAS OF CATALONIA USING ON-LINE SPE-LC-MS/MS

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Water is an essential resource for humans and all living organisms. However, the availability of freshwater resources is threatened due to a constant increase of its demand by the growing world's population and its pollution by anthropic activities. Agriculture, apart from being a highly water demanding activity, is a diffuse source of pesticide pollution. After use, pesticides are likely to leach into groundwater or to be washed off into surface waters [1]. Considering this and the harmful properties of pesticides, the European Commission has established Environmental Quality Standards (EQS) for their presence in both groundwater (2006/118/EC) and surface water (2013/39/EC).

To increase the knowledge on regulated and non-regulated medium to highly polar pesticides in water resources, the present work aimed at developing and validating a fast and simple analytical methodology able to detect in a single run up to 52 of these substances in surface and ground waters. The validated methodology, based on an isotope dilution approach, allows quantification of these compounds at levels below EQS in the investigated matrices. Its main advantage over other analytical methods previously developed for the same purpose is the use of a fully automated approach based on solid phase extraction coupled in series with liquid chromatography – tandem mass spectrometry (on-line SPE-LC-MS/MS). This allows minimizing sample manipulation and increasing analytical throughput and method sensitivity and precision. The developed method was applied to the analysis of various water samples collected in two agriculture-impacted areas of Catalonia in order to evaluate the occurrence of the target pesticides and compliance with the EQS.

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OY22. ENANTIOMERIC DETERMINATION OF CATHINONES IN ENVIRONMENTAL WATER SAMPLES

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Enantiomeric determination of chiral drugs in the environment is of emerging concern in environmental field as their enantiomers often exhibit stereoselectivity in environmental occurrence, fate and toxicity [1]. In this study, a method based on liquid chromatography and high resolution mass spectrometry is developed, validated and applied for enantiomeric determination of 6 cathinones in effluent and river water. The enantioseparation was carried out using Chiralpak CBH column in reversed-phase mode, and optimized by evaluating the effects of mobile phase pH, organic modifier, buffer concentration and flow rate. Under the optimal conditions, good enantioseparations ($R_s \geq 1.0$) were achieved for all the analytes. For the analysis of environmental water samples, solid-phase extraction on Oasis WCX sorbent was used for sample clean-up and pre-concentration. It is worth noting that sample matrices can clearly influence the retention times of analytes. The method was validated with river water samples, and its overall performance was satisfactory [2].

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POSTERS



P1. ADVANCED MEMBRANE ASSISTED SOLVENT EXTRACTION (MASE) FOR ISOLATING NEW PSYCHOACTIVE SUBSTANCES FROM URINE BEFORE HPLC-MS/MS DETERMINATION

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The global market for new psychoactive substances (NPSs) continues to expand, and the range of drugs available on the market has probably never been wider. The data reported for 2016 show that one of the major identified NPSs were synthetic cathinones (SCs). SCs share certain parts of the molecular structure of amphetamine. The psychoactive power of these new substances is therefore similar, or even higher, than that exhibited by traditional drugs and they act as stimulating substances. However, these new substances have certain structural differences when compared to regulated traditional drugs; thus, they go unnoticed during routine toxicological-forensic analysis.

In the current communication, Membrane Assisted Solvent Extraction (MASE) has been novelty proposed for the simultaneous extraction of synthetic cathinones (SCs) from urine. MASE device consists of polypropylene (PP) non porous membranes. Isolated SCs were further determined by high performance liquid chromatography – tandem mass spectrometry (HPLC- MS/MS). Optimized sample pre-treatment procedure consists of using 400 μ L of hexane as an extracting solution placed inside the polypropylene (PP) membrane and 5 mL of urine (pH adjusted at 11.8) with a previous acondicioning step (NaF 0,15%). Extraction was assisted by ultrasounds system at room temperature for 40 min. After hexane extract evaporation to dryness, the residue was re-dissolved in 100 μ L of mobile phase, which leads to a pre-concentration factor of 50.

Method validation showed analytical recoveries between 80% and 120% for most SCs, and repeatability (inter-day and intra-day assays) with RSD values lower than 15%. The proposed method was found to be selective and sensitive and limits of quantification (LOQs) lower than 1 ng mL⁻¹ were achieved. Despite absence of SCs cut-off values, LOQs were lower than cut-off values established by the Food and Drug Administration (FDA) for traditional drugs. Finally the method was applied for several real samples.



P2. ANALYTICAL CHALLENGES ASSOCIATED TO ESSENTIAL OILS AND THEIR INDIVIDUAL COMPONENTS IN COSMETICS

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Plant extracts and essential oils are added to cosmetic products due to their well-recognized properties. Several studies have shown their efficacy [1,2] avoiding for the necessity of additional chemical preservatives. This is known as self-preservation. However, 28 natural extracts have been listed as contact allergens established in humans [3]. In addition, the individual chemical components also belong to the 82 declared allergenic substances [3] and, in general, their names and concentrations do not appear on product labels.

The objective of the proposed analytical approach is to develop appropriate methodologies (sample preparation and analytical determination using chromatographic techniques) with selective phytomarkers for each natural extract or essential oil in order to determine their presence in cosmetics.

This work shows the first experimental tests carried out. At first, a prior assessment of the allergen content is necessary in order to determine the real level of allergens in cosmetics. We search for possible marker substances in groups of essential oils/natural extracts according to their origin similarity (e.g. obtained from flowers), using gas chromatography methods with MS detectors. The ideal would be to achieve a systematic approach that facilitates common analytical developments, and then to further develop a multicomponent methodology that would allow the global determination of the main phytomarkers.

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P3. COMPARISON OF TWO CHEMOMETRIC APPROACHES FOR THE IDENTIFICATION BY LC-QTOF BASED UNTARGETED METABOLOMICS OF POTENTIAL PLASMA BIOMARKERS IN PEDIATRIC CHRONIC KIDNEY DISEASE

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The identification of plasma biomarker that would enable early diagnosis of Chronic Kidney Disease (CKD) in pediatric patients is essential in order to avoid irreversible kidney damage [1]. With this aim, LC-QTOF based untargeted metabolomics has been applied to plasma samples from healthy children and pediatric patients suffering from different degrees of CKD [2]. Concerning that different chemometric approaches used could affect the number of entities obtained, two different chemometric approaches have been used. Data analysis carried out by means of Mass Profiler Professional (MPP) software returned seven significant entities, while eight entities showed to be significant with Matlab R2015a software. Five of the entities were found to be coincident in both data analysis procedures. Four of these entities were fully identified as n-butyrylcarnitine, cis-4-decenoylcarnitine, bilirubin and sphingosine-1-phosphate. Even though the fifth metabolite could not be identified, taking into account the high polarity of the feature and its MS/MS spectra it is suggested that it could correspond to some kind of amino acid derivative similar to aminoadipic acid.

A partial least squares discriminant analysis model built with these five entities enabled to classify correctly 96% of the samples. A higher performance was obtained when considering only early CKD patients against controls, shedding new light on the early diagnosis of pediatric CKD.

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P4. TIME-SERIES PROTEOMIC STUDY OF DIABETIC NEPHROPATHY IN HUMAN PROXIMAL TUBULAR HK-2 CELLS BY UHPLC-ORBITRAP MS/MS

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Diabetic nephropathy is the leading cause of end-stage renal disease. This disease is derived from high glucose/hypoxia diabetic exposition, which can result in chronic loss of kidney function. It is now evident that proximal tubular cells play a critical role in the development of the disease. Therefore, a better understanding of the molecular mechanism involved in its development is needed. The aim of this work was to perform a time-series proteomic study to analyze the effect of high glucose/hypoxia induced changes in HK-2 cells. To achieve it, cells in control conditions for up to 48 h were compared to cells simultaneously exposed to high glucose (25 mM) and hypoxia (1% O₂). A relative protein quantification was performed using the dimethyl-labeling methodology in combination with UHPLC-Orbitrap high-resolution mass spectrometry analysis. Our results demonstrated that the simultaneous exposition to these conditions did not affect cell proliferation during the first 24 h, but it decreased the total number of cells after 48 h. Moreover, the proteomic analysis allowed the identification of an average of 317, 296 and 259 proteins at 5, 24 and 48 h, respectively. The multivariate analysis showed a good separation among the three time points, and the time expression profiles indicated that some proteins involved in the *Glycolysis* and the *Cytoskeletal regulation by Rho GTPase pathways* were increased and decreased, respectively, with time. In overall, our data suggest that some effects are specific for the combination of high glucose/hypoxia exposition.

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P5. ANNOTATION OF OXIDIZED PHOSPHOLIPIDS IN METABOLOMICS GUIDED CANCER STUDY

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Phospholipids (PLs) represent the major lipid constituents of cell membranes and perform an important role in many processes. Oxidative stress, which significantly affects the native phospholipids, generates hundreds of structurally diverse PL species¹. Some of these oxidized PLs (OxPLs) have an important range of different biological activities, and their alterations lead to dramatic consequences related to different types of pathologies or diseases such as cancer². With the aim of searching for biomarkers in a cancer metabolomics study, plasma samples from controls (n=68) and cases (n =77) (Hospital 12 de Octubre) were analyzed through a non-targeted LC-MS approach in positive and negative mode. Samples are deproteinized following plasma protocols and to characterize plasma analysis is carried out using LC-QTOF/MS from Agilent Technologies. After data treatment, an initial tentative identification based on the m/z of the compounds showing significant differences in class separation was performed by CEU Mass Mediator tool³. To confirm the identity of compounds, LC-MS/MS analysis were carried out repeating the experiment with the same chromatographic conditions to the primary analysis. As a result, thirty-one identified lipids were confirmed by LC-MS/MS and three of them were OxPLs. Metabolite identification in non-targeted metabolomics studies is an important bottleneck. In this study we apply a specific workflow to annotate oxidized lipids through their mass changes and fragmentation patterns in a manual MS/MS interpretation. Modifications that PLs suffer change their native characteristics regarding retention time and the shift in m/z of fatty acids, adducts, neutral losses and fragments formation¹. Due to a very limited availability of commercial standards and the lack of oxidized lipid spectra in the databases, a very profound study of the MS/MS spectra is mandatory. These findings will support the investigation of the role of oxidized lipids in the initiation and progress of cancer.

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P6. VISIBLE LIGHT PHOTOCATALYTIC DEGRADATION OF TRAZODONE USING DIFFERENT MODIFIED TITANATE NANOWIRES

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Heterogeneous photocatalysis is one of the most promising technologies for the removal of pollutants, since many of these compounds are not completely removed by conventional processes at wastewater treatment plants [1]. Catalysts based on nanomaterials have been used for the degradation and can be doped with metals to improve the performance of photocatalysis [2]. In this work, the photocatalytic activity of undoped titanate nanowires (undoped-TNW), cobalt modified nanowires (Co-TNW), iron modified titanate nanowires (Fe-TNW) and ruthenium modified nanowires (Ru-TNW) was evaluated for the removal of the antidepressant trazodone (TRA) under visible light radiation. Fe-TNW was the best catalyst in the degradation of TRA by surface area of the sample (124.7 m²/g) with the totally removal in 8 h of experiment. In addition, thirteen transformation products (TPs) were elucidated by the analysis of MS/MS spectra obtained by UHPLC-QTOF-MS, these TPs resulted in distinct time profiles (formation/degradation) for each catalyst. This study demonstrated the differences in photocatalytic performance and the importance of considering the formation of TPs during the photodegradation of pollutants.

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P7. ANALYSIS OF PALYTOXIN AND ITS ANALOGUES (OVATOXINS) PRODUCED BY *OSTREOPSIS* CF. *OVATA* MICROALGAE

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Since the end of 90s, marine unicellular *Ostreopsis* cf. *ovata* microalgae blooms were detected in some Mediterranean beaches and it have been linked to mild respiratory disorders in people exposed to marine aerosols [1,2]. This alga produces palytoxin, one of the most potent marine biotoxins so far known, and its analogues (ovatoxins). Some studies suggest that the palytoxin could be responsible of the health impact, but the direct cause-effect relationship has not been clearly established yet due to sampling and analytical limitations.

Under this frame, the main objective of this project is to develop and evaluate an analytical strategy based on mass spectrometry (MS) to estimate these complex marine biotoxins in microalgae samples. An ultra-high-performance liquid chromatography (UHPLC) system was coupled to a Q-Exactive Orbitrap Fourier-Transform Mass Spectrometer (FTMS) equipped with a heated-electrospray ionization source (H-ESI) operating in positive ion mode to analyze palytoxin and its ovatoin analogues. The chromatographic separation was performed in a Hypersil GOLD™ C18 column (100 mm x 2.1 mm id., 1.9 µm particle size) packed with totally-porous silica particles, under a gradient elution using an acetonitrile:water (0.1% formic acid) mobile phase.

The developed UHPLC-HRMS method has been applied to study the growth and toxin production of this microalga grown under different nutrient availability. Experiments were run in a commonly used for coastal marine algae f/2 seawater enriched culture medium (883 µM Nitrate; N:P ratio 24), in f/10 (105 µM N; N:P ratio 16) and in nitrogen (N) and phosphor (P) deficient media, with N:P ratios of 5 and 92, respectively. Nitrogen appeared to be the most limiting nutrient for growth by *Ostreopsis* cf. *ovata*. Additionally, the UHPLC-HRMS method has also been applied to monitor the biotoxins in microalgae collected during the 2018 summer in the catalan coast.

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P8. MICROPLASTICS ADSORPTION CAPACITY AND TRANSPORT OF COCONTAMINANTS IN SEAWATER.

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Microplastics (MPLs) are defined as plastic fragments of less than 5 µm of diameter, and nanoplastics (NPLs) those that are in the nano-scale range. Nowadays, the occurrence of MPLs and NPLs in the environment, in particular, in coastal areas, are recognised as a relevant environmental problem, because of their potential impact on living organisms and their potential to adsorb and transfer to biota other contaminants present in the same compartments^[1]. Several factors like the physicochemical properties of the polymers involved and the cocontaminants as well as the medium e.g., organic material content or salinity will strongly influence the affinity and adsorption/desorption between the plastic particles and other contaminants^[2].

In this context, our aim is to study and characterize the interaction between selected persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and perfluoroalkyl substances (PFASs) in two different types of microplastics: Polyethylene (PE) and Polystyrene (PS). In this work, we show the sorption isotherms of selected contaminants and MPLs studied in microcosms emulating relevant environmental conditions along 50-60 days (20°C, cocontaminants concentrations work range between 1-20 µg/L). The study of these interactions under controlled conditions were assessed and quantified at 0, 4, 7 and 50 days by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) in the case of selected PCBs and by liquid chromatography coupled to mass spectrometer in tandem (LC-MS/MS) in the case of PFASs.

The results showed that PE and PS can act as carrier materials for those POPs in the environment; and in both cases, the model isotherm can be adjusted to a Freundlich model, which is the most suitable for these kind of interactions.

Acknowledgement

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P9. CHIRAL ANALYSIS OF AMPHETAMINE-RELATED DRUGS IN WASTEWATER

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Recently, wastewater-based epidemiology (WBE) has arisen as a very useful complementary tool to follow patterns of drug abuse at local level. WBE is based on the determination of specific biomarkers (i.e. metabolites or the active drug ingredient itself) in wastewater. A disadvantage of WBE when applied to amphetamine-like substances is that the biomarker employed is the active drug itself. Therefore, concentrations in wastewater cannot differentiate human abuse from eventual direct disposals of the drug into wastewater or from human consumption due to therapeutic application. This may be solved by performing a chiral analysis of these drugs, since human metabolism renders an enrichment of the non-active enantiomer (the R form in most cases). The aim of this work was the development of an analytical method capable of performing the chiral analysis of amphetamine, methamphetamine and MDMA in wastewater. Thus, a method based on solid-phase extraction on mixed-mode cartridges followed by chiral liquid chromatography-tandem mass spectrometry (using a Phenomenex Lux AMP column, 150 x 3.0 mm, 3 µm chiral column with a basified eluent) was developed and validated with wastewater samples, affording recoveries in the 83-110% range and LODs in the 0.8-3.2 ng/L range. The method was finally applied to several samples of wastewater from Spain where the enantiomeric fractions of the three substances were calculated. It was found that amphetamine was slightly enriched in the S form, MDMA in the R form and methamphetamine was exclusively present in the S form.

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P10. CAPSULE PHASE MICROEXTRACTION WITH MIXED-MODE PROPERTIES TO SELECTIVELY DETERMINE ACIDIC OR BASIC COMPOUNDS FROM ENVIRONMENTAL WATER

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Among the different extraction techniques, capsule phase microextraction (CPME) has been recently introduced to overcome the limitations of other microextraction techniques. CPME is based on the principle of equilibrium extraction that uses a miniaturized microextraction capsule (MEC) as the extraction medium. The MEC involves two fused porous tubular polypropylene membranes, one to accommodate the sorbent through sol-gel technology, while the other encapsulates a magnetic metal rod. These MECs accommodate different coatings, such as poly(dimethylsiloxane), C₁₈ or Carbowax 20M. CPME have been applied to extract personal care products from environmental water samples [1] and sulfonamides from milk [2].

In this study, two different MEC have been developed and evaluated to extract acidic and basic analytes. One MEC combines C₁₈ with a quaternary amine as cation-exchange group and the other one with a sulfonic group as anion-exchange group, establishing both hydrophobic and ionic interactions with the analytes. The extraction parameters such as sample pH, sample/elution volume, extraction/elution time and elution solvent were thoroughly investigated and optimized. The extraction optimum conditions were used to determine acidic and basic model compounds in environmental water samples.

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P11. APPLICATION OF MULTIVARIATE ANALYSIS TO IDENTIFY PEPTIDES RESPONSIBLE FOR THE HYPOCHOLESTEROLEMIC CAPACITY OF PROTEIN HYDROLYSATES RELEASED FROM OLIVE SEEDS

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The incidence of hypercholesterolemia in developed countries has urged the development of synthetic molecules to treat this disease that, in many occasions, also results in undesirable side effects. Recently, our research group has demonstrated the in vivo capability of peptides released from olive seeds proteins to reduce blood cholesterol levels in mouse [1]. In order to identify those peptides responsible for this capacity, protein hydrolysates obtained from twenty different olive varieties were studied and peptides were identified using RP-HPLC-MS/MS. Four different methods that evaluated the capacity of hydrolysates to inhibit the absorption of diet cholesterol and to inhibit cholesterol biosynthesis were employed. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to study similarities among varieties. Moreover, correlations between identified peptides and studied bioactivities were also evaluated. A total of 31 of 103 identified peptides showed a strong correlation with at least one of the capacities. Some of these peptides displayed opposite coefficients with the capacities to reduce the endogenous and exogenous cholesterol. Moreover, capacity of peptides to bind bile acids and to reduce micellar cholesterol solubility were positively correlated while a negative correlation was observed between the capacity to inhibit cholesterol esterase and the HMG-CoA R enzymes. Finally, four different groups of hydrolysates with similar peptide composition and hypocholesterolemic capacity were suggested according to the PCA and HCA.

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P12. EVALUATION OF VERMICOMPOST BIOFERTILIZATION ON THE AROMA AND POLYPHENOLIC COMPOSITION OF ALBARIÑO MUSTS AND WINES

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The synergy of two industrial processes, in particular, a vermicomposting process of grape bagasse [1] and a winemaking process, is good strategy for valorization of wineries residues and for their transition to circular economy. In this sense, the effect on Albariño musts and wines of adding a fertilizer treatment enriched with vermicompost to *Vitis Vinifera* plants has been evaluated (humus being added in a complementary way to the usual fertilization). For this evaluation, parameters such as colour, total polyphenol index and antioxidant activity of both control products (must and wine produced from conventionally fertilized grapes) and "humus" products (obtained from biofertilized grapes) have been monitored. In addition to these quality parameters, several aroma markers have been selected, mainly volatile compounds belonging to the families of alcohols, fatty acid esters, acetates and terpenols. The analytical monitoring of the wine aromas has been carried out by the green technique of headspace solid phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME-GC-MS). On the other hand, the polyphenolic profiles have been obtained by liquid chromatography with diode array detector (HPLC-DAD) and liquid chromatography-mass spectrometry (LC-MS). The results have allowed knowing the similarities and differences between the wines of conventional production (control) and the "humus" wines.

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P13. ISOTOPIC PATTERN AS FILTERING RULE FOR THE SCREENING OF AUTHORIZED GREEN COLORANTS IN FOODS

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The color impression represents among 60 to 90% of the final acceptance/rejection choice made by consumers [1]. Consequently, color additives are attribute standards for our daily life in any market and in any culture. However, the relation between additives and consumers has not been always peaceful. As a consequence, international food safety agencies and policy makers (EFSA, USDA, FDA) are committed to improve the legal framework to guarantee not only the quality, security and safety of food additives but also the confidence of the consumers. With this aim, different official calls have been launched for development of analytical methods to characterize green food colorants [2]. Currently, authorized natural green food colorants comprise several copper-chelated chlorophyll derivatives [3]. Both the raw material and the manufacturing process for the acquisition of these green food colorants are still unclear. Hence, each producer applies its own know-how to obtain 'signature' green colorant products. Indeed, the chlorophyll profile of these products is partially known and may substantially differ among batches, while their identification just by HPLC-UV-Vis is not satisfactory [4]. We propose a fast and specific method for natural green food colorant identification based on the isotopic pattern of the copper chlorophyll derivatives present in the authorized food colorants. We apply a specific HPLC-ESI/APCI-HRTOF-MSⁿ assisted with powerful post processing software. The present approach can detect the presence of authorized green food colorants, but it can also be applied for other coloring foodstuff as zinc chlorophylls.

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P14. DUAL RETENTION BEHAVIOR OF BENZALKONIUM CHLORIDE IN CORE-SHELL COLUMN WITH BIPHENYL GROUPS

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A dual retention behavior with “U shaped” has been described in the analytical literature for some polar and ionizable analytes on tri-functional phases and on hydrophilic interaction liquid chromatography (HILIC) phases [1, 2]. Thus, reversed-phase (RP) and HILIC mechanisms have been found, depending on the content of organic modifier in the mobile phase. In this work, a “U shaped” behavior was also observed for benzalkonium chloride (BAK) on a *core-shell* column with biphenyl groups using a mixture of acetonitrile and buffer as mobile phase. On biphenyl *core-shell* column, different conditions of mobile phase were tested for the separation of four BAK homologs with different alkyl chain length (C12, C14, C16, and C18). The BAK retention was also examined using a cyano column in which hydrophobic and ionic interactions are also found. Unlike cyano column, excellent separation was achieved on *core-shell* column in all tested conditions. The nature and concentration of the salt or of organic additive, pH and % acetonitrile have influence on BAK retention. For two retention behaviors, an increase of salt concentration and decrease of pH induced a lower retention. All homologs showed the same tendency with studied variables but quantitative differences were observed between them, except for the highest concentration of acetonitrile (95%). The experimental data were adjusted to a polynomial retention model.

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P15. STATISTICAL COMPARISON OF THE RETENTION MECHANISM OF POLYCHLORINATED BIPHENYLS IN DIFFERENT GAS CHROMATOGRAPHY STATIONARY PHASES

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Topological or electrotopological descriptors have been frequently used in statistical studies trying to predict the gas chromatographic (GC) retention of selected analytes in specific stationary phases. In the case of families of numerous isomers, the practical use of these approaches requires very accurate predictions due to the close-related structure of the compounds and the frequent presence of co-elutions. Thereby, for isomers mixtures, the statistical estimation of the effects of simple structural descriptors of the target analytes on their GC retention can be considered a more interesting approach, as it allows to compare the interactions of studied compounds with different stationary phases and, consequently, to describe the specific phase selectivity toward groups of compounds sharing a specific substructure.

In this study, a mixture of 59 polychlorinated biphenyls (PCBs), covering different groups of homologues and chemical substructures, was used for the statistical valuation of the retention behavior of these analytes in seven GC stationary phases with widely divergent polarities and compositions (i.e., from 5% diphenyl-methylpolysiloxan-type to ionic liquid-based phases). The proposed model used structural descriptors as independent variables and retention time values as dependent variables. However, it was also demonstrated to provided satisfactory regression values ($R > 0.996$) when using retention indexes instead, and both, under pseudo-isotherm and programmed GC conditions (i.e., $R > 0.99$). Its application to PCB retention data obtained using extremely polar (SLB-IL60 and SLB-IL76), polar (SuplecoWax-10), semi-polar (DB-17) and non-polar (DB-5 and HT-8) GC stationary phases provided satisfactory-to-acceptable regression fittings. More importantly, the statistical comparison of the PCB retention behavior in these stationary phases demonstrated that those based on ILs showed a completely differentiated selectivity through PCBs from those observed in the other investigated stationary phases.

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P16. APPLICABILITY OF ATMOSPHERIC PRESSURE PHOTOIONIZATION FOR THE DETERMINATION OF DECHLORANE PLUS AND ANALOGUES BY GC-HRMS

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Dechlorane Plus (DP) is a chlorinated flame retardant which has been used as additive in a wide range of consumer products, such as electrical hard plastic connectors, computer and television monitors, wire coatings and furniture [1]. Although it has been produced for almost half a century, its occurrence in the environment was not reported until 2006 [2]. After that, DP and analogues have been detected in different environmental matrices, becoming a worldwide contaminant, which are susceptible to long-range atmospheric transport. DP stereoisomers and their analogues (Dec 602, 603 and 604) are currently determined by GC-MS using electron capture negative ionization (ECNI) [3]. However, the mass spectra of these compounds show a high fragmentation, generating for some of them non-selective ions (e.g. $[\text{Br}]^-$), which could lead to mass interferences and identification problems. Recently, the introduction of soft atmospheric pressure ionization sources allows to prevent the fragmentation of molecular ions, becoming an excellent alternative for the GC-MS analysis of this family of compounds.

Here, the ionization behaviour of DPs and related compounds by atmospheric pressure photoionization (GC-APPI) has been studied using both ionization modes, achieving the best results in negative-ion mode. Different classical and novel dopants (i.e., toluene, acetone and diethyl ether) and source parameters (i.e., temperatures and make-up gas flow) have been investigated in order to maximize the ionization efficiency, to control the nature of the ions generated and to provide a reliable GC-APPI-HRMS method for the determination the analytes. The developed method provided a high sensitivity and selectivity, and its applicability has been demonstrated by analysing selected environmental samples in a wide range of DP concentration levels.

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P17. COMBINING *IN VITRO* BIOANALYSIS AND HIGH RESOLUTION MASS SPECTROMETRY FOR A REFINED NON-TARGET SCREENING WORKFLOW USING SURFACE WATERS FROM MEDITERRANEAN LITTORAL (SPAIN) AS A CASE STUDY

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In vitro bioanalyses permit the establishment of a toxicological fingerprint of a sample, and comparison of toxicological activities between different samples. Also, the combination of *in vitro* bioanalysis and chemical analysis allows to identify the toxicity driver. Non-target screening analyses, with the aim of identifying all chemicals present in a sample, are gaining in popularity in environmental research. However, dealing with such large data sets with many features is often time-consuming or even unaffordable, and therefore, prioritization strategies are essential for this task. Usually, many of these strategies require a comparison between 'reference' and real samples. But, since environmental analyses often lack reference samples, the application of bioanalysis can reveal these type of samples. In this study, the application of eight *in vitro* bioanalyses (aryl hydrocarbon receptor (AhR), estrogen receptor (ER), nuclear factor erythroid 2-related factor 2 (Nrf2), androgen receptor (AR) and vitamin D receptor (VDR)) for the establishment of toxicological fingerprints was combined with non-target and suspect screening by means of state of the art high resolution mass spectrometry techniques for the development of a prioritization strategy using binary comparison between samples. In total, 11 surface waters from coastal lagoons from the Mediterranean coast of Spain have been selected as a study case for this approach.

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P18. $\delta^{13}\text{C}$ AND $\delta^{18}\text{O}$ ISOTOPE SIGNATURE OF *SPONDYLIOSOMA CANTHARUS* OTOLITHS

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The black seabream, *Spondyliosoma cantharus*, is an economically important species with a vast distribution along the eastern Atlantic, exploited in European waters. Despite its vast distribution and commercial importance there is no information on black seabream population structure along the eastern Atlantic. Stable isotope ratios in fish otoliths can provide valuable information for origin signature and ecological studies of individuals and populations¹. Otolith's stable isotopes ratios are strongly influenced by factors such as diet, environmental conditions, growth rates and ontogenetic and physiological processes. In the present study, otoliths collected from seven areas covering the geographical distribution of the black seabream, were compared through their carbon and oxygen isotopic ratios ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$).¹ Fishes were collected from: English Channel (EN), Bay of Biscay (BI), Galicia, north Spain (GL), Peniche, west coast of Portugal (PN), Algarve, south coast of Portugal (AL), Canary Islands (CN) and Angola (AN). For each sample area, twelve left otoliths were selected from fish with ages between 4 and 6 years. After removing the organic matter, each otolith was ground with a pestle and mortar into a fine powder. Subsamples of about 50 μg from this homogenised material were used for oxygen and carbon stable isotopes determinations ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) using Dual-Inlet Isotope Ratio Mass Spectrometer (IRMS-DI) hyphenated with a Multiprep carbonate reaction device. The isotopic values were normalised to the international standard NBS-19 and reported to V-PDB (Vienna - Pee Dee Belemnite). The precision was better than 0.1 per mil for both carbon and oxygen isotopes. The spatial variation in the stable isotopes of black seabream was evaluated with PERMANOVA (Permutational ANOVA). Results of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ can discriminate the origin of black seabream for some of the studied geographic areas, ranging from -0.73 ‰ to +0.92 ‰ for $\delta^{18}\text{O}$ and from -6.65 ‰ to -1.20 ‰ for $\delta^{13}\text{C}$.

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P19. HORMONES AND THEIR METABOLITES AS PREHISTORIC SHEPHERDS' ACTIVITIES AND MILK STORAGE BIOMARKERS

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New Archaeology tries to explain the human activities of the past [1]. The search of new biomarkers which explain prehistoric shepherds' activities and food handling and storage is a new challenge. One of those biomarkers can be hormones and their metabolites.

On one hand, those compounds are accumulated in animal manure, and at appropriate conditions, they can be preserved over time. Neolithic shepherds used caves and rock-shelter as pen. One of those caves is El Mirador Cave (Sierra de Atapuerca, Spain) where *fumier* structures are found (archaeological sediment deriving from animal dung, typically burnt). Different *facies* are observed through the *fumier* structures and one corresponds to unburned dung where hormones and their metabolites could be preserved. Thus, the quantification of those compounds could explain shepherd's activities such as pregnant sheep/goat and lamb separation from the flock. Thus, a new method based on microwave assisted extraction and gas chromatography tandem mass spectrometry has been developed in order to determine shepherds' activities from unburned *facies* from el Mirador Cave.

On the other hand, milk produced by sheep, goats or cows was storage and handled in ceramic artifacts. That milk contains hormones and they could be accumulated either on ceramic surface or in ceramic pores. Again, at appropriate conditions, those compounds can be preserved over time. This way, a new method based on ultrasonic extraction and gas chromatography mass spectrometry has been developed to determine the possible milk handling or stored in ceramic artifacts from a medieval Jewish located in Pancorbo (Burgos, Spain).

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P20. DETERMINATION OF MELATONIN AND ITS METABOLITES BY DIFFERENT QUANTIFICATION APPROACHES USING LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Melatonin (MEL), N-acetyl-5-methoxytryptamine, derived from tryptophan is the major night product of the pineal gland. Beside its role on the physiological adaptation to circadian rhythms, this indole displays potent antioxidant and cytoprotective actions, with important neuroprotective and anti-cancer roles. Free radical scavenging activity of MEL results in the formation of a series of bioactive metabolites, including N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK), N¹-acetyl-5-methoxykynuramine (AMK) and cyclic 3-hydroxymelatonin (c3OHM). In addition to the classical radioimmunoassay (RIA) techniques, some HPLC and ELISA assays for MEL detection have been reported. However, simultaneous detection of MEL and the abovementioned bioactive metabolites has not been experimentally approached with advanced analytical techniques. Detection of all these compounds in biological samples is essential to understand the cellular and molecular mechanisms involved in the neuroprotective and anti-tumor role of melatonin.

We evaluate here several analytical approaches for the quantification of MEL and its metabolites in PC3 cell cultures. One and two-dimensional UPLC-ESI-MS/MS methods were proposed for the separation and determination of MEL, AFMK, AMK and c3OHM. Two different quantification approaches based in a calibration curve using internal standards and isotope dilution mass spectrometry (IDMS) using a newly synthesized ¹³C-MEL as tracer were tested and compared. The 2D-UPLC method allowed a suitable separation of the analytes using trifluoroacetic acid (TFA) as mobile phase modifier in the first dimension and formic acid (FA) was used in the second dimension to prevent the ion suppression due to TFA reaching the ESI source. For the 1D-UPLC method FA was used as modifier leading to coelutions of some of the metabolites. The IDMS based quantification approach provided a better precision and accuracy in the quantification of melatonin. The commercial availability of labelled standards for the rest of metabolites is currently the only limitation to apply IDMS for their determination in cell cultures.



P21. DEVELOPMENT OF A MS/MS SPECTRAL LIBRARY OF PHENOLIC COMPOUNDS USING ELECTROSPRAY IONIZATION AND TRIPLE-QUADRUPOLE MASS SPECTROMETER

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Structural identification through Electrospray Ionization (ESI) tandem mass spectrometry (MS/MS) has rapidly become the method of choice in analytical studies, particularly in metabolomics, foodomics and proteomics. Structurally informative spectra may be obtained either by collision-induced dissociation (CID) in the source region or by tandem mass spectrometry (MS/MS). However, variations in collisional stabilities and the different designs of instrumentation make it difficult to obtain 'library searchable' spectra by this approach. Recently, several MS/in-source CID, and MS/MS libraries have been constructed for natural products. However their scope is often limited and it is very important to accumulate an inter-laboratory MS/MS spectra collection. The aim of this study was the development of a MS/MS library of phenolic standards. Seventy compounds were analyzed by ESI, mainly phenolic acids and flavonoids. Several phenolic compounds, such as glucuronide and sulphate derivatives were also synthesized and characterized by NMR. MS spectra were acquired in positive or negative ionization modes. Several cone voltages and collision energies were assayed in order to optimize analytical conditions and maximize the transition signals [precursor ion > product ion]. All MS spectra were analyzed and the best ionization modes, cone voltages and collision energies were chosen for each compound. A library of spectra was created in which a molecule can be characterized in terms of its fragmentation pattern and product ions. This knowledge was applied to metabolite profiling in a vast number of samples. With such a library, a variety of metabolites could be routinely identified in a single HPLC-MS/MS experiment.

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P22. BUILDING A COLLISION CROSS SECTION (CCS) DATABASE FOR ENVIRONMENTAL ORGANIC MICROPOLLUTANTS SCREENING UNDER TRAVELLING WAVE ION MOBILITY SPECTROMETRY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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Recently developed Traveling Wave Ion Mobility Spectrometry coupled to High Resolution Mass Spectrometry (TWIMS-HRMS) instruments provide an extra valuable information for their application in target, suspect and non-target screening approaches. After the liquid chromatographic separation, the introduction of an ion mobility cell orthogonally separates isobaric ions depending, mainly, on their shape. Therefore, it allows to separate the compound of interest from the co-eluting matrix interferences, as well as to, in some cases, resolve isomers. The drift time, or the time that it takes to an ion to travel through the mobility cell, can be then translated into the Collision Cross Section (CCS) for this particular ion. The objective of this transformation is to obtain a comparable value between different analyses using the same instrument or even between different TWIMS-HRMS instruments. In this study, a CCS library has been built containing approximately 1,000 entries about pesticides, pharmaceuticals, illicit drugs, new psychoactive substances, mycotoxins and hormones for both positive and negative ionization modes. The data collected within this database comprises information regarding CCS for protonated adducts, sodiated adducts, water loss, ammonia loss, deprotonated adducts, chlorinated adducts and formiated adducts. Additionally, for those molecules that more than one adduct was observed, the difference in CCS value between different adducts was calculated (protonated vs sodiated, protonated vs deprotonated, deprotonated vs chlorinated, and deprotonated vs formiated). In this sense, no specific trend was observed for any of the pairs studied which highlighted the fact that the tridimensional chemical structure play a key role in the accommodation of the ion in accordance with its volume and charge. The built database is of great importance for future target screening applications using liquid chromatography coupled ion mobility - high resolution mass spectrometry.

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P23. GC MEETS VION IMS-QTOF. FACILITATING ION MOBILITY MEASUREMENTS FOR GC-AMENABLE COMPOUNDS IN FOOD AND ENVIRONMENTAL ANALYSIS

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In the last years, ion mobility separation coupled to high resolution mass spectrometry (IMS-HRMS) instruments provide an extra valuable information for its application in screening approaches. After chromatography, the introduction of an ion mobility cell orthogonally separates isobaric ions depending, mainly, on their shape. This allows to separate the compound of interest from the co-eluting matrix interference and/or resolve isomers. The drift time can be translated into the collision cross section (CCS) for a particular ion, a comparable parameter between instruments used for identification purposes.

Until now, only liquid chromatography (LC) has been coupled to IMS-HRMS with promising results [1]. Gas chromatography (GC) coupled to HRMS is a powerful technique that combines excellent separation power of GC with improved identification based on accurate mass measurements of parent and/or fragment molecules. These features designate GC-HRMS as the first choice for identification and structure elucidation of unknown (semi)volatile compounds. Recently, and thanks to the atmospheric pressure chemical ionization source (APCI) designed for GC, the meeting between GC and IMS-HRMS has occurred. Oppositely to electron ionization (EI) sources, the soft ionization promoted by APCI allows a rapid and wide-scope screening based on the investigation of the molecular ion and/or protonated molecule. This approach, has been revealed in the last decade as an advantageous alternative to the existing GC-EI-TOF MS methods.

In this work, a CCS library has been built containing approximately 300 entries including pesticides, polycyclic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), flame retardants (brominated and phosphorated), and different emerging contaminants considering insect repellents, musks and UV-filters among others. Favoring the formation of a highly abundance (quasi) molecular ion in the source has been pursued. The addition of water as modifier has been considered as a way to promote the generation of protonated molecules.

The data collected within this database comprises information regarding CCS for molecular ion and/or protonated molecules and some fragments. The built database is of great importance for future target screening applications using GC-IMS-HRMS.

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P24. OPTIMIZATION AND VALIDATION OF A METHOD BASED ON CAPILLARY ELECTROPHORESIS COUPLED TO LASER INDUCED FLUORESCENCE DETECTION (CE-LIF) FOR CHIRAL ANALYSIS AND ABSOLUTE QUANTITATION OF 3 AMINO ACIDS: L AND D-ASP, L AND D-GLU AND L AND D-SER. APPLICATION TO OSTEOCYTE AND OSTEOBLAST CELL LINES

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Amino acids have a biological role not only as metabolites, but also several as neurotransmitters. The improvement of analytical techniques has allowed a better insight of the importance of D-amino acids as neurotransmitters, particularly D-Asp and D-Ser.

The presence of three amino acid pairs, L/D-Asp, L/D-Glu and L/D-Ser has been studied in two biological samples: mouse osteocytes (MLOY4) and osteoblasts (MC3T3-E1) by using Capillary Electrophoresis coupled to Laser Induced Fluorescence detection (CE-LIF). 4-fluoro-7-nitro-2,1,3-benzoxadiol (NBD-F) was the derivatizing agent, suitable for an Argon ion laser source. The background electrolyte (BGE) in the optimized method consists of borate buffer and β -cyclodextrins as chiral selector. The analysis time is less than 30 min.

The method was validated for linearity, accuracy, precision, selectivity and sensitivity for osteocyte and osteoblast samples. Concentration range was 0.25-2.5 μ M and 2.5-25.0 μ M for D- and L-amino acids respectively. Linearity was confirmed with $r > 0.995$ for all compounds. Accuracy was between 81.9% and 111.7%. Intra-day precision was 1.8% and 10.9%. Experimental LOQ was 0.25 μ M for lysed cells. The method was applied for the absolute quantitation in real samples (n:6) and statistical differences were found. The highest differences were for L- and D-Glu. This application could play a fundamental role in the study of therapeutic targets in the treatment of bone diseases.

Acknowledgement

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P25. PYROLYSIS COMPOUND-SPECIFIC NITROGEN ISOTOPE ANALYSIS ($\delta^{15}\text{N}$ PY-CSIA): NOVEL ANALYTICAL APPROACH FOR ARCHAEOLOGICAL STUDIES

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The measurement of stable isotopes has become an important tool within the field of archaeology. The isotopic trace of human and animal tissues and components (bone, collagen, keratin, muscle, fat etc.) allowed insight into the diet of our ancestors in a specific period of time, as well as its relationship with various human pathologies. Furthermore, this technique informs about food origin and possibly also their commercial routes, as well as population migrations. Pyrolysis-compound specific isotope analysis (Py-CSIA) is a cutting-edge analytical approach able to provide, not only a precise identification of organic compounds in different complex matrices, but also additional valuable information about nature and origin of the materials based on their isotope composition. This technique is based on the coupling of a micro-furnace pyrolysis unit to a gas chromatograph equipped with an isotope ratio mass spectrometer (IRMS) as detector. The individual volatile pyrolysis products separated by gas chromatography are directed to a combustion or pyrolysis micro-reactor (GC-Isolink system) and finally the isotope composition of the gases produced measured in a continuous flow IRMS via a interface unit. With this technique it is possible to make direct determinations of stable isotope ratios (i.e. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$) of specific compounds with minimum sample handling and pre-treatment, thus minimizing the chance of contamination and artefacts productions. In this communication, we introduce the Py-CSIA technique into the field of archaeology by studying the direct determination of the isotopic composition of human skeletons buried in medieval necropolises from Center and South of Portugal.

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P26. OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION OF BIOACTIVE CARBOHYDRATES FROM ALFALFA

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Alfalfa (*Medicago sativa* L.) is a perennial flowering plant of the Fabaceae family, widely used as forage. Several beneficial effects on human health, such as antioxidant activity, lowering cholesterol levels, etc., have been also attributed to the consumption of sprouts and leaves of this plant [1]. Although phenolic compounds are the most studied bioactive constituents of *M. sativa* [1], the presence of bioactive carbohydrates in its leaves and seeds such as cyclitols and α -galactooligosaccharides (α -GOS) has been also described [2]. The potential use of these bioactive compounds as ingredients of functional foods makes interesting the search for new extraction methods. Microwave assisted extraction (MAE) is an efficient technique that usually provides a significant reduction in extraction times and solvent consumption as compared to conventional solid-liquid extraction (SLE).

Thus, the aim of this work was to optimize a MAE method, following a Box-Behnken experimental design, for the extraction of bioactive carbohydrates from alfalfa leaves and seeds. MAE yields were compared with those obtained by SLE and the stability of the extracts was also evaluated. Characterization of carbohydrate composition of alfalfa extracts was carried out by gas chromatography coupled to mass spectrometry, previous to their derivatization to the corresponding trimethylsilyloximes.

The optimized MAE method (0.3 g/10 mL water, 5 min and 40°C for leaves and 0.1 g/10 mL, 5 min and 80°C for seeds) resulted to be a good alternative to conventional SLE for the production of extracts enriched in bioactive carbohydrates from alfalfa. Cyclitols (*myo*-inositol and methyl-inositols such as pinitol and ononitol) were the most abundant carbohydrates of alfalfa leaf extracts, whereas seed extracts were mainly constituted by α -GOS (raffinose, stachyose and verbascose), glycosyl-inositols and glycosyl-methyl inositols. Regarding stability, no significant differences were observed in the carbohydrate content at different storing days (0, 5, 12, 19 and 26 days). These studies reveal the utility of *M. sativa* MAE extracts as a potential source of bioactive carbohydrates for their use as functional ingredients.

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P27. EFFECT OF THE PURIFICATION STEPS OF GLYCOPROTEINS FROM BIOLOGICAL FLUIDS ON THEIR RECOVERY

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Glycoproteins are very complex compounds which play relevant roles in numerous fields. Every glycoprotein can present heterogeneities including post-translational modifications (PTMs), such as glycosylation among many others. These changes give rise to different proteoforms of that glycoprotein. The complexity of the mixture of proteoforms of the glycoprotein make their analysis very attractive and challenging. Additionally, alterations in proteoforms can be linked to different factors, such as pathophysiological conditions of individuals, production conditions of glycoprotein drugs, or biological fluid handling, just to name a few.

Capillary electrophoresis (CE) allows separating peaks (called isoforms) containing one or more proteoforms of a glycoprotein, thus allowing to detect alterations on glycoprotein proteoform mixture [1, 2]. To perform the CE-UV analysis, the glycoprotein needs to be first isolated from the complex matrix. The higher is the purification, the higher is the CE analysis reliability.

In this presentation, the effect of several steps involved in the sample pre-treatment as well as in the purification including affinity chromatography, on glycoprotein recovery is shown.

The effect of different factors studied are mainly exemplified using prostate specific antigen (PSA). Using centrifugal filtration devices for solvent-exchange and concentration of the glycoprotein, the protein solvent, the final volume and the way of performing recovery from the device influence the results. The procedures employed for constructing and using the HPLC affinity columns required to perform combined steps of affinity purification of the glycoprotein effect the outcome.

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P28. DEVELOPMENT OF A RAPID METHOD TO CONFIRM CONTAMINATION BY MINERAL OILS THROUGH SPE-GC-MS

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Mineral oil hydrocarbons (MOH) are complex mixtures of chemical substances formed by hundreds of isomers, which have been grouped according to their chemical structure in MOSH and MOAH. MOSH are open-chain and cyclic saturated hydrocarbons, often branched. MOAH are mono or polycyclic and branched aromatic molecules, with or without sulphur. The MOAH fraction represents a danger to human health, since they can act as mutagenic agents and / or carcinogenic agents [1]. The chemical complexity of mineral oils makes the analysis very difficult. MOSH and MOAH are fractionated through a column of silica impregnated with silver nitrate (0.33%) and subsequently quantified by GC-FID or the analysis is carried online using LC-GC-FID[2]. The result of this analysis is a hump of unresolved peaks, which is quantified using an internal standard and integrating the entire signal interval with subtraction of the peaks that are not part of the mineral oils. The poor definition of this hump can give false positives. Objective of this research is the development of an alternative and complementary method to those currently used that confirm the presence of MOAH in food samples and materials in contact with food. For this purpose, a series of compounds have been selected as markers, representing different families that make up MOAH and an extraction method has been optimized by SPE and determination by Gas Chromatography with mass detector (GC-MS).

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P29. APPLICATION OF DISPERSIVE LIQUID LIQUID MICRO EXTRACTION (DLLME) FOR DETERMINATION OF AFLATOXINS B1, B2, G1 AND G2 IN FISH

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The application of the dispersive liquid liquid micro extraction (DLLME) technique for extraction the aflatoxins of B1, B2, G1 and G2 in fish has been evaluated. After extraction of the aflatoxins from fish flesh with a mixture of acetonitrile: buffer (0.1M/0.1M $\text{KH}_2\text{PO}_4\text{-NaOH}$ at pH 6) (6:4 V/V), and the analyte were transferred from extract to another small volume (400 μL) of organic solvent, chloroform by DLLME. Aflatoxins were determined using ultrahigh performance liquid chromatography tandem mass spectroscopy (UHPLC-MS-MS). Parameters affecting DLLME procedure such as extraction solvent pH, volume of the organic solvent, vortex speed and time, salting out effect were systematically investigated and optimized to achieve the best extraction efficiency. Under the optimized conditions (extraction solvent pH-7, volume of the organic solvent-400 μL , vortex speed and time-2000 rpm for 1 min, and 0.5% NaCl) the whole analytical method provide detection limit of 0.12 $\mu\text{g}/\text{kg}$, 0.93 $\mu\text{g}/\text{kg}$, 0.25 $\mu\text{g}/\text{kg}$, and 1.04 $\mu\text{g}/\text{kg}$ for B1, B2, G1 and G2 which are lower than the EU/EC regulation limits. The proposed method was shown to be an accurate and precise method through the relative deviation of intraday and inter-day tested below 20% and analytical recoveries in the range of 80-100% and it was successfully applied to the analysis of aflatoxins in fish with having high repeatability and recoveries.

Key words: Aflatoxins, fish, dispersive liquid liquid micro extraction, tandem mass spectroscopy



P30. APPLICATION OF MOLECULARLY IMPRINTED POLYMER MICRO SOLID PHASE EXTRACTION (MIMSPE) FOR DETERMINATION OF AFLATOXINS B1, B2, G1, G2 AND M1 IN FISH

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The application of the molecularly imprinted polymer micro solid phase extraction technique (MIMSPE) for extraction the aflatoxins of B1, B2, G1, G2 and M1 in fish has been developed. Molecularly Imprinted Polymer (MIP) as an adsorbent has been proposed as a highly selective micro solid phase extraction procedure for isolating aflatoxins B1, B2, G1, G2 and M1 from fish. 5,7- demethoxycoumarin (DMC), methacrylic acid (MAA), divinylbenzene (DVB) and 2,2'-azobisisobutyronitrile (AIBN) was used as a template, functional monomer, crosslinker and initiator for MIP beads synthesis. After extraction of the aflatoxins from fish flesh with a mixture of acetonitrile: buffer (0.1M/0.1M KH₂PO₄-NaOH at pH 6) (6:4 V/V), the fish extract (1.5 mL) was transferred to the micro solid phase extraction device, and elute was injected to the ultrahigh performance liquid chromatography tandem mass spectroscopy (UHPLC-MS-MS) for quantification. Parameters affecting for MIMSPE procedure such as extraction solvent pH, vortex time and speed, elution solvent ratios, elution speed and time were investigated and optimized to achieve the best extraction efficiency. The best extraction solvent pH was pH 7, and 1500 rpm for 3 min were the selected settings as the best vortex speed and time. Moreover, the 97.5:2.5 (Acetonitrile/Formic Acid) ratio of the elution solvent was selected and 2000 rpm for 4 min selected as the best elution speed and time based on the analytical recovery. The proposed method provides the detection limit for aflatoxin B1, B2, G1, G2 and M1 which are lower than the EU/EC regulation limits.

Keywords: Aflatoxins, fish, molecularly imprinted micro solid phase extraction, tandem mass spectroscopy



P31. IDENTIFICATION OF PEPTIDES IN THE OLIVE SEED RESPONSIBLE FOR *IN VITRO* AND *IN VIVO* HYPOLIPIDEMIC CAPACITY

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Olive stones are an important by-product from the olive industry. Usually, these residues are employed in low-cost applications such as pellet production or animal feeding. However, the seed inside olive stones is an important source of proteins, which represent 16-28% of the total seed weight [1]. Our research group has demonstrated that proteins from olive seeds are able to release bioactive peptides with capacity to disrupt micellar cholesterol transport and to inhibit enzymes involved in the absorption of cholesterol [2]. The main objective in this work was to evaluate the hypolipidemic capacity of olive seeds at physiological level and to identify those peptides responsible for this activity by HPLC-MS/MS. Moreover, the capacity to reduce the biosynthesis of the endogenous cholesterol by the inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme and peptides cytotoxicity were also evaluated. The olive seed hydrolysate showed a high capacity to reduce cholesterol biosynthesis compared with the positive control. Moreover, two *in vivo* assays using different peptides concentrations were designed. Both low and high peptides concentrations demonstrated to have a cardioprotective effect, reducing total cholesterol and increasing HDL cholesterol in mice. Identification of peptides enabled to observe a high ratio of hydrophobic amino acids being peptides ADLY, FLPH, KLPLL, and TLVY the most abundant in the olive seed hydrolysate.

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P32. MOLECULARLY IMPRINTED POLYMERS FOR SOLID-PHASE EXTRACTION OF ARYLOXYPHENOXYPROPIONATE HERBICIDES FROM WATER SAMPLES

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Aryloxyphenoxypropionate are post-emergence herbicides commonly used in agriculture to control the growth of perennial grassy weeds [1]. Their widespread use contributes to their presence in surface and ground water, which, together with their high toxicity, makes the analysis of these pesticides mandatory. Due to the complex nature of the matrices in which the target compounds are present and their low concentration level, an adequate sample preparation step is needed in the analytical procedures employed for their determination.

Molecularly imprinted polymers (MIPs) are synthetic materials, which mimic the interaction between antigen-antibody, prepared in presence of a template. Given its advantages of high selectivity, easy preparation, and low cost, these polymeric networks have been extensively investigated as molecular recognition media in solid-phase extraction (SPE) [2].

In this study, several MIPs, with diclofop methyl as template, were prepared using methacrylic acid or 4-vinylpyridine as functional monomers. The corresponding non-imprinted polymers were also prepared using the same conditions but in the absence of template. The polymeric sorbents were employed for the concentration of diclofop methyl by SPE prior to its determination by HPLC with an UV-vis diode array detector, and the recoveries of all the sorbents were comparatively discussed. Moreover, the performance of the polymeric sorbents over other aryloxyphenoxypropionate compounds was studied.

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P33. DEVELOPMENT AND APPLICATION OF A MOLECULARLY IMPRINTED POLYMER FOR THE EXTRACTION OF PHENOXY HERBICIDES FROM WATER SAMPLES

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Sensitivity and selectivity are some of the main goals in trace analysis of real samples and they are especially important in sample pre-treatment. Furthermore, sample preparation is a compulsory step on several analytical procedures for extraction, enrichment and clean-up of trace compounds in aqueous samples: solid-phase extraction (SPE) is by far the most usually employed pre-concentration technique. Selectivity of SPE can be enhanced in a powerful, easy way by using imprinting polymers (MIPs) with template molecules [1].

In this study, a MIP that allows selective extraction of five phenoxy herbicides has been synthesized. The MIP was synthesized by thermal polymerization using 2-methyl-4-chlorophenoxyacetic acid (MCPA) as template, 4-vinylpyridine as functional monomer, ethylene glycol dimethacrylate as cross-linker, and acetonitrile as porogenic solvent. The same polymer synthesized in the absence of the pesticide was used as reference polymer. Variables that affect extraction efficiency (porogenic solvent, functional monomer and contact time) were optimized with the aim of achieving a selective extraction of MCPA. On the other hand, the optimum characteristics of SPE procedure (washing solvent, type and volume of eluent, preconcentration capacity, reusability) were also established. Its performance was also studied by extracting other phenoxy herbicides. Finally, the MIP was applied to the extraction of these herbicides in water samples from different sources.

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P34. DEVELOPMENT OF STIR BAR SORPTIVE EXTRACTION DIRECTLY ON POLYTETRAFLUOROETHYLENE MAGNETS MODIFIED WITH MONOLITHS

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The sample preparation step plays an important role in the development of analytical methodologies. It consumed a great part of the analysis time, and determines the accuracy and precision as much as the measurement step. Within extractive technologies, stir bar sorptive extraction (SBSE) is a great tool for solventless extraction and enrichment of compounds from different matrices. However, the limited selectivity of these coatings together with their damage during stirring process are common drawbacks of this type of devices. In this sense, the development of novel housing supports as well as coatings with enhanced features is still desirable [1,2]. Monoliths can be used as attractive media in some extractive technologies in sample treatment [3]; however, its extension to SBSE field has been scarce.

In this study, we propose the use of polytetrafluoroethylene (PTFE) magnets as housing support for SBSE using polymeric monoliths. The developed strategy consisted in the following steps. First, a chemical modification of the commercial PTFE stir bars was done in order to anchor the monolith. For this purpose, the stir bar was treated with FluoroEtch[®] to introduce hydroxyl groups on the PTFE surface. Then, these groups were subsequently modified by methacryloylation to obtain a vinylized surface. Special attention was also paid in the development of a suitable mold in order to obtain a uniform coating of monolithic polymer with an adequate thickness. To demonstrate the usability of this new extraction approach, a polymer monolith was prepared and its potential as extractive sorbent was evaluated using several small solutes with environmental impact in aqueous matrices.

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P35. POTENTIAL OF CATIONIC CARBOSILANE DENDRIMERS FOR SUSTAINABLE PROTEIN SAMPLE PREPARATION

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Protein sample preparation is the bottleneck in the analysis of proteins and usually requires the use of organic solvents and polluting reagents. Nanomaterials have open new possibilities in this step. Anionic carbosilane dendrimers (sulphonate- and carboxylate-terminated) have already demonstrated their interaction with proteins and their potential in protein sample preparation. The aim of this work was to evaluate the feasibility of carbosilane dendrimers functionalized with cationic groups for this purpose. Interactions between positively charged carbosilane dendrimers and different model proteins were studied under different pHs, dendrimer concentrations, and dendrimer generations. Amino- and trimethylammonium-terminated carbosilane dendrimers presented, in some cases, weak interactions with proteins. Unlike them, carbosilane dendrimers with terminal dimethylamino groups could interact, in many cases, with proteins. Moreover, dendrimer precipitation was observed at all pH values, although just 2G and 4G dendrimers resulted in the formation of complexes with proteins. Under experimental conditions promoting dendrimer-protein interactions, 2G dimethylamino-terminated dendrimers were proposed as sustainable alternative for the purification of proteins in complex samples.

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P36. IMMOBILIZATION OF PROTEOLYTIC ENZYMES ON SILICA SUPPORTS FUNCTIONALIZED WITH CARBOSILANE AND PAMAM DENDRIMERS AND EVALUATION OF THEIR POTENTIAL IN SUCCESSIONAL HYDROLYSIS

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Enzymes immobilization enables their stabilization and recycling making more sustainable their use. The immobilization of enzymes on material surfaces covered with multipoint molecules promotes the attachment of enzymes with respect to monofunctional linkers. This work proposes the immobilization of two different proteolytic enzymes (thermolysin and alcalase) on dendrimer-silica supports. Two different dendrimers (PAMAM and carbosilane) with two different generations (zero (G0) and first (G1) generations) were employed and immobilization and hydrolysis conditions were optimized in each case. Optimal conditions for the immobilization of each enzyme were applied to the digestion of a protein. Results demonstrated that thermolysin and alcalase could be immobilized on dendronized silica supports and that the immobilized enzyme could be reutilized for three times. In general, the highest hydrolysis degrees were obtained with G0 PAMAM. The enzyme activity decreased after the first digestion cycle probably because peptides released during the digestion of proteins remained stucked on the own support encapping the enzyme.

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P37. MINIATURIZED SOLID-PHASE MICROEXTRACTION FOLLOWED BY GC-MS FOR THE IDENTIFICATION OF COMPOUNDS WITH ORGANOLEPTIC CHARACTERISTICS IN HONEY

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Honey is a natural product mainly composed by sugars, water and other components like minerals, pollen grains, enzymes, waxes, amino acids, proteins, organic acids and phytochemical compounds¹. Its composition is strongly dependent on the plant species from which the nectar or honeydew was collected, and on other factors such as environmental conditions and climate². For this reason, it is important to identify specific markers to guarantee both the authenticity and quality. A method based on miniaturized solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) has been developed for the determination of volatile and semi-volatile compounds in honey. For preliminary studies, (type of column, dilution, temperature...) multifloral honey was used, concluding that the miniaturized SPME was the best option to conduct extractions. After that a fractional factorial design $3 \times 2^{3-1}$ plus 4 central points giving rise to 16 experiments was applied. The extraction time profile of the process was also studied. The selected method showed satisfactory sensitivity, repeatability, and reproducibility. Finally, real Galician's honey samples were analysed using the developed method.

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P38. ULTRASOUND ASSISTED EXTRACTION FOR THE DETERMINATION OF TOXIC SUBSTANCES IN SURFACES MADE OF RECYCLED RUBBER BY GC-MS/MS

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Tire rubber is a material which is reused to manufacture sport surfaces, children's playgrounds, pavers for different urban uses (e.g. retention of tree roots) and as infill in synthetic turf football fields. This represents a risk to human health because rubber contains toxic substances¹. Due to the concern generated in recent times by the use of recycled rubber in recreational sport surfaces, this study aims to assess the presence of forty-two organic compounds including polycyclic aromatic hydrocarbons (PAHs), plasticizers (phthalates and adipates), antioxidants and vulcanisation additives. These target compounds were selected according to previous studies on synthetic turf football fields² and children's playgrounds³. Ultrasound assisted extraction (UAE) was the technique chosen to extract the target compounds, obtaining satisfactory results, and analysis was performed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). Samples from different places of A Coruña's district (Spain) were collected. They were taken from outdoor and indoor facilities, and some of them were acquired in specialized stores. The analysis confirmed the presence of a large number of toxic substances including, PAHs, phthalates, adipates, antioxidants and vulcanisation additives, including carcinogenic compounds such as chrysene, benzo[a]pyrene and benzo[a]anthracene. These contaminants were detected in all samples analysed, excluding two of them: a cork infill of a football field and a sand playground taken from an urban area with high traffic density.

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P39. DEVELOPMENT AND APPLICATION OF POLYMERIC SORBENTS WITH ZWITTERIONIC CHARACTER

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Mixed-mode ion exchange sorbents integrate both effective reversed-phase chemistry with ion-exchange groups so as to interact with ionic species [1,2]. Nevertheless, the problem of extracting analytes with acidic and basic properties simultaneously within the same cartridge led to introduce novel ion exchange sorbents, like zwitterionic polymers, which incorporate cation and anion exchange moieties in the same functional group attached to the polymeric network.

In this study, two hypercrosslinked zwitterionic polymers have been synthesised and evaluated by solid-phase extraction (SPE) for the retention of acid, basic and amphoteric analytes simultaneously [3]. The weak anion- and weak cation-exchange polymer (HXLPP-WAX/WCX) has a tertiary amine and a carboxylic acid as the weak anionic- and cationic-exchangers, respectively. A secondary amine and a sulfonic acid are the weak anion- and strong cation-exchangers incorporated in the weak anion- and strong cation-exchange polymer (HXLPP-WAX/SCX).

The SPE parameters were optimized to exploit the ionic interactions between compounds and the functional groups and the pK_a values of both the analytes and the functional groups of the sorbents should be taken into consideration. The optimum conditions involve a washing step to eliminate the compounds retained by reversed-phase, thus increasing the selectivity. The method developed was applied to environmental samples, like river water and wastewater, and good performance was achieved.

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P40. MASS SPECTROMETRIC IMMUNOASSAY OF PROTEOFORMS OF APOLIPOPROTEIN A AND C IN INDIVIDUALS WITH OBSTRUCTIVE SLEEP APNEA

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Obstructive sleep apnea (OSA), a common sleep-related breathing disorder is often associated with series of comorbidities of which the most common are diabetes and cardiovascular diseases. It is known that OSA can mediate pathological alterations of cholesterol and triglycerides¹ and is associated with insulin resistance². In this work we performed analysis of Apolipoprotein A (ApoA) and Apolipoprotein C (ApoCs) using targeted proteomics approach-Mass Spectrometric Immuno Assay (MSIA)³ in morning and evening serum samples of 84 male individuals of which 20 was regular snorers and 64 was diagnosed with OSA. Intensity of expressed protein variants are measured, followed by Statistical analysis. Data mining disclosed individual protein variants of ApoA and ApoCs specifically linked with OSA. In combination with selected clinical parameters a multivariable decision tool was created. The validation process, performed using bootstrap samples, showed that decision trees successfully distinguish snorers of individuals with sleep apnea.

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P41. CARBON ISOTOPIC FRACTIONATION OF URINARY METABOLITES IN TRANSGENIC MICE DURING THE DEVELOPMENT OF PROSTATE CANCER

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Cancer is one of the most important worldwide health problems, being one of the leading causes of morbidity and mortality, and this problem is expected to grow due to several factors as the increase of the aging population and the obesity. The development of early detection tools is desperately needed since like the improvement in their treatments mean that the survival rate of individuals diagnosed with cancer increases. Metabolites are the end products of cellular metabolism and it is well known that the cancer cells are characterized by different metabolic perturbation. [1,2]. The metabolic analysis in non-invasive biofluids as urine may help to obtain a complete molecular picture of the biochemistry and ultimately the discovery of new cancer biomarkers. [3]

The urinary analysis was made using Gas Chromatography Isotope Ratio Mass Spectrometry following on-line combustion (GC-C-IRMS) as an analytical method to find out isotopic fractionation in some metabolites This analysis has been performed in an animal model to detect potential biomarkers that could help in the early detection of prostate cancer, which is the second most frequent type of cancer in men [4]. The comparison between healthy and diseased mice was carried out analyzing urine samples from Wild-Type (WT) mice and Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice of C57/BL6 strain and at different ages to study the development of the disease. The aim of this study is to find differentiation in isotope ratio between healthy and sick mice and propose those metabolites as a possible biomarker.

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P42. BIOSYNTHESIS AND CHARACTERIZATION OF ISOTOPICALLY LABELLED DNA AND RNA METHYLATED NUCLEOSIDES FOR THE QUANTIFICATION OF GLOBAL DNA AND RNA METHYLATION BY ISOTOPE DILUTION LC-ESI-MS/MS

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Epigenetic modifications are reversible modifications on the cellular genetic material affecting gene expression without altering the DNA sequence. The most important epigenetic modifications are related to the methylation pattern in DNA cytosine (5-methylcytosine) and RNA adenine (N6-methyladenosine). This plays critical roles in a variety of cellular processes as cell differentiation, gene regulation, stress/immune response and carcinogenesis [1, 2].

LC-ESI-MS/MS is one of the best techniques for the detection and quantification of modified nucleobases due to its remarkable sensitivity and accuracy. Most of the methods perform a previous enzymatic hydrolysis to obtain nucleosides, which are further separated by Liquid Chromatography (LC) coupled to MS detection with an electrospray ionization source (ESI) [1]. The use of isotopically labeled analogs is of outstanding importance as ESI is seriously affected by matrix effects. Moreover, it also allows the application of Isotope Dilution to obtain highly accurate and precise determinations. However, the simultaneous quantification of DNA and RNA methylation using Isotope Dilution Mass spectrometry has not been performed so far.

Here, we present a cost-effective procedure to obtain ¹⁵N-labelled nucleic acids and their application to simultaneously quantify the global DNA and RNA methylation. Microalgae *Chlamydomonas reinhardtii* CC503 were grown in a media containing ¹⁵NH₄Cl as the only nitrogen source. Then, the biosynthesized ¹⁵N-DNA and ¹⁵N-RNA were extracted and hydrolyzed. The isotope labelled nucleosides were characterized in terms of purity, concentration and isotopic enrichment. Finally, the isotopically labelled nucleosides were used to quantify DNA and RNA methylation in samples with a known level of methylation for validation purposes.

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P43. SURVEY OF α -SYNUCLEIN PROTEIN VARIANTS USING EXTREME RESOLUTION FOURIER-TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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α -synuclein is the key protein in synucleinopathies¹ of which the vast majority of cases are sporadic. Since mutations only appear to play a very minor role in some familial cases, other hypothesis must be sought after to explain idiopathic origin, detect early on-set of the disease and lead the way towards effective therapeutic options. Enzymatic and non-enzymatic post translational modifications such as glycation, play a crucial role in protein structure and stability. Indeed, we and others have shown that glycation is broadly involved in amyloid diseases from Alzheimer's to Parkinson's and transthyretin amyloidosis². A detailed description of all proteoforms of a protein like α -synuclein call for an integrative approach based on non-biased enrichment methods, extreme resolution and high dynamic range mass spectrometry. Moreover, effective gas-phase ion activation methods capable to preserve modified amino-acid side chains while providing extensive interrogation of protein sequence are needed. Here we report the extensive coverage of primary structure of human α -synuclein proteoforms, at the extreme resolution provided by a dynamically harmonized cell equipped FT-ICR mass spectrometer and its multiple techniques and applications.

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P44. STUDY OF AN *IN VITRO* MODEL OF HIGH GLUCOSE-INDUCED CHANGES IN HUMAN PROXIMAL TUBULAR HK-2 CELLS USING A CE-MS METABOLOMIC STRATEGY

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Diabetic nephropathy is the major cause of end-stage renal disease and currently there is no cure. In this sense, metabolomics approach provides crucial information which helps developing new therapies. It is now evident that functional and structural changes in proximal tubular cells play a critical role in the development of the disease. Therefore, the purpose of this work was to study an *in vitro* model of high glucose-induced metabolic alterations in human proximal tubular HK-2 cells. Thus, an untargeted metabolomic strategy based on capillary electrophoresis-mass spectrometry (CE-MS) was developed to find the metabolites which were affected under high glucose conditions. It is important to highlight that this is the first time that this study is carried out using a CE-MS platform. Three different groups of cells, high glucose, low glucose (glucose control) and mannitol (osmotic control) were analyzed. Both intracellular and extracellular fluids were studied for expanding the metabolite coverage. The differences among the three experimental groups were presented by principal components analysis and partial least square discriminant analysis. Moreover, molecular features were discovered thanks to the combination of variable importance in the projection values and Mann Whitney U univariate test. Some metabolites, mainly amino acids were identified in both fluids.

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P45. A LC-MS METABOLOMIC-BASED STRATEGY FOR THE SEARCH OF POTENTIAL MARKERS OF COCOA POWDER ADULTERATION

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Cocoa powder is an important ingredient in the manufacture of numerous foods and beverages due to its characteristic and pleasant flavor and aroma. Lately, its demand has increased while its supplies have tightened because of climatic changes or deforestation, among other reasons. Consequently, the addition of low-cost raw material for an economic profit is a food fraud that affects both producers and consumers. Until now, MS-based metabolomics approaches have been used to search markers of geographical origin, growing region, and fermentation process of cocoa [1-3] but not to investigate cocoa powder adulteration. Thereby, the aim of this work was to develop an untargeted LC-MS metabolomic approach for the search of adulteration markers of cocoa powder samples. Different groups of samples were analyzed including cocoa powder without adulterant materials and with low and high levels of chicory, carob and soy flour. Principal component analysis and partial least square discriminant analysis were performed to find differences among the experimental groups. The variable importance in the projection (VIP) values were employed to select the most relevant metabolites which can be considered potential markers of cocoa powder adulteration.

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P46. AN EXTENSIVE PHOSPHOPROTEOMIC ANALYSIS SUGGESTS AN UP-REGULATION OF SFK-RELATED SIGNALLING PATHWAYS IN PLATELETS FROM OBESE PATIENTS

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Central obesity corresponds to an excess of intra-abdominal adipose tissue. It is well known that obesity-associated alterations constitute a relevant risk factor for suffering cardiovascular diseases (CVD), especially, the development of atherothrombosis. Indeed, recent studies focused the attention on the pathogenetic role of platelet hyperactivation and reduced sensitivity to antiaggregating therapy in obese patients.

The aim of this study was to identify potential biomarkers and altered activation pathways associated to the risk of suffering atherothrombosis in obesity.

We performed a comparative phosphoproteomic analysis of platelets from obese patients and their age- and sex-matched controls. Platelets were obtained from a cohort of 24 individuals (12 obese patients and 12 lean controls). Phosphopeptides were enriched with TiO₂ and analyzed by Label free LC-MS/MS. Differential analysis was done by Progenesis QI software.

Regarding the proteomic analysis, we identified a total number of 3765 phosphopeptides. From those, we found that 325 were differentially regulated between conditions corresponding to 11 pTyr, 57 pThr and 257 pSer. Interestingly, all of them were up-regulated in obesity. These phosphopeptides described at least 181 proteins; most of them related to platelet activation, vesicle transport and cytoskeleton organization. Some of the altered pathways identified were integrin α IIb β 3 and GPVI, which are mediated by Src-kinase family (SFKs) and play an important role in platelet activation. Our results show an up-regulation of key phosphosites from relevant signaling proteins. Among others, integrin β 3 tail (**Thr**⁷⁸⁸) is increased in obesity, which is necessary for SFKs binding upon activation. Indeed, we also found increased levels of SFKs **Tyr**⁴¹⁹ residue, which supports kinase full activation. Other phosphoproteins up-regulated in obesity are involved in GPVI downstream signalling such as PKC θ (**S**⁶⁹⁵) and Filamin-1 (**S**¹⁴⁵⁹), which support calcium liberation and cytoskeleton reorganization.

In summary, our results show an up-regulation of the SFK-related signaling pathways, such as integrin α IIb β 3 and GPVI, in platelets from obese patients, which could be essential to further understand platelet hyperactivation in obesity.



P47. DISCOVERING CHEMICAL PROCESSES DRIVING WINE REDUCTIVE PROBLEMS USING A UPLC-QTOF-BASED METABOLOMIC APPROACH

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Wine suffers important changes during aging in presence of oxygen but also in anoxic conditions. Reactions promoted by O₂ have been deeply studied but changes responsible for the most evident effects of reduction (decrease of redox potential and release of volatile sulfur compounds, VSCs) are not so well-known. The modern winemaking techniques tend to favor anoxic conditions therefore, reduction problems occur more frequently. The objective of this work was study changes that happened in the metabolome of wines stored under different redox conditions. Twelve wines (8 reds and 4 whites) of four grape varieties were selected. Aliquots of each wine were distributed in vessels with controlled oxygen permeability to induce different oxidation levels and were stored at 25°C during 3 months. Aliquots of the same wine were stored at the same temperature during 1, 2 and 3 months and also at 35°C for 3 months in complete absence of oxygen. All samples were analyzed using an UPLC-HRMS-QTOF instrument following a metabolomic strategy. Target analysis of VSCs was carried out by GC-SCD. The measured redox potential confirmed that different redox states of samples were achieved with our approach. More than 10000 features were detected by LC-MS analysis which were reduced to 150 markers applying different filters. Behavior of some markers is remarkable, for example some anthocyanins glucosides disappear in oxidative and also in reductive conditions while concentration of some pigments decreases under oxidation but, increases under reduction. Overall, results bring new light into chemical changes taking place in the absence of oxygen, revealing the existence of an interesting and unexplored chemistry which may explain why wine can become spontaneously enriched in H₂S and mercaptans when stored in complete anoxia.

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P48. POSTTRANSLATIONAL MODIFICATION DIFFERENCES IN *BRACHYSPIRA HYODYSENTERIAE* AND *B. PILOSICOLI*. A PROTEOMICS STUDY.

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Post-translational modifications (PTMs) of bacterial proteins were almost unknown until the advent of mass spectrometry. Mass spectrometry-based proteomics has revealed that prokaryotes are able to modify their proteins with a high number of PTMs which have influence on bacterial physiology and virulence [1]. Acetylation is the most studied PTM in prokaryotic organisms [2]. Proteins involved in metabolism are the main group of acetylation targets. This modification has been related to the degree of virulence, adaptation to environmental conditions, and persistence in several bacterial species. In this study, acetylomes of *Brachyspira hyodysenteriae* and *B. pilosicoli* have been characterized. These species are known gut pathogens; *B. hyodysenteriae* is the causative agent of swine dysentery and *B. pilosicoli* causes colonic spirochaetosis in pigs and is responsible for a human form of the disease.

Bacterial acetylomes were prepared by immunoprecipitation with specific anti-acetyl Lys antibody and analysed in an Orbitrap Fusion Lumos™ Tribrid coupled to a nanoLC system. Raw data were processed with Proteome Discoverer and filtered at 0.1% FDR using Percolator. Localization probability for each site was calculated using ptmRS.

3142 and 5496 acetylated peptides (3221 and 5579 sites) were identified in *B. hyodysenteriae* and *B. pilosicoli*, respectively. The two sets of acetylated proteins were found to be enriched in proteins involved in metabolic pathways and the biosynthesis of secondary metabolites. We have reported a different degree of proteome acetylation in these species, being *B. pilosicoli* proteome the most acetylated one. We have shown that the main targets of acetylation were proteins involved in metabolism and that the differences observed are reflected in the different composition of the components involved in the Acetyl-CoA/Acetate metabolic pathway.

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P49. ESTABLISHMENT OF URINARY VOLATILOME OF DISTANT HUMAN POPULATIONS. A USEFUL APPROACH TO DETECT POTENTIAL LUNG CANCER BIOMARKERS

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Urine is used as diagnostic tool for different clinical conditions since ever. In the past, the urine smell was the primary source for such diagnosis, but with time such qualitative diagnosis was abandoned and substituted by rigorous analytical determination of different metabolites. However, several works have shown that the urinary volatome constitute a diagnostic tool with great potential for the non-invasive diagnosis of different diseases, namely cancer. In this work, we have analysed urine samples from LC patients and controls of two distinct geographic regions in Portugal and India by using a headspace solid-phase microextraction gas chromatography-mass spectrometry that we previously optimized. The study involved control subjects and lung cancer patients recruited in both countries, from both sexes, smokers and non-smokers. This creates a substantial variation in the volatome data obtained and this was used to assess the influence of the external variables considered in this study in the robustness of the statistical models obtained. Overall, given the high number of variable (urinary volatiles identified), it became clear that the fidelity of the statistical models obtained is dependent on the number of subjects recruited, both increasing in the same proportion. Moreover, the study also showed that the application of the same experimental layout in both research groups, limiting all experimental variations, including the hardware, which was the same and using the same operation parameters, it is possible and desirable to extend this volatome strategy to larger cohorts.

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h ESTIMATION OF THE MOST APPROPRIATE URINE SAMPLING PERIOD BASED ON THE URINARY VOLATOMIC PROFILE

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The worldwide high incidence and cancer mortality justify the development and implementation of effective and non-invasive strategies leading to early diagnosis. Biological fluids such as urine are rich in several metabolites including volatile organic metabolites (VOMs) that can reflect imbalances of all biochemical pathways. Theoretically, urinary volatonic profiles could be useful to characterize the disease itself, as well as disease progression and response to therapy. However, the presence and concentrations of VOMs may depend not only on biochemical or pathologic processes but also on physiological parameters. As urine sampling can be done in different periods (e.g. fasting, after or before food ingestion), there is a lack of comprehensive studies on the impact of sample collection period on volatile composition of human urine. Therefore, in order to overcome this weakness, we aimed to investigate the influence of the sampling period through the urine collection at different periods - first urine morning, before/after lunch and afternoon, from healthy volunteers, over a period of 25 days. For that, the variability of VOMs was analysed by means of HS-SPME/GC-MS combined with multivariate statistical tools in order to find if the volatile urinary fingerprint is affected by the period of urine collection. The preliminary results are very promising, showing significant differences between some sampling periods which might allow an in-deep and comprehensive understanding regarding the best collection period as a tool to select the more adjusted sampling period and reduce the presence of external confounding factors.

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P51. APPLICATION OF NOVEL AUTOMATED DATA DEPENDENT ACQUISITION (DDA) IN UNTARGETED METABOLOMICS

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The untargeted metabolomic approach has become widespread in different fields of applications such as medicine, nutrition and agriculture. An untargeted metabolomics study usually starts by sample analysis, where the coupling of chromatographic techniques with high resolution mass spectrometry has gained importance; and ends with the characterization of the relevant markers highlighted in the statistical process. For this end, second-injection is habitually required to obtain their fragmentation spectra (MS^2) [1]. To save time, it would be necessary to obtain both HRMS and MS/MS for all the metabolites of interest within a single analysis. The main objective of the present work was the implementation, optimization and application of a novel methodology for the automatic but “intelligent” MS^2 acquisition metabolomics workflow on a last-generation Fusion Orbitrap for the large-scale analysis of human plasma metabolites. The AcquireX intelligent MS^2 data acquisition strategy (Thermo Scientific), uses an automated iterative exclusion and inclusion lists to obtain fewer redundant data dependent MS^2 features produced by the background, as well as to perform exhaustive precursor selection and obtain more relevant MS^2 spectra. Then, such lists are automatically imported into the data dependent acquisition (DDA) method before the first LC- MS^2 acquisition of the sample and updated prior the next LC- MS^2 run, bypassing precursors already fragmented to the exclusion list. MS^2 spectra are acquired for compounds remaining on the inclusion list. This approach enables to obtain complete data and cover a wider range of compounds (including the lower-abundance ones) that, until now, the traditional methods of DDA have not achieved. This would provide valuable qualitative information for compound characterization at great depth.

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P52. DEVELOPMENT OF A QUECHERS-BASED METHOD FOR THE ANALYSIS OF PPCPS IN VEGETAL MATRICES AND AGRICULTURAL SOILS

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Pharmaceuticals and personal care products (PPCPs) are a diverse group of substances, mostly considered contaminants of emerging concern (CECs). Because of their wide and daily basis use, these compounds are continuously released into the environment. Current wastewater treatment technologies are not efficient at removing such contaminants and thus, they are again discharged into the aquatic environment. Because of their pseudo-persistence, these CECs could be up-taken by crops or absorbed by fish. Considering that both are used for human consumption, their occurrence may put the human health at risk. Many studies have reported occurrence data of PPCPs in the environment. However, studies describing the use of reclaimed wastewater for irrigation of plants and fruits are very scarce. One study determined 19 PPCPs in crops, although the vegetables were grown under hydroponic conditions [1]. Another reported no data from the analysis of 118 PPCPs, because all the investigated analytes were below LOD [2]. However, this could be improved by applying more efficient extraction procedures. In 2003, a new extraction method called QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was developed [3]. This procedure has frequently been used for the extraction of pesticides from food matrices [4]. In the present work, the QuEChERS approach is used as extraction technique in the HPLC-ESI (QTRAP)-MS/MS analysis of 62 PPCPs in different crop products, such as lettuces and soils irrigated with both, WWTP effluent and renaturalized water by infiltration through reactive barriers. The influence of the PPCPs accumulation on the different soils (sandy, clayey), as well as the irrigation system (drip, sprinkler) used are also evaluated. The validation and optimization of the method will be presented and discussed and the applicability to determine the selected PPCPs in agricultural products will be also shown.

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P53. COMPATIBILITY AND PHYSICAL-CHEMICAL STABILITY OF INTRAVENOUS MIXTURES FOR MULTIMODAL ANALGESIA BY UHPLC-DAD

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Nowadays, multimodal analgesia is a common practice recommended by clinical practice guides for the treatment of post-surgery pain. This practice is based on the combination of different drugs and analgesia techniques in order to achieve more efficient and less secondary effects.

The main objective of this project is to evaluate the physical and chemical compatibility of drug mixtures used in postoperative treatment in a tertiary hospital. The six target drugs are Ketamine, Tramadol, Ondansetron, Ketorolac, Methadone and Dexketoprofen. The stability of the mixtures prepared in physiological saline solution is evaluated at room temperature for a short period of time (0, 24, 48 hours) and refrigerated for a longer period (7, 14, 30 days).

In order to achieve this goal, two UHPLC-DAD methods have been developed to analyse the 6 target drugs in a single chromatogram. This milestone has excellent operational advantages, although only three or four drugs would be into each analgesia mixture treatment. Acquity BEH and HSS C18 (100x2.1 mm) columns, acetonitrile and HCOOH/NH₃ buffer in gradient mode and DAD (215, 230, 254, 305 nm) are used. In the designed procedures, the 6 drugs, having different pKa, elute in a significantly different order depending on the mobile phase pH. One method is used to quantify the drugs in samples and the other developed method is used to corroborate the results of the previous one. Using two orthogonal methods, the interference risk of the degradation products to the quantification of the active substances is clearly being reduced.

The quality assays confirm that the methods are stability indicating methods (SIM). As the degradation products are not accessible, forced degradation must be performed. With the aim of achieving degradation, standards of 6 target drugs have been subjected to acid (HCl 1 M, 80 °C), basic (NaOH 1 M, 80 °C) or oxidative (H₂O₂ 15 %, room temperature) treatment. The established criteria of no more than 20 % of active substance degradation, ensures that only primary degradation products have been formed, not secondary ones.

The quality of the proposed protocol has been confirmed by studying a first mixture of three analgesics (Ketamine, Tramadol and Dexketoprofen) in saline solution.

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P54. COMPARATIVE DEGRADATION OF TWO HIGHLY CONSUMED ANTIHYPERTENSIVES IN WATER BY SONOCHEMICAL PROCESS

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In 2016, around 250 million people in Latin America and the Caribbean zone were affected by hypertension. Pan-American health organization (PAHO) created programs on the prevention and control of this illness and mechanisms to make antihypertensive drugs more available and affordable within the Americas [1]. Consequently, compounds used for hypertension treatment (antihypertensives) are frequently detected in environmental waters. Indeed, antihypertensives such as losartan (LOS) and valsartan (VAL), which act as angiotensin II receptor antagonists, have been found in surface water and groundwater, respectively [2].

This work compares the sonochemical degradation of losartan and valsartan (antihypertensives) in water. Initially, the suitable operational conditions of ultrasonic power density and frequency were established. Under such conditions, losartan was eliminated in a higher percentage than valsartan, which was associated to differences in their hydrophobicities. Additionally, degradations in presence of isopropanol and ferrous ions confirmed that losartan was closer to cavitation bubble than valsartan. Afterwards, the structures of primary products indicated that sonogenerated hydroxyl radical attacked biphenyl tetrazole moiety (common nucleus of both pharmaceuticals). Then, theoretical calculations were applied to the products to estimate the toxicity, degree of oxidation and probable routes of aerobic biodegradation suggesting a beneficial action of sono-degradation. Finally, the sonochemical degradation of the antihypertensives was carried out in two simulated complex matrices (i.e., seawater and hospital wastewater) and an actual wastewater. Interestingly, the losartan and valsartan eliminations in such waters was similar to the observed in distilled water. This fact indicates the high potentiality of ultrasound to degrade losartan or valsartan in waters containing other substances, even at higher concentrations than these pollutants.

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P55. DEVELOPMENT OF A MODIFIED QuEChERS PROTOCOL TO IMPROVE EXTRACTION EFFICIENCIES OF PHARMACEUTICALS FROM LETTUCE IRRIGATED WITH RECLAIMED WATER

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Edible plants, like lettuce, grown in contaminated soils irrigated with reclaimed water have been found to uptake and accumulate pharmaceuticals in their tissues through roots posing threats to the public health. In order to ensure the safety of the edible plants, there is a strong need to increase the knowledge on the behavior of pharmaceuticals in crops developing reliable analytical methodologies. Their detection is usually performed by LC-MS using both low and high resolution. Sample preparation is often a key parameter in the development of new analytical methods. Several techniques of sample preparation for the extraction of pharmaceuticals from crops has been used such as solid-liquid extraction and pressurized liquid extraction (PLE), ultrasonic liquid extraction (ULE) and soxhlet extraction (SE) followed by an extract clean-up with solid phase extraction cartridges. One alternative to the previous methods can be the use of the versatile QuEChERS protocol, initially developed for the extraction of pesticides from vegetables, which includes extraction and purification steps in one kit. The selectivity of extraction can be improved, among others, by the selection of the most efficient extraction solvents. For the extraction of pesticides, solvent modifications have been proposed, such as acidified solvents.

Therefore, the present study involved, primarily, the development and validation of an extraction method for pharmaceuticals using QuEChERS-based method using a modified extraction solvent and liquid chromatography coupled to quadrupole time of flight high resolution mass spectrometry. Secondly, the application of proposed method to assess the potential uptake of pharmaceuticals by lettuce tissues irrigated with both real and fortified wastewaters. To validate the optimized method, recovery tests were performed at five spiking levels. Satisfactory recoveries were achieved. The MRM-HR mode was used to determine the occurrence of 42 compounds (pharmaceuticals and some of their metabolites). All samples analyzed in triplicate were positive for 20 compounds. The compounds that accumulated the most in lettuce leaves irrigated with real reclaimed water were Carbamazepine followed by Fluconazole and Clarithromycin. The lowest concentrations were observed for Climbazole with average values below 6ng/g. Acidic compounds, such as Diclofenac and Sulfamethoxazole were not detected at all. This could be attributed to a low potential for uptake, translocation, or accumulation of these compounds by lettuce plants, due to plant physiology itself, compound degradation in the soil or preservation in plant roots. The results revealed that most of the target compounds can potentially accumulate in different rates in lettuce plants irrigated with both real and fortified wastewaters.

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P56. DEVELOPMENT AND VALIDATION OF A LC-ESI-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF AFLATOXINS B₁, B₂, G₁, AND G₂ IN MEDICINAL PLANTS

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Aflatoxins are secondary metabolites produced by filamentous fungi of the genus *Aspergillus*, particularly *A. flavus*, *A. parasiticus* and *A. nomius* in determined stress conditions of temperature and humidity. Aflatoxins B₁, B₂, G₁, and G₂ are the most common and studied aflatoxins due to their genotoxic and carcinogenic effects, and natural occurrence as contaminants in agricultural products such as plants. Medicinal plants are widely used as home remedies and raw materials for pharmaceutical industries. The European Pharmacopoeia is the only one organism with a maximum permitted levels of aflatoxins in herbal drugs (NMT 2 µg·kg⁻¹ of aflatoxin B₁ and 4 µg·kg⁻¹ for the sum of aflatoxins B₁, B₂, G₁, and G₂). Due to the increasing consumption of medicinal plants and the lack of a global legislation that establish maximum permitted levels of each aflatoxin in herbal drugs their use has become a problem of public health concern. There are also no official methods available for quantifying aflatoxins in low concentrations (ppb) in complex matrices such as plants.

In this study, a new analytical method has been developed and validated for the simultaneous analysis of aflatoxins B₁, B₂, G₁, and G₂ in different medicinal plants acquired from local herbal stores (*Menta piperita*, *Osmarinus officinalis* and *Eucalyptus globulus*). The extraction and purification steps were established by optimizing the extraction mode, temperature, solvent ratio, sample weight, and SPE and immunoaffinity (IAC) columns. The optimized procedure was based on accelerated solvent extraction (ASE) using MeOH:H₂O (3:2), followed by IAC clean-up. The extracts were analyzed by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), achieving a limit of quantification of 1 µg·kg⁻¹. No trace level has been found in studied samples.



P57. DETERMINATION OF CREATININE AND CREATINE IN HUMAN SERUM BY TWO DIMENSIONAL LIQUID CHROMATOGRAPHY AND DOUBLE-SPIKE ISOTOPE DILUTION LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Serum creatinine determination is widely employed in the diagnosis of kidney diseases. However, the possible interconversion between creatine and creatinine during sample preparation may lead to significant errors particularly in samples with high creatine content. We have previously developed a methodology [1] capable of correcting and quantifying the creatine-creatinine interconversion occurring during the analytical determination of both compounds in human serum samples. The methodology was based on the use of minimally labeled ¹³C analogues (¹³C₁-Creatinine and ¹³C₂-Creatine) and the measurement of the isotopic distribution of creatine and creatinine by LC-MS/MS. This method was based on a cation-exchange chromatographic separation in which a significant ionization suppression affecting the creatine determination was observed in some serum samples.

This work describes an improvement of the previous methodology [1] based on the application of two dimensional liquid chromatography (2D-LC) that allows to work in a multiple heart cutting mode. A reversed phase separation is applied in the first dimension and combined with a cation exchange chromatography in the second dimension. The on-line isolation of the fraction in which creatine and creatinine coelute from the first dimension allows a faster chromatographic separation in the second dimension by cation exchange with less matrix effects than previously reported [1]. The analytical characteristics of the method are presented as well as validation experiments with the analysis of fortified serum samples and certified reference materials.

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P58. DETERMINATION OF NEW PSYCOACTIVE SUBSTANCES BY US-DLLME AND ULTRA PERFORMANCE LIQUID CROMATOGRAPHY

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In the recent years, new psychoactive substances (NPS) have appeared on the illicit market as substitutes for other drugs, such as MDMA (ecstasy). Some of these NPS are not regulated by the United National Psychotropic Drugs Convention or their regulation is limited to certain countries. Synthetic cathinones have become important, owing to they can be more potent and toxic than other drugs. For example, methyldone is an analog of MDMA [1].

On the score of the appearance of these NPS, it is necessary to develop fast and reliable analytical methods for their determination in different biological matrices. Miniaturization and green chemistry principles are the goals of the microextraction techniques. An example is the dispersive liquid-liquid extraction (DLLME). Reduced volumes of sample and reagents are used, as well as minor time of extraction and costs. Oral fluid is a biological sample very use in road traffic, since it presents advantages, such as no invasive collection, stock up and transportation [2].

A new analytical method has been validated and optimized for the extraction of 4 “second generation” synthetic cathinones in oral fluid. US-DLLME was used as an extraction technique of cathinones and their subsequent determination by ultra-performance liquid chromatography coupled to diode array detector.

Acknowledgement

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**P59. MULTITARGET ANALYSIS BY GC-MS/MS FOR DOPING SUBSTANCES IN URINE
SAMPLES: EVALUATION OF DETECTION TIMES OF DIFFERENT MESTEROLONE
METABOLITES**

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The most common technology to detect the misuse of substances in sports is LC-MS. However, some anabolic androgenic steroid (AAS) metabolites are not effectively ionized under electrospray ionization conditions. Therefore, GC-MS/MS constitutes a reliable alternative for the detection and quantification of those substances in urine samples. The aim of the present work was to develop and validate a GC-MS/MS method capable of reaching the minimum required performance limits (MRPLs) established by the World Antidoping Agency (WADA), not only for anabolic steroids, but also for other doping agents such as hormone antagonist and modulators, β 2-agonists and stimulants. A multiple reaction monitoring (MRM) GC-MS/MS method (containing 134 compounds with two specific transitions for each one) was developed. Urine samples were subjected to a standard sample preparation procedure consisting on an enzymatic hydrolysis with β -glucuronidase from *Escherichia Coli* followed by a liquid-liquid extraction and a derivatization step to form the trimethylsilyl (TMS) derivatives of hydroxy and keto functional groups. The extraction of ten blank urine samples, six samples fortified at low concentrations equivalent to the 50% of MRPL, six samples fortified at higher concentrations and six samples spiked after the extraction procedure for recovery calculations, was done in order to test and validate the new method. No interferences were detected at the corresponding retention times, confirming the specificity of the method. For those AAS metabolites that are not commercially available, urine samples obtained after administration of the AAS to healthy volunteers were analyzed. Finally, the method was applied to evaluate the detection times of different mesterolone metabolites in urine by analyzing samples from an excretion study. Thus, a single dose of 25 mg of mesterolone was orally administered to two healthy volunteers and urine samples were collected up to 31 days. The best marker to detect mesterolone administration was evaluated.



P60. METABOLIC PROFILING OF THE NOVEL INDANYL-CATHINONE 5-PPDI AFTER HUMAN HEPATOCYTE INCUBATION

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Synthetic cathinones represent a tremendous health risk worldwide. For most of the 130 reported compounds, information about toxicology or metabolism is not available, which hampers their detection (and subsequent medical treatment) in intoxication cases [1]. In this sense, the forensic analytical chemistry and the use of powerful instrumentation are needed for establishing the most proper biomarkers for these substances. Human metabolic fate of synthetic cathinones can be assessed by the analysis of urine and blood obtained from authentic consumers; however, this type of samples are very limited and difficult to access.

In this work, the metabolic behaviour of the recently reported cathinone 3,4-trimethylene- α -pyrrolidinobutiophenone (5-PPDi) [2] has been evaluated by incubation with pooled human hepatocytes and metabolite identification by high-resolution mass spectrometry (HRMS), using a hybrid quadrupole-Orbitrap instrument. This *in vitro* approach has previously shown its feasibility for obtaining excretory human metabolites. Firstly, samples were analysed using Data-Dependent Acquisition (DDA) mode and processed using Compound Discoverer 2.0 software, searching for expected biotransformation based on compound structure. Once identified potential metabolites based on DDA MS/MS fragmentation (collision energy at 25 eV), samples were re-analysed by Parallel Reaction Monitoring (PRM), acquiring fragment ions at three different collision energies (10, 30 and 50 eV). PRM data allowed the elucidation of the metabolite structure. In this way, twelve phase I metabolites were elucidated, all of them produced by hydroxylation and/or oxidation processes. The oxidative indanyl ring-opening and pyrrolidine ring-opening metabolites were the major ones detected in terms of response. Nevertheless, 5-PPDi presented at the end of the incubation around 67% of its initial response, illustrating, again, that synthetic cathinones have a high resistance to hepatic metabolism. Up to our knowledge, this is the first metabolic study of an indanyl-cathinone.

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P61. IDENTIFICATION OF DRUGS OF ABUSE IN BIOLOGICAL FLUIDS BY DIRECT ANALYSIS USING ATMOSPHERIC SOLIDS ANALYSIS PROBE-HIGH RESOLUTION MASS SPECTROMETRY

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The use of drugs of abuse (DOAs) represents an important health and social problem worldwide. According to the most recent report from the European Monitoring Centre for Drug and Drug Addiction (EMCDDA), the consumption of illicit drugs has increased in the last years, especially MDMA and cocaine [1]. The control of DOAs in biofluids is routinely performed by test strips, which provide a rapid result to the authorities. Nevertheless, positive samples should be lately re-analysed by validated and high-discriminatory analytical methodologies [2]. For this purpose, chromatographic techniques coupled to mass spectrometry (MS) are among the most powerful and used equipment. However, these techniques have a high cost per sample technique and are quite time consuming. Within this context, there is an emerging need for the continuous development of fast and reliable analytical methods suitable to directly determine these compounds in biological samples. Recently, the atmospheric solids analysis probe (ASAP) ambient source has proved its applicability for the direct determination of tobacco markers in saliva [3] or amphetamine in urine [4]. In this work, the capabilities of ASAP-HRMS (QTOF MS) have been explored for a rapid identification and quantification of 10 selected DOAs and metabolites in urine and saliva. A modified glass capillary has been used, based on an open capillary tube instead of the sealed one traditionally used in the ASAP source. ASAP-HRMS determination was performed in TOF-scan and TOF-MRM acquisition modes. The results have showed that both techniques allowed the identification of the illicit compounds in only 3 min of analysis. The developed methodology will be useful for an initial and rapid screening of biofluids suspect to contain illicit compounds. In a second step, only those positive samples would be analysed by chromatography-mass spectrometry for an unequivocal identification and accurate quantification. Finally, this methodology could be implemented in small-size mass spectrometers, to create a “portable” instrument that could be used, for example, in driving under drug influence (DUID) controls.

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P62. AN OVERVIEW OF SYNTHETIC CANNABINOIDS CONSUMPTION PATTERNS IN VALÈNCIA OVER 2018

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The consumption of synthetic cannabinoids is an increasing problem. Although, in Spain the number of seizures and intoxications related to this type of compounds is lower than in the rest of Europe [1], the analysis of herbs sold in smartshops and web pages reflect that these products are available in our streets [2].

In this work, the consumption pattern of synthetic cannabinoids in the city of Valencia (Spain) and surroundings is studied through the analysis of herbal blends, cigars and urine samples collected in different Units of Addictive Behaviours (UCAs) of the València city. Analyses were performed by ultra-high performance liquid chromatography coupled to ion mobility separation high resolution mass spectrometry (UHPLC-IMS-HRMS).

Different products available in a local smartshop were analysed, as well as herbs seized in the UCAs. The analyses revealed the presence of the synthetic cannabinoids XLR-11 and UR-144. The urine of a patient, attended by cannabis consumption and which yield a negative result to routine controls, was also analysed. In this case, enzymatic hydrolysis with β -glucuronidase was performed prior to UHPLC-IMS-HRMS analysis, in order to detect phase I metabolites. Metabolites of XLR-11/UR-144, previously reported in the literature, were found in different urine samples from this patient, illustrating the consumption of synthetic cannabinoids.

After the summer, new samples of weed and cigars were seized in the UCAs, now identifying the dangerous cannabinoid 5F-ADB. The main metabolites reported for this cannabinoid were also detected in the urine samples of the same patient collected after summer. This change in consumption perfectly fits with the Spanish ban of XLR-11 in July 2018, illustrating how the regulation of these compounds alters their availability and, therefore, their consumption pattern.

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P63. DEVELOPMENT AND VALIDATION OF AN UHPLC-HRMS MULTI-SCREENING METHOD IN URINE FOR DOPING CONTROL PURPOSES

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Doping analysis is subjected to constant evolution. Laboratories have to manage a rising number of samples and continuously lower the required analytical performance level in routine daily operations. Moreover, there is a need to expand the scope of the established methods by including new compounds every year. Therefore, even more powerful assays than those already employed in sports drug testing are required.

In this study, Ultra-High Performance Liquid Chromatography (UHPLC) coupled to High Resolution Mass Spectrometry (HRMS) was performed in order to develop a multi-target approach assay for different compounds such as stimulants and hypoxia inducible factor (HIF) stabilizers as stated in the World Anti-Doping Agency (WADA) list of prohibited substances. The best UHPLC-HRMS conditions to evaluate 16 different compounds in the same assay (6 stimulants, 7 HIF agents, 1 metabolic modulator, 1 masking agent, and 1 ethanol metabolite) were established. Several acquisition methods such as full scan, targeted-Single Ion Monitoring (t-SIM), and full scan with in-source collision-induced dissociation (CID) were combined in positive and negative ionization modes for the determination of all the studied compounds at the required sensitivity. Moreover, an All-Ions Fragmentation (AIF) scan was also included to provide additional ions for identification of the compounds.

Under optimum conditions, the method was validated with regards to limits of detection, selectivity, matrix effects, and intra-day precision. Method validation was performed at or below minimum required performance levels specified by WADA technical documents for all the analytes, hence showing the potential of the optimized methodology for these compounds in doping control analyses.



P64. POTENTIAL OF COLLISION CROSS-SECTION MEASUREMENTS FOR STRUCTURAL CHARACTERIZATION OF CONJUGATED ANABOLIC STEROIDS

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Endogenous and exogenous anabolic steroids are banned in sports by the World Anti-Doping Agency as a consequence of their potential use to increase athlete's performance. Generally, the unequivocal identification of these compounds is deemed challenging, considering their large variety and the similarity in their structures. For this purpose, anti-doping laboratories normally perform analytical methods based on gas or liquid chromatography coupled to mass spectrometry to obtain the retention time and characteristic ions of the prohibited analytes. Unfortunately, some difficult cases need further information to ensure the correct identification of the steroid.

Recently, ion mobility spectrometry (IMS) has been applied in different analytical fields to determine Collision cross-section (CCS). As it is undertaken in gas phase and is not generally affected by the sample matrix, the CCS values could be taken into account as an additional parameter for steroid characterization.

In this work, the CCS determination of 103 anabolic steroid metabolites (including unconjugated and sulfate and glucuronide conjugates) was performed by liquid chromatography coupled to traveling wave IMS. A time of flight mass analyzer was used to perform the determination of the analytes at high-resolution. The chromatographic separation was carried out with a C18 column and a binary mobile phase of acetonitrile:water both with 0.01% formic acid and 1mM ammonium formate. Under these conditions, all analytes were ionized under electrospray in positive and negative mode, forming $[M+H]^+$, $[M+NH_4]^+$ or $[M-H]^-$ as main ions. High reproducibility was observed for the CCS determination, obtaining RSD lower than 0.3% in all cases. In general, CCS values were dependent on the ion mass, and allowed differentiating between glucuronides, sulfates and free steroids, although they did not show significant differences among steroids of the same group. However, more specific information was obtained observing differences in the CCS value of isomeric couples with regards to conjugation position or α/β conformation. These features could be useful in the structural elucidation of new steroid metabolites.



P65. ANALYTICAL COMPARISON OF INFLIXIMAB AND RELATED BIOSIMILARS

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Monoclonal antibodies (mAbs) encompass a rapidly growing therapeutic market. While new antibodies are continually being discovered, expiring patents of the earliest antibodies have prompted generics, or biosimilars, to emerge. Because mAbs are such large proteins, creating an exact replica is nearly impossible. While the amino acid sequence remains largely the same, post-translational modifications (PTMs), like glycosylation, will vary depending on the cell line and manufacturing processes used. Because of these variations, it is necessary to fully characterize these new biosimilars. Here, we show an in-depth characterization of an antibody therapeutic mAb that recently came off patent, Infliximab, and other biosimilars. Finally, we demonstrate the utility of Phenomenex bioZen LC columns for the characterization of these antibodies.

It can be concluded that: Intact Mass: Deglycosylated intact mass shows differences in the primary amino acid sequence, Peptide Mapping: Adequate sequence coverage was obtained. Post-translational modifications were characterized and quantified Released Glycan: Glycan quantitation was done for each of the Infliximab antibodies SEC: Differing levels of aggregate were detected for each antibody, and Charge Variant: Differences in acidic and basic variants were observed for each antibody.



P66. MICROSAMPLING TECHNIQUES FOR IMMUNOSUPPRESSANTS' TDM

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Introduction: Therapeutic drug monitoring (TDM) of immunosuppressants (ImmSupp) is required to prevent organ rejection and toxicity because of their narrow therapeutic ranges [1]. Microsampling techniques are getting interest due to their many advantages (self- and home-sampling, less invasive, cost-saving, stability, less biological risk) [1-2]. However, correlation between venous and capillary blood must be established before their application to TDM [3].
Aim: To develop analytical methods for ImmSupp in venous blood, dried blood spots (DBS) and Mitra VAMS™ to assess these microsampling techniques for ImmSupp's TDM. **Methodology:** Chromatographic separation was performed using an XBridge® Shield RP18 column (2.1mm×100mm, 3.5µm) at 65°C, and a gradient with 2mM ammonium formate and 0.1% formic acid (A) and acetonitrile (ACN) (B). Detection was performed with a Quattro Micro API ESCI triple quadrupole. Venous blood extraction was optimized as follows: 0.2 mL sample were mixed with 0.8 mL water, and subsequently precipitated with 0.1 M ZnSO₄ and ACN. After centrifugation, the supernatant was acidified to perform SPE. For DBS, 4 cards (FTA DMPK-A, DMPK-B, DMPK-C and Whatman 903), 4 extraction solvents (MeOH:H₂O, 80:20; ACN:ZnSO₄, 50:50; MeOH:ACN, 80:20; MeOH) and different punch sizes were assessed. For Mitra devices, 2 volume tips (10 and 20 µL), the previous described extraction solvents, and SPE were assessed. **Results:** The method in venous blood was fully and satisfactorily validated according to FDA and EMA guidelines. For DBS, optimum extraction conditions were achieved using Whatman 903 or DMPK-C cards and ACN:ZnSO₄ as extraction solvent. For Mitra, the best results were obtained using MeOH. **Conclusion:** A method for the determination of ImmSupp was fully validated in venous blood, and optimized for its application to DBS and Mitra devices.

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P67. LC-MS/MS IDENTIFICATION OF CITOSTATIC DRUGS IN WIPE SAMPLES FOR SURFACE CONTAMINATION DETECTION

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Introduction: Use of anticancer drugs is continuously increasing¹. The personnel involved in handling of these drugs may be accidentally exposed and at risk of suffering adverse effects².

Objectives: To develop and validate an analytical method for the detection of 12 antineoplastic drugs (gemcitabine, dacarbazine, methotrexate, irinotecan, doxorubicinol, doxorubicin, epirubicin, cyclophosphamide, vinorelbine, etoposide, docetaxel and paclitaxel) on surfaces, and to apply it to real samples.

Methodology: To prepare calibrators and quality control samples, a 100-cm² surface area in a stainless-steel plate was contaminated with the corresponding reference standard solution, and air-dried. Surfaces were sampling using a tissue wetted with 0.5 mL of 0.1% formic acid:acetonitrile (80:20), which was subsequently extracted with 0.1% formic acid:acetonitrile 80:20. The solvent was evaporated, reconstituted in mobile phase and 20 µL injected into the LC-MS/MS. This method was applied to 12 real samples from different locations of the oncology ward of the University Clinical Hospital Complex of Santiago de Compostela, Spain.

Results: The method was quantitatively validated for gemcitabine, dacarbazine, methotrexate, irinotecan, cyclophosphamide, vinorelbine, etoposide and docetaxel, and qualitatively for doxorubicinol, doxorubicin, epirubicin and paclitaxel. The following parameters were evaluated: linearity (0.005-0.1 to 5 ng/cm²), selectivity (no interferences), limits of detection and quantification (0.005-0.1 ng/cm²), accuracy (89.0%-112.5%), imprecision (≤22.1%), matrix effect (-58.3% to 303.1%), recovery (10.8%-75%), autosampler stability and after 1 week at 4°C (no loss). Six out of the 12 real samples were positive for gemcitabine, irinotecan, cyclophosphamide or paclitaxel.

Conclusion: The method was successfully validated, and its applicability was proved by the analysis of real samples.

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P68. EMERTOX - EMERGENT MARINE TOXINS IN THE NORTH ATLANTIC AND MEDITERRANEAN

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Episodes of human poisoning caused by Harmful Algal Blooms have been commonly recorded in the last century mostly because of the lack of regular monitoring programs. Today, the cases of human poisonings are sporadic, usually because of violations of national health authorities' regulations imposing the closure of harvesting areas and seafood commercialization. Nevertheless, the occurrence of emergent toxins and the respective producing organism in the North Atlantic and Mediterranean, such as tetrodotoxins, ciguatoxins, palytoxins and a diversity of congeners, is being increasingly reported either by scientific studies or by events of human intoxications. The project EMERTOX - Emergent Marine Toxins in the North Atlantic and Mediterranean: New Approaches to Assess their Occurrence and Future Scenarios in the Framework of Global Environmental Changes, funded by H2020 under the RISE program, will create a robust and sustainable network of experts with excellent complementary competencies on marine algal toxins and the detection of the organisms producing these toxins. The network will collaborate not only with national authorities but also with European ones, such as the European Food Safety Authority (EFSA), for the assessment and management of risks associated to emerging toxins and the species that produce them. Current risks assessment relating to emerging harmful algae and predicting future scenarios will be fundamental for EFSA, which will recommend whether these emerging toxins should be monitored in Europe, and for the development appropriate strategies to protect human health. EMERTOX - aims to map the actual situation in emergent marine toxins and the producing organisms, develop new approaches to assess their occurrence and predict the possible future scenarios in the framework of global warming. The consortium, formed by a multidisciplinary team, will produce a joint research and innovation project that will exploit the complementary expertise of the participants and will create synergies among them. The main objectives are: i) to assess the current situation on potentially harmful algae and bacteria and the relevant emerging toxins in 9 countries belonging to different but geographically connected areas (Mediterranean Sea and North Atlantic); ii) to develop innovative approaches to sample, and analyses the producing organisms and their toxins by chemical and biological methods including immunoassays and sensors; iii) to predict different future scenarios based on molecular data (routes of dispersion) and modelling.

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P69. STUDY OF THE PHOTODEGRADATION OF PBDES IN WATER BY UV-LED TECHNOLOGY

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Polybrominated diphenyl ethers (PBDEs) have been one of the most employed family of flame retardants and are considered as persistent organic pollutants. For that reason, this project assesses the photodegradation of PBDEs in water samples applying advanced oxidation processes (AOPs). The combination of AOPs and UV-LED radiation can be an effective treatment against a wide range of macropollutants, microorganisms and micropollutants such as phenols, pesticides or pharmaceuticals. To study the photodegradation of PBDEs in water samples, irradiation was applied using the PearlBeam T255/265/285 device, which provides three different wavelengths: 255 nm, 265 nm and 285 nm. First set of experiments were performed with spiked purified water samples in order to determine the best degradation conditions (wavelength, time). Then, the optimized methodology was applied to real water samples from different sources: superficial continental, marine, influent and effluent samples from two different wastewater treatment plants and a greywater sample.

For the analyses of both spiked and real samples, three consecutive LLE extractions of the samples were performed with hexane and followed by two purification columns: an anhydrous sulphate column and an acidic silica column. Extracts were concentrated to 25 µl and the analyses were performed by GC-HRMS. Best PBDE degradation results studied in spiked samples were achieved after irradiating samples at 285 nm during 240 min, reaching degradations from 50 to 87 % for all the congeners. Moreover, results of the repeatability studies pointed out that main contribution of the whole process variability came from the irradiation step (18 % < RSD < 50 %) in comparison to the analytical contribution (3 % < RSD < 10%). Real water samples were spiked and exposed to 4 hours of irradiation at 285 nm. Successful photodegradation of PBDEs ranging 51 % to 97 % was achieved for all the PBDE congeners in the different water samples with the exception of the coastal one, in which only a 31 % of degradation was achieved.

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P70. USE OF AN ELECTRONIC NOSE (PURGE & TRAP, GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY) FOR THE EARLY DETECTION OF VOLATILE COMPOUNDS IN RAW WATER

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Rivers can be affected by unexpected spills containing malodorous compounds [1,2] that can reach the drinking water treatment plants (DWTP) located downstream. These volatile compounds could contaminate or even damage the inner DWTP's infrastructures. Reverting this situation involves operative processes modification as well as an increase of operational and maintenance costs. For this reason, the early detection of these discharges before entering the DWTP, poses a challenge for water utilities. To overcome this problem, a new automated methodology using a purge & trap extraction coupled to a gas chromatograph and ion mobility spectrometer (*P&T- GC-IMS*) that works as an electronic nose, has been tested and optimized. This GC-IMS combination offers a two dimensional separation that allows a higher selective detection of volatile compounds compared to other systems based on mass spectrometry. This system can perform on-line analysis and allows automated processing and reporting of data. This, together with the possibility to be operated in-field, offers a continuous monitoring of the river water so that unpleasant odour compounds can be detected before they can get into the DWTP. A method for the unequivocal identification of 15 concerning odorous compounds, some of them with low odour thresholds such as 2-ethyl-5,5-dimethyl-1,3-dioxane (OTC 0.6 ng/L), has been optimized in the laboratory thanks to three-dimensional fingerprint analysis. From December 2018 to April 2019, 1700 river samples, 200 of them spiked with standards, have been analysed. Algorithms based on odour index and volatile compounds pattern of these samples have been developed. The system has been trained to classify water samples by means of algorithm application. The electronic nose capability to distinguish from raw water samples considered as acceptable and non-acceptable in terms of odour threshold has been demonstrated. Nowadays, the electronic nose is installed in the DWTP and has been equipped with adjustable alarm thresholds that allows automated notification when a non-acceptable water sample has been detected, sending out a warning to prevent the pollutant from entering the DWTP.

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P71. APPLICATION OF WASTEWATER ANALYSIS TO THE ESTIMATION OF HUMAN EXPOSURE TO PLASTICIZERS: DEVELOPMENT OF AN ANALYTICAL METHODOLOGY.

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Plasticizers are large-scale chemical compounds used in many industrial processes and consumer-oriented products, which causes a wide variety of harmful effects. Therefore, it is necessary to develop novel methodologies that allow to quantify the exposure to these substances in a population in a rapid and effective way, so that authorities may adopt control measures within the shortest timeframe. In this context, wastewater-based epidemiology (WBE) is a very promising methodology that can complement human biomonitoring [1]. WBE is based on the fact that xenobiotic chemicals, e.g. plasticizers, are excreted as metabolites in the urine. Then, the combined urine of a given town gets through the sewage system to the wastewater-treatment plant (WWTP). Analysing that wastewater and applying the flow rate of the WWTP, the population served and the excretion rate of the metabolites, it is possible to calculate the average exposure of that population in a very inexpensive way, as compared to regular biomonitoring [1]. Thus, we developed an analytical method for the extraction and determination of 21 plasticizer metabolites in wastewater. The method covers phthalates, terephthalates and di-isononyl cyclohexane-1,2-dicarboxylate (DINCH) metabolites, since several phthalates are currently being replaced by these later plasticizer alternatives. Firstly, the detection by tandem mass spectrometry (MS/MS) and chromatographic separation by ultra-high performance liquid chromatography (UHPLC) were optimized. Subsequently, a solid phase extraction (SPE) protocol was optimized by using Oasis MAX mixed-mode sorbents, and the stability of both the metabolites and the precursor plasticizers in wastewater was assessed. The method was validated in terms of recovery, repeatability and detection and quantification limits. Finally, it was used to analyse 24 h composite raw wastewater samples from Santiago de Compostela, where several phthalate metabolites, as well as dimethylterephthalate, diethylhexylterephthalate and DINCH metabolites were detected.

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P72. DETERMINATION OF PHARMACEUTICALS AND PESTICIDES/TRANSFORMATION PRODUCTS IN THE MIJARES RIVER (SPAIN) BY UHPLC-MS/MS

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Organic contaminants such as pharmaceuticals and pesticides are present in different bodies of water, causing pollution in the environment. The main source of water contamination by pesticides is due to agricultural practices. Pharmaceutical products mainly enter to the water bodies due to the inefficiency of their elimination by the wastewater treatment plants after their consumption and excretion[1,2].

In this work, the contamination of the Mijares River, sited in the Mediterranean area of Spain, has been investigated. Surface water samples were sampled in different points in the river from its birth until its mouth and were collected in three different campaigns (June 2018, September 2018 and February 2019) in order to distinguish possible differences between seasons. Analytical methodology was focused on the determination of 41 pharmaceuticals (analgesics, antibiotics, antidepressants, antiepileptics and benzodiazepines, antihypertensives and beta-blocker agents, hypolipidemic and anti-inflammatory agents and others pharmaceuticals) and 24 pesticides/transformation products (fungicides, herbicides and insecticides).

After adding the isotopically-labeled internal standard (ILIS) mixture, samples were analyzed using direct injection into the liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), using a triple quadrupole (QqQ) analyzer. Selected Reaction Monitoring (SRM) mode was employed with the acquisition of at least two transitions per analyte (one for quantification and one or two for confirmation). In every batch of samples, quality controls (QCs) were included. QCs consisted of three real samples, each fortified at three concentration levels: 10, 100 and 1.000 ng/L. Most of QC recoveries were satisfactory with values between 60 and 140% (SANTE, 2015), which provided reliability to the results obtained.

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P73. SURVEY OF POLY- AND PERFLUOROALKYL SUBSTANCES (PFASs) IN CONSUMER PRODUCTS AVAILABLE IN SPANISH MARKETS

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Poly- and Perfluoroalkyl substances (PFASs) are anthropogenic organic compounds synthesized to resolve a wide diversity of societal and industrial issues. Unfortunately, it has been demonstrated that many of them pose characteristics to be considered as Persistent Organic Pollutants (POPs) ¹. In order to minimize potential harm to human health and environmental damage its application in some uses has been restricted or banned, namely textiles, non-stick cooking ware, paper –microwave popcorn bags and baking paper-, carpets and firefighting agents. However, the range of applications where still PFASs can be found include waterproof agents, lubricants, paints as well as electric and electronic devices. Thus, we proceed with the screening of consumer products according to the decision of SC-8/18 ².

Selected samples included articles with high consumer demand purchased in common malls from Madrid, and based on its properties to repel water, oil and dust, in accordance to prior studies ³⁻⁶. Sample treatment and extraction followed that described in Van der Veen et al. 2016 with minor modifications ⁷. Identification and quantification of ionic PFASs was performed using a Waters Acquity Ultra Performance Liquid Chromatography system coupled with a Waters XEVO TQS, triple-quadrupole mass spectrometer (UPLC-MS/MS) operating in the multiple-reaction-monitoring (MRM) mode and equipped with an electrospray ionization (ESI) source. Perfluorooctanoic acid (PFOA) was detected in almost all studied products, while perfluorooctane sulfonic acid (PFOS) was only found above the limit of detection (LOD) in four articles (carpet, mountain jacket, sofa cover and frying pan). PFASs with longer perfluorinated chains (C9-12) have also been found.

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P74. PCBs IN FISH SPECIES FROM THE MEDITERRANEAN SEA

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Polychlorinated biphenyls (PCBs) are toxic and persistent chemicals able to biomagnify along the food web, posing a potential risks for humans and wildlife. Despite their apparent decrease resulting from the wide ban in the mid 80's, some authors still report high concentration levels in top predators inhabiting some well-known contaminated area, such as the Mediterranean Sea¹. At the same time, the slight reduction reported for some fish species appears to be less noticeable in last years², suggesting a potential remobilization or new inputs and raising the question about the effectiveness of actual European and global mitigation efforts.

The aim of this study was to assess the degree of PCB contamination in three important commercial fish species of the Mediterranean Sea: European sardine, European anchovy and bogue specimens obtained in 2017 from Tuscany local markets (Italy). Samples were analyzed for dl-PCBs and the 7 indicator PCBs using GC-HRMS. Quantification was carried out by the isotopic dilution technique.

All target contaminants were detected in all samples analyzed. Sardine showed the greater concentrations, while bogue and anchovy showed average levels about three times lower, in line with previous studies³. Sardine and bogue exhibited the same profile PCB-77>126>169>81, which is commonly found in other studies⁴. Instead, the pattern of non-ortho-PCBs was quite different in anchovy (126>77>169), likely due to dissimilarities in toxicokinetics between species⁵. TEQ values are in compliance with the regulated levels in diet. At the same time, our results are often not different from values reported for the same species in the last years, which underpins the idea of a virtual halt or very slow decline in PCB concentrations in the Mediterranean Sea.

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P75. STEREOSELECTIVE SEPARATION OF A GROUP OF AGROCHEMICALS BY ELECTROKINETIC CHROMATOGRAPHY

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A broad number of agrochemical compounds contain chiral centers and are marketed and employed as racemates [1]. However, the behavior of the enantiomers of a chiral molecule can be very different giving rise to a different activity, toxicology or degradation patterns which affect to their persistence in the environment and this is why some agrochemicals are being marketed as pure enantiomers. Due to the importance that the stereochemistry may have from an environmental point of view, the development of analytical methodologies for the enantiomeric separation of agrochemicals has received considerable attention in last years. In this work, analytical methodologies based on the use of Electrokinetic Chromatography (EKC) were developed enabling the stereoselective separation of three chiral agrochemicals: tetramethrin (a pyrethroid insecticide), fipronil (a phenylpyrazole insecticide), and prothioconazole (a triazole fungicide). Although some works have reported the chiral separation of these compounds by other separation techniques, the use of EKC has never been described with this aim. To select the optimal separation conditions, a screening of cyclodextrins was carried out and the influence of different experimental parameters, such as pH, chiral selector concentration, temperature, and separation voltage was also studied. The developed methodologies were successfully applied to the analysis of commercial agrochemical formulations.

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P76. BASIN-SCALE MONITORING OF EMERGING CONTAMINANTS IN SOUTH AMERICAN COASTAL LAGOON USING LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY

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Emerging contaminants (ECs) such as pharmaceuticals, personal care products, abuse drugs and polar pesticides are under particular attention due to their high consumption, frequent detection in the environment and reported ecotoxicological effects. This study investigates the occurrence and distribution of multiclass contaminants of emerging concern in surface waters at basin scale of two Atlantic coastal lagoons of Uruguay, South America. For this purpose, a target screening approach covering up to 364 compounds was employed using nanoflow liquid chromatography - high resolution mass spectrometry (nanoLC/HRMS). Fifty-seven compounds were identified including five banned pesticides in the European Union: atrazine, carbendazim, chlorpyrifos ethyl, diazinon, and ethion. Pharmaceuticals, hormones and abuse drugs showed maximum detection frequencies and concentrations downstream cities. The highest occurrence of pesticides was found in lagoons and streams with neighboring agricultural activity. ECs were also found in coastal sea. Environmental risk assessment revealed that the hormones 17 α -ethinylestradiol and 17- β -estradiol showed the highest risk to aquatic organisms in these basins. This study represents the first monitoring of ECs in superficial waters at basin scale encompassing streams, lagoons, and coastal seas in Uruguay, South America.

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P77. ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN BIOCHARS PRODUCED FROM CROP RESIDUES: IMPACT OF PYROLYSIS CONDITIONS ON THEIR POTENTIAL HAZARD

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Biochar, an emerging highly aromatic porous material produced by pyrolysis of organic residues¹ is considered a good amendment for degraded soils. Thus, the interest in using biochar as soil or manure conditioner is continuously increasing during the last decade. Nevertheless, during the pyrolysis process persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) are formed due to incomplete combustion². These PAHs may enter the environment when the biochar is applied as soil conditioner. The aim of this study was to discern how the pyrolysis conditions affect the risk of PAHs exposure from biochars, thus we examined the total and individual contents of the 16 US EPA PAHs in biochars produced using three different pyrolytic reactors from biomass of rice husks, wood, wheat and sewage sludge at temperatures ranging from 400 to 600 °C. Results of this study show that pyrolysis conditions are the key factor in the total amount of PAHs present in biochars. The maximum amount of PAHs was observed for the biochar produced in the batch reactor at 400 °C and decreased with increasing temperature. Increasing the residence times had not significant effect on the PAHs. Looking for a more reliable risk assessment of potential exposure to PAHs in biochar than the thresholds solely based on the Σ PAHs, the total toxic equivalent concentrations (TEC) of the biochars were calculated. TEC values confirmed the need of separating the syngas and bio-oil from the solid phase³. Results of this study constitute valuable information in the development of strategies for producing biochars with minimum risk of PAHs contamination.

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P78. CHEMISTRY QUALITY OF GROUNDWATER OF A LIMIA REGION (NW OF SPAIN)

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Anthropogenic activity (agriculture, livestock, industry, etc.), germs and chemical substances contaminate [1] groundwater. In this work the concentration of several major cations and anions, as well as heavy metals, has been analyzed to evaluate the quality of well waters located in the A Limia region (province of Ourense, Spain), many of which are used for human consumption.

Water samples (75 wells) were collected in May 2019 and conserved at 4°C for the analysis of six major anions (fluorides, chlorides, nitrites, nitrates, phosphates and sulphates) by ion chromatography and various elements by inductively coupled plasma mass spectrometry (ICP-MS) were analyzed.

Many of the samples had high nitrate contents exceeding the limit value of 50 mg/L [2] established by the legislation as maximum amounts for drinking water. Low levels of nitrates occur naturally in plants, for which it is a key nutrient, but high contents due to the agricultural activity (mainly with the fertilizers) and animal manure can be reached penetrating through the underground water [3]. With respect to the toxic metals, several samples showed As, Mn and Cr contents higher than those allowed legislation.

Acknowledgement

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P79. INVESTIGATION OF PESTICIDES AND THEIR TRANSFORMATION PRODUCTS IN THE JÚCAR RIVER HYDROGRAPHICAL BASIN (SPAIN) BY WIDE-SCOPE HIGH- RESOLUTION MASS SPECTROMETRY SCREENING

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The Water Framework Directive 2000/60/EC implemented by the European Union established as the main objectives to achieve a “good ecological and chemical status” of the surface water and a “good quantitative and chemical status” of groundwater bodies. One of the major pressures affecting water bodies comes from the use of pesticides and their potential presence in the water ecosystems[1]. For this purpose, the reliable determination of pesticides and their transformation products (TPs) in natural waters (both surface and groundwater) is required. The high number of compounds potentially reaching the aquatic environment makes extraordinary difficult, if not impossible, to investigate all these compounds even using the most powerful analytical techniques. Among these, LC coupled to HRMS is emphasized due to its strong potential for detection and identification of many organic contaminants thanks to the accurate-mass full spectrum acquisition data. This work focuses on wide-scope screening of many pesticides and their TPs in surface water and groundwater samples in the Júcar River Hydrographical Basin, Spain. For this purpose, a home-made database containing more than 500 pesticides and TPs was employed. Analyses performed by LC-QTOF MS allowed the identification of up to 27 pesticides and 6 TPs. The most detected compounds in groundwater were the herbicides atrazine, simazine, terbuthylazine, and their TPs atrazine-desethyl, terbumeton-desethyl and terbuthylazine-desethyl. Regarding surface water, the fungicides carbendazim, thiabendazole and imazalil, the herbicide terbutryn and the TP terbumeton-desethyl were also detected. These results illustrate the wide use of these compounds (in the present or in the recent past) in the area under study and the vulnerability of the water bodies, and are in accordance with previous findings in other water bodies of the different Spanish Hydrographic systems.

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P80. DEVELOPMENT OF A NEW GC-APPI-HRMS METHOD FOR THE DETERMINATION OF SHORT-, MEDIUM- AND LONG-CHAIN CHLORINATED PARAFFINS

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Chlorinated paraffins (CPs) comprise a complex mixture of polychlorinated n-alkanes with a variable carbon chain length and a total chlorine content ranging from 30 to 70% (w/w) [1]. Among them, short-chain chlorinated paraffins (SCCPs, from C₁₀ to C₁₃) has been recently classified as persistent organic pollutants by the Stockholm Convention. On that way, the restrictions on the use of SCCPs mainly result in a replacement by medium-chain (MCCPs, from C₁₄ to C₁₇) and long-chain chlorinated paraffins (LCCPs, > C₁₈), but information about their occurrence in the environment is still very scarce. CPs are currently analysed by GC-MS, but the chromatographic separation of this complex mixture is not workable and the ionization by negative ion chemical ionization (NICI) generates mass internal interferences between homologue groups, which leads to quantification errors.

In this work, a GC-APPI-HRMS method based on anion attachment has been developed for the simultaneous determination of SCCPs, MCCPs and LCCPs. On that way, different strategies based on mixtures of halogenated additives (carbon tetrachloride and bromoform) with a dopant (acetone) were evaluated to reduce in-source fragmentation and to avoid internal interferences of congeners with different chlorination degree and carbon chain lengths. This GC-APPI-HRMS method avoids the complex mass spectrum deconvolution required for the quantification of CPs by APCI-HRMS [2]. Moreover, the method allowed the quantification of the analytes without considering the chlorination content of the sample because the response of CPs by anion attachment APPI was not strongly affected by the number of chlorine atoms. Finally, the feasibility of the proposed GC-APPI-HRMS method was investigated by analysing selected environmental samples such as seagull eggs and marine sediments.

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P81. A SIMPLE METHODOLOGY FOR THE ANALYSIS OF LINDANE IN INDOOR AIR

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Lindane is a persistent and toxic insecticide, which accumulates in the human body by inhalation, ingestion and through the skin. It was completely forbidden in the EU in 2008. In Galicia, Northwest Spain, there are some focal points where lindane residues have been accumulated in the soil for decades, generating a big social alarm among local populations.

Some of the lindane isomers are especially volatile. Thus, its control in soils and groundwater are necessary but also in air, requiring effective tools so eventual indoors pollution could be well-characterized. Since analytical sampling and extraction methods for the determination of lindane in air are usually long-time consumption and laborious, the main goal of this work is the development of an environmentally friendly simple and fast methodology based on Solid-Phase Extraction (SPE) followed by Ultrasound Assisted Extraction (UAE) for the determination of 4 lindane isomers. Experimental parameters affecting both air sampling and extraction have been optimized to achieve the highest efficiency. The analytical determination was performed by gas chromatography-tandem mass spectrometry (GC-MS/MS). The method was successfully validated in terms of linearity, repeatability, accuracy and precision. The limits of detection and quantification (LODs and LOQs) were also evaluated and they were at the low ng m³ concentration level. Finally, to show the suitability of the proposed methodology, the SPE-UAE-GC-MS/MS method was applied to the air analysis of a storeroom containing contaminated soil samples.

The simplicity, fastness and low-cost of the proposed sampling and extraction methodology make it an interesting alternative to other methods being used and could be easily implemented in routine indoor air monitoring analysis.

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P82. STUDY AND RECOVERY OF PHENOLIC COMPOUNDS DURING CORK PROCESSING

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Cork industry performs a key role in the economy of several Mediterranean countries. The cork manufacturing process involves several stages with waste generation, mainly industrial water or cork boiling wastewater (CBW) which is generated by boiling the cork planks in order to improve their properties. There are several phenolic compounds in crude cork, mainly low molecular weight phenolic compounds and ellagitannins (1), that can be extracted during the cork boiling cycle. As many of these compounds have useful properties, this work studied the main phenolic compounds extracted and their concentration in CBWs for further recovery. CBWs samples were collected from several cork factories and analyzed with an innovative methodology: direct analysis by HPLC/DAD/FLD. On the other hand, these samples were also treated with calcium in alkaline media according to a methodology developed by CICYTEX (2) what led to the precipitation of most of phenolic compounds, along with the isolation and/or concentration of the remaining ones. Up to 26 phenolic compounds presented in crude cork were analyzed after and before the treatment and the results compared. The analysis showed low molecular weight phenolic compounds similar to those quoted by the bibliography (3). Besides, some ellagitannins (castalagine and vescalagine) were detected and/or quantified. With reference to treated CBWs: some phenolic compounds remained practically without precipitating and even with a higher concentration. In conclusion, with this methodology a wide range of phenolic compounds, including ellagitannins, are detected and quantified in a fast and reliable way. Besides, these by-products can be recovered due to its potential use for several industrial sectors, generating added-value for cork sector.

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P83. ORGANIC GEOCHEMISTRY OF RIBETEHILLO PEAT BOG (DOÑANA N.P., SW-SPAIN)

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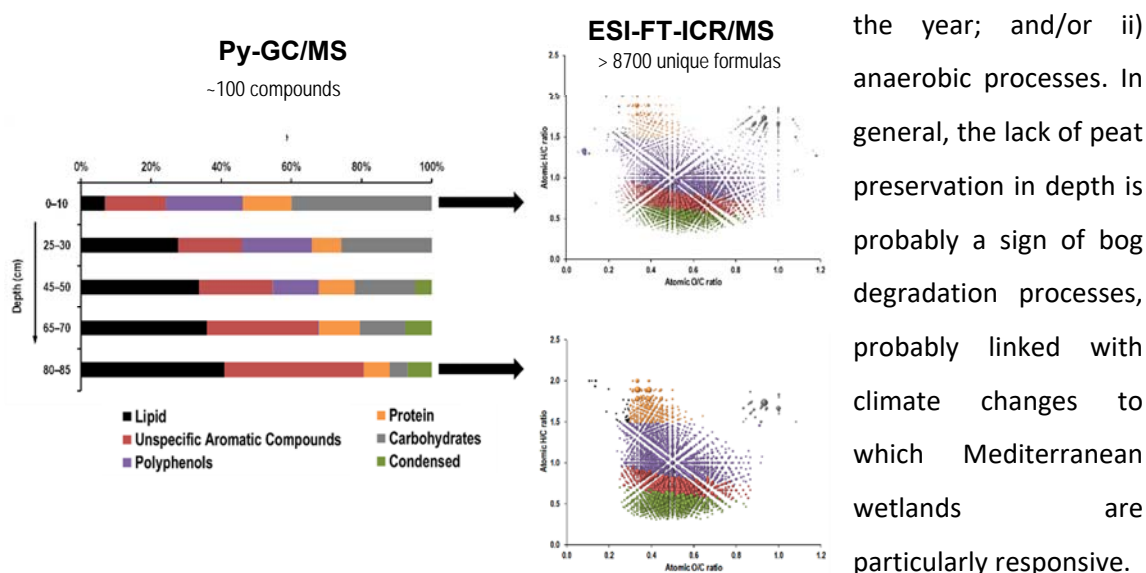
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In less than 50 years the area occupied by peat bogs in the Gulf of Cádiz (SW-Spain) has been reduced by more than 90%. The peat bog studied here may well be considered as relict and one of the last and more Meridional still in existence in the Northern hemisphere. The vertical molecular composition of a 85 cm peat bog from Ribetehilo lagoon (Doñana National Park; SW-Spain 37° 7'30.81"N; 6°37'50.19"O) is studied using analytical pyrolysis (Py-GC/MS) and ultra-high resolution mass spectrometry (ESI-FT-ICR/MS).

The results obtained by the two techniques were similar, showing as shallow OC composition was dominated by fresh material from upper vegetation cover, while more transformed and humified material was accumulated in the bottom layers. This may be due to i) intense microbial activity combined with large fluctuations of the water table occurring during



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P84. DETERMINATION OF FLUOROQUINOLONES AND SULPHONAMIDES ANTIBIOTICS IN DIFFERENT WASTEWATER SAMPLES

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Nowadays, pharmaceuticals are considered as environmentally emerging contaminants. Water contamination is a growing, due to the increasing use of these substances by the population and because of the veterinary practices in livestock farming and diseases treatment in aquaculture¹. One of the most interesting groups are antibiotics, which produce microbial resistance because of their irresponsible and irrational use². The pollution caused by antibiotics makes necessary to develop analytical methods for their identification and quantification at low levels, particularly in wastewater samples from WWTPs (Waste Water Treatment Plant), which are the most important source of these pollutants. In consequence, a quantitative analytical method based on simultaneous extraction of eight fluoroquinolones and sulphonamides antibiotics was developed. The method was based on solid phase extraction (SPE) followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). *Non-target* screening, based on a data independent workflow, was also carried out to identify unknown compounds in the samples using liquid chromatography coupled to high-resolution TOF mass spectrometry (LC-TOF-HRMS).

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P85. PROPAGATION OF PESTICIDES FROM REGENERATED WATER TO THE CROPS BY IRRIGATION

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Water scarcity will increase in the future because of climate change. In this way, water resources will decrease, so we will need to find new water sources. It is known that agriculture is the biggest consumer water, and nowadays treated wastewater are being an alternative to fight and to avert water scarcity. However, this treated wastewater can incorporate many emerging contaminants (ECs), as pesticides or drugs, so that, it is very important to evaluate the propagation and absorption of these in crops and the foods produced with their use.

Thus, the overall objective of this work was to estimate the potential use or limitations of the reuse of reclaimed water in the production of vegetable foods. For that, this study assesses the propagation and the accumulation of pesticides in soils and several crops irrigated with reclaimed water doped at concentrations similar than those expected in agricultural irrigation systems. To achieve these objectives, a multiresidue method based on QuEChERS (Quick, Easy, Cheap, Effective and Rugged) extraction was optimized and validated to extract plant materials and soil. A solid-phase extraction method was also validated to analyse reclaimed water samples. The determination of pesticides was carried out using an ultra high performance liquid chromatography system coupled to triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS). Finally, this methodology was applied to evaluate the presence and the absorption of pesticides in a crop irrigated with two types of regenerated wastewater (secondary and tertiary treatment), by two types of different irrigation (drip and sprinkle), and cultivated in two types of soil (with and without clay content).

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P86. DISTRIBUTION AND TRANSFER OF PESTICIDE RESIDUES IN THE BEEHIVE COMPARTMENTS TO THE HONEYBEE BROOD

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Honeybee (*Apis mellifera*) playing an essential role in the agricultural production and the natural ecosystems, therefore honeybee is the most important plant and crop pollinizer. Nowadays, the number of honeybee colonies is decreasing worldwide, which has motivated extensive research into the factors affecting their development and survival. In this work is evaluated the distribution of pesticides inside the beehive, their persistence and their migration to the bee brood. Samples of beeswax, bee bread (processed pollen) and bee brood were extracted and analyzed by GC-MS/MS and by LC-MS/MS using a multiresidue method previously developed and validated by our research group [1,2]. The highest concentrations of pesticide residues were amitraz in beeswax, followed by coumaphos and tau-fluvalinate, with total concentrations of up to 16858, 7102 and 1775 µg/kg. Other veterinary treatments were also found to accumulate in the beeswax and migrate to other beehive matrices such as bee bread and bee brood. Phytosanitary products were also found in the beehive matrices, especially in the bee bread. In bee brood samples were found acrinathrin, amitraz, coumaphos, cypermethrin and tau-fluvalinate at concentration levels up to 4.4 % migration with regard to the total concentration in the beehive. These findings reveal that bee brood reared in field conditions is exposed to pesticides through direct contact with contaminated wax and bee bread.

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P87. PRESENCE AND BEHAVIOUR OF PERFLUORINATED COMPOUNDS IN MARINE

FISH

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Perfluorinated compounds (PFCs), also known as perfluoroalkyl substances (PFAS), are organofluorine compounds containing only carbon-fluorine bonds (no C-H bonds) and C-C bonds but also other heteroatoms. Nowadays these manufactured organic compounds have been produced for decades mainly as surface agents in products such as fast food packaging, waterproof clothing, water-based foams against fires and paint-resistant in paints and molds. PFCs have emerged as significant global environmental pollutants with persistent, bioaccumulative and toxic properties. The aim of this study was to develop an analytical method based on ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) to determine PFCs in marine fish samples. The PFCs studied were heptafluorobutyric acid or perfluorobutyric acid (HFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), pentadecafluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), nonafluoro-1-butanesulfonic acid (PFBS) and perfluorooctanesulfonic acid (PFOS). Chromatographic conditions were optimized considering the chemical structure and chromatographic behavior of the analyzed compounds. The best performance was obtained with an AcQuity BEH C18 column (2.1 x 100, 1.7 μm), which allowed the separation of the 8 compounds in 10 min. In addition, the performance of LC-MS/MS was studied in terms of linearity, sensitivity, intra- and inter-day precision, and overall robustness. Finally, fish samples were analyzed obtaining levels in the range of ng L^{-1} .



P88. FIRST DETECTION OF PINNATOXINS IN SHELLFISH IN ATLANTIC AND CANTABRIAN COASTS OF SPAIN

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Pinnatoxins (PnTXs) are marine biotoxins belonging to the Cycles Imines (CI) group which are produced by the dinoflagellate *Vulcanodinium rugosum*. To date, no case of human poisoning has been unequivocally associated to PnTXs, probably because these toxins show the same clinical effects in humans like other drugs and natural toxins [1].

This is the first report of the presence of pinnatoxins (PnTXs) in shellfish from the North-Northwest coasts of Spain (Galicia, Asturias, Cantabria and Basque Country) [2]. Only two PnTXs have been detected in this study, pinnatoxin G (PnTX G) and pinnatoxin A (PnTX A), being the most prevalent toxin was PnTX G.

Currently, PnTXs are not regulated in European Union (EU) nor there is an official method for analyzing them in shellfish. This work shows the possibility of monitoring these toxins by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) by fine-tuning the EU reference method of lipophilic toxins analysis [3]. Recoveries for PnTX G were evaluated in three different mollusc species with average values between 83 and 87%. Therefore, they can be considered quantitative. The obtained linearity was good ($R^2 \geq 0.99$) and the LOQ and LOD were 0.28 and 0.08 $\mu\text{g}\cdot\text{Kg}^{-1}$ respectively.

The method was applied to 872 samples including: mussels (raft-cultures and wild), razor shells, clams, cockles and oysters. The PnTX G was present in 261 samples (30%) with levels between 0.36 and 14.98 $\mu\text{g}\cdot\text{Kg}^{-1}$.

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P89. ANALYSIS OF ETHOXYQUIN RESIDUES IN ANIMAL FEED USING QUECHERS AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Ethoxyquin (EQ) is a quinolone that is both an agrochemical and an additive. As an antioxidant additive, it is used in spices to reduce color loss due to carotenoids oxidation and to slow the rancidity process of fats, preventing lipid autoxidation in animal feed [1]. It was approved as a feed additive and it was listed in the EU register on feed additives (Reg. 1831/2003/EC) as E324. As a fungicide, it is used in pre- and post-harvest applications to reduce scald. In 2015, the European Food Safety Authority concluded that they could not guarantee the consumers and the environment safety related to EQ, due to the toxicity of one of its metabolites Ethoxyquin quinone imine (EQI) and its impurity called p-Phenetidine as a mutagenic agent. For these reasons, REGULATION (EU) 2017/962 suspended the authorization of EQ as a feed additive for all animal species and categories. The aim of this study is to accomplish this regulation. The EQ was extracted from animal feed by a QuEChERS protocol in which an ascorbic acid buffer was added to minimize the degradation of the EQ [2]. The analysis was performed with GCQQQ. The method was validated according to the European Commission guidelines. Residues of EQ were detected in 90% of the feed samples analysed previous to validation. For this reason, validation was carried out with 20 different feed samples manufactured in the laboratory from a mixture of several cereals used in animal feed. A matrix-matched calibration was established and a recovery was calculated with spiked samples. Satisfactory results were obtained in the validation of the method, excellent linearity and good sensitivity as well as recoveries between 70 and 120% at the quantification limit of 0,01 mg kg⁻¹.

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P9 . DETERMINATION OF MOSH AND MOAH BY GCXCG-TOFMS

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Nowadays across the European Union food contamination by mineral oils from production processes and packaging is becoming a serious problem for public health institutions and governments.

In this study comprehensive two-dimensional gas chromatography (GCXGC) in combination with time-of-flight mass spectrometry was evaluated, in order to find a robust one run analytical method.

LECO GCxGC system takes advantage of a dual-stage, quad-jet thermal modulator positioned between the two columns and a secondary oven allows independent temperature control of the second dimension column, combined with high acquisition rate, full range TOF mass spectra.

The combination of two different polarity columns led to effective separations between compound families, identifications within families were easily reached by high acquisition rates TOFMS systems and ChromaTOF software classification capabilities defined chromatogram regions to locate clearly each compound family.



P91. AUTHENTICATION OF PAPRIKA BY HPLC-UV AND CHEMOMETRICS

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Paprika, or chilli pepper, is a red powder seasoning with a characteristic flavor obtained from the drying and grinding of certain varieties of red peppers. The two most known varieties of paprika in Spain, and the only ones with product designation of origin (PDO), come from the region of La Vera in Cáceres (Extremadura) and from Murcia. There are three important varieties: sweet, bittersweet, and spicy paprika, and the Vera ones are characterized by their smoky aroma produced during the drying process by the smoke of oak woods. Polyphenols are among the most interesting bioactive compounds found in paprika, and their distribution may be attributed to their different red pepper varieties. Nowadays, the interest in developing analytical methodologies for the determination of polyphenols as well as to guarantee PDOs as an important and remarkable product quality factor is raising [1].

In the present work, C18 reversed-phase HPLC-UV was employed for the analysis of paprika [2]. HPLC-UV fingerprints were evaluated as potential chemical descriptors to address paprika sample characterization and classification. The plot of scores obtained after linear discriminant analysis (LDA) using non-targeted HPLC-UV fingerprints revealed patterns that were perfectly related to different sample characteristics such as PDO and flavor variety.

Furthermore, the proposed methodology was evaluated for the identification and quantitation of paprika frauds by means of LDA and partial least squares (PLS) regression. The score plots obtained evidenced clear patterns related to the degree of adulteration with clear clusters for the pure classes, and classification rate over 81% for the different levels (1 to 80%). In case of the quantitative PLS model, correlation coefficients were higher than 0.97 for the test subset.

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P92. CHARACTERIZATION AND CLASSIFICATION OF TURMERIC AND CURRY SAMPLES BY UHPLC-ESI-HRMS AND CHEMOMETRICS

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Turmeric (*Curcuma longa*) is a plant related to ginger family that has been used for centuries as a remedy in traditional Asian pharmacy and is appreciated as a condiment in cuisine. The benefits in human health are associated to its antioxidant, anti-inflammatory and antineoplastic activities. Curcumin is the most relevant molecule of turmeric providing color and pharmacological activity. Besides, other curcuminoids and polyphenols are important as well [1].

In the present work, C18 reversed-phase ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) using a q-Exactive Orbitrap analyzer was employed to address the characterization and classification of different commercial turmeric and curry samples. Both, profiling (targeted approach by monitoring curcumin, curcuminoids and polyphenolic compounds) and fingerprinting (non-targeted approach by monitoring relevant signals as a function of m/z and retention time) strategies were employed as a source of potential sample chemical descriptors to achieve sample authentication by means of principal components analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA). The plot of scores obtained after PCA and PLS-DA using non-targeted UHPLC-HRMS fingerprints revealed patterns that were perfectly correlated to the different characteristics of the samples. Moreover, sample discrimination and authentication tend to be related to polyphenolic and curcuminoid content, as suggested by the UHPLC-HRMS profiling study.

The proposed PLS-DA methods using both profiling and fingerprinting strategies were validated for the authentication of this type of samples in order to detect consumer frauds, showing good classification rates.

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P93. CLASSIFICATION OF COFFEE VARIETIES BY UHPLC-MS/MS AND UHPLC-HRMS

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Coffee is today one of the most popular beverages in the world. Intake of coffee is associated with a reduced risk of several diseases probably due to its antioxidant activity. Polyphenols are among the most interesting bioactive compounds found in coffee. In fact, coffee is the major source of chlorogenic acids in the human diet and there is plenty of evidence of their important antioxidant activity. Thus, polyphenolic coffee content could be related to features such as the coffee variety (Arabica and Robusta), production region and climate conditions, among other parameters [1].

In the present work, C18 reversed-phased targeted UHPLC-MS/MS (triple quadrupole) and UHPLC-HRMS (Orbitrap) polyphenolic profiling methods, as well as non-targeted UHPLC-HRMS (Orbitrap) fingerprinting method were employed as a source of potential chemical descriptors to address coffee sample classification by means of principal component analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA).

The plot of scores obtained after PCA and PLS-DA using the proposed methodologies revealed patterns that were perfectly correlated to different characteristics of the coffee samples such as the coffee variety (Robusta vs Arabica), the production region and the roasting degree. Moreover, patterns strongly dependent on polyphenolic content were observed.

The proposed PLS-DA methods using both profiling and fingerprinting strategies were validated for the authentication of this type of samples in order to detect consumer frauds, showing good classification rates.

Acknowledgement: Projects PGC2018-095013-B-I00, 2017SGR-171 and 2017SGR-310.

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P94. HPLC-UV FINGERPRINTING AND CHEMOMETRICS TO AUTHENTICATE HEN EGGS

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In the last years, public organizations, such as the European Union (EU), have established rigorous legislation as far as labelling is concerned, in order to guarantee food origin, quality and traceability. Moreover, products with value-added due to specific particularities, such as organic production, have recently gained special attention from consumers. In this line, hen eggs, which are well-known for their high nutritional value, are classified into 4 groups according to their production method: organic, free-range, barn or caged. Thereby, the misrepresentation of a high quality egg with a lower one is an illegal practice worldwide. Chromatographic fingerprinting strategies, where non-selective signals related to a range of potential discriminating compounds are recorded, have emerged as promising approaches when combined with chemometrics to address food authenticity issues [1].

In the present work, high performance liquid chromatography with ultraviolet detection (HPLC-UV) fingerprints were proposed as a source of potential chemical descriptors to achieve hen eggs classification according to their labelled type. Thus, chromatographic data was processed by means of principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). Results by PCA showed particular trends for organic and caged hen eggs; PLS-DA models to discriminate among each egg category in front of those with lower quality achieved a classification rate of at least 82.6%, proving the applicability of the method. Moreover, differences in egg phytochemical content among samples with different size independently of their type, as well as different manufacturers between samples from the same class, were also observed.

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P95. DETERMINATION OF NINE CYANOTOXINS IN SALINE WATER SAMPLES BY USING SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION AND UHPLC WITH UV AND MS/MS DETECTION

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In this study two different analytical approaches have been developed to determine the presence of several cyanotoxins in saline water samples from a continental salt marsh. A salting-out assisted liquid-liquid extraction (SALLE) has been used in combination with ultra-high performance liquid chromatography-tandem mass spectrometry and UV-diode array detection (UHPLC-MS/MS and UHPLC-DAD). The target analytes are eight microcystins named MC-RR, MC-YR, MC-LR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF and nodularin (NOD), covering a wide range of polarities. The separation was achieved using a Zorbax Eclipse Plus RRHD C18 column (50 × 2.1 mm, 1.8 μm) in less than 7.5 and 5.5 min for UV and MS/MS detection, respectively. The mobile phase used consisted of water (solvent A) and acetonitrile (MeCN) (solvent B), both containing 0.01% of formic acid for DAD and 0.4% of formic acid for MS/MS detection, at a flow rate of 0.4 mL min⁻¹. The temperature of the column was set at 25°C and 20 μL of sample were injected. The main parameters affecting the SALLE procedure were studied and the following optimum values were obtained: neutral pH, 2 mL of acetonitrile as extraction solvent and 1.2 g of ammonium sulfate as salting-out agent for 4 mL of water sample. The validation protocols for both methods were accomplished with real water samples obtaining LODs ranging from 1.0 to 3.4 μg L⁻¹ and 0.02 to 0.11 μg L⁻¹ for DAD and MS/MS respectively. Although the SALLE-UHPLC-DAD methodology is easier and cheaper than UHPLC-MS/MS significantly better detection limits were achieved with tandem mass spectrometry as well as allowing for unambiguous identification. Extraction recoveries were higher than 77.0% (except for MC-RR and NOD which were 53.2% and 54.3, respectively) with satisfactory inter-day and intra-day precisions (RSD below 13.3%).

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P96. OPTIMIZATION OF A SPME GC-MS METHOD FOR AUTHENTICATION OF *Garcinia Cambogia* SUPPLEMENTS

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The consumption of food supplements for overweight control (FSOC) is attracting increasing interest due to the high incidence of obesity in developed countries and the consumer's perception of their use as a natural remedy. Among the different FSOC commercially available, those based on *Garcinia cambogia* are one of the most demanded. However, the limited availability of *Garcinia* fruits mainly associated with their restricted cultivation area make these food supplements a frequent target of adulteration [1]. The main bioactive in *G. cambogia* supplements is hydroxycitric acid. Industries extract this compound from *G. cambogia* fruit pericarps previously subjected to a drying process (oven-drying or traditional smoking) that greatly affects their volatile composition [2]. Therefore, the main objective of this work was the optimization of a SPME GC-MS method for evaluating the authenticity of *G. cambogia* supplements based on their volatile profile. To that aim, the effect of the most important SPME operating factors (fibre coating, sample amount, equilibrium/extraction temperature and time) on isolation of volatiles was considered. Under optimized conditions (DVB/C/PDMS fibre, 0.1 g, T=40 °C, t_{eq} =15 min, t_{ext} =15 min, t_{sp15s} =0.5 min), a large number of volatiles (mainly sesquiterpenoids) were identified in the GC-MS profiles of the different *Garcinia* reference samples (5 x *G. cambogia*, 3 x *G. indica* and 4 x *G. mangostana*). Irrespective of the specie considered, α -copaene was the major compound detected; high levels of this volatile and δ -cadinene were found in *G. mangostana*. *G. cambogia* showed α -amorphene and α -gurjunene as major components, whereas the main volatiles in *G. indica* were α -muurolene and β -caryophyllene. Aldehydes such as octanal, nonanal, etc. and alcohols as 2-phenylethanol were also detected in all the samples under study. Regarding the analysis of FSOC, most of samples under study showed a GC-MS profile with a lower volatile content and only 4 out of 17 *G. cambogia* supplements gave rise to a profile resembling that of corresponding reference sample in terms of terpenoid markers.

SPME followed by GC-MS is shown as an affordable, fast and solvent-free technique which can be performed with low sample amount and be easily implemented for authentication of *G. cambogia* supplements.

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P97. DETERMINATION OF ORGANOPHOSPHATE FLAME RETARDANTS IN FISH SAMPLES USING QUECHERS EXTRACTION FOLLOWED BY GC-MS/MS

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Organophosphate flame retardants (OPFRs) are chemical additives incorporated in materials susceptible to ignition to prevent or delay the fire to start. They appeared as an alternative to the highly used brominated flame retardants and are now in the spotlight as they are also believed to present potential adverse health effects. Their ubiquity in aquatic organisms has led to an urge to develop methods capable of quantifying their presence so as the risk originated by the ingestion of fish could be assessed. Previous studies used extraction techniques such as pressurized liquid extraction (PLE) [1], ultrasound-assisted extraction (UAE) [2] or solid-liquid extraction (SLE) [3] followed by gas chromatography coupled to mass spectrometry. To the best of our knowledge, this is the first developed method for the determination of organophosphate flame retardants in fish samples that uses QuEChERS extraction procedure as a quicker, easier and cheaper alternative. The extraction procedure optimization includes the optimization of the QuEChERS salts used and the clean-up strategies for two different fish species differing in lipid content: cod (low lipid content) and salmon (high lipid content). A convenient matrix clean-up is necessary to reach low concentrations of the target compounds. Therefore, various clean-up procedures including LLE (hexane as solvent) and d-SPE (PSA, florisil, activated charcoal and coco charcoal) have been tested to evaluate their ability to clean the obtained extracts. The final extracts are analyzed using gas chromatography coupled to tandem mass spectrometry.

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P98. DEVELOPMENT OF A SIMPLE AND FAST HPLC-MS/MS METHOD FOR α - AND β -ODAP QUANTIFICATION IN *LATHYRUS SATIVUS* (GRASS PEA) SAMPLES

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Grass pea (*Lathyrus sativus*) is the most important and more cultivated *Lathyrus* species [1]. However, this species have been implicated as causes of a motor neuron degeneration syndrome, called neurolathyrism, in both men and animals, due to the presence of the neurotoxin oxalyl-L- α , β -diaminopropionic acid (β -ODAP) [1]. In *Lathyrus* seeds, β -ODAP is accompanied by lower concentrations of its non-toxic α -isomer [1]. Therefore, it is worthy to quantify both ODAP isomers in different samples, in order to study their potential toxicity. It is a challenge to analyse ODAP using direct HPLC methods due to its high polarity and weak UV absorption. Hence, α - and β -ODAP quantification has been performed essentially by liquid chromatography after sample derivatization, which is often laborious and time consuming [2]. However, hydrophilic interaction chromatography (HILIC) is suitable for analysing compounds in complex systems that always elute near the void volume (highly polar) in reversed-phase chromatography, and it can be conveniently coupled to mass spectrometry (MS) [3]. In this work, a procedure proposed by McKeown (2015) for the selection of the best HILIC-type column was followed [4], and a simple and fast HPLC-MS/MS method was developed without sample derivatization, using a chemically bonded diol phase HILIC column. This method was applied to the quantification of both ODAP isomers in twenty *Lathyrus sativus* varieties. The content of the neurotoxic isomer in the analysed samples ranged from 0.45 to 4.99 mg.g⁻¹. The developed method is suitable for the prompt identification of contrasting accessions in what concerns α and β -ODAP contents.

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P99. FUNCTIONALIZED MESOSTRUCTURED SILICA IN SAMPLE PREPARATION FOR THE ANALYSIS OF HYDROXYMETHYLFURFURAL IN CEREAL AND INSECT BARS

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In the last years, Food Safety Authorities and Scientifics have paid great attention to food processing contaminants. One of these chemical compounds is 5-hydroxymethylfurfural (HMF), which is formed during thermal processing of carbohydrate- and protein-rich foods. HMF is a potential genotoxic and carcinogenic compound. The main mechanism of HMF formation is the Maillard reaction (considered as an early marker of this reaction), but also caramelisation, so it is found in different types of processed foods such as breakfast cereals, cookies and muesli, as well as in several types of bakery products. These foods have very complex composition (sugars, honey, cocoa, dry fruits, nuts) that difficult its analysis. In this sense, it is interesting to develop new extraction and clean up approaches to eliminate matrix interferences, including the application of new sorbents. Therefore, in this work a mesostructured silica (pore-expanded SBA-15) was synthesized and functionalized with aminopropyl- (SBA-15-PE-NH₂) and octyl- (SBA-15-PE-C8) groups. The prepared materials were evaluated as sorbents in solid phase extraction (SPE) for the determination of HMF in cereal and insect bars. Analyses were carried out by high performance liquid chromatography coupled to a triple quadrupole mass spectrometry detector with an ESI in positive ion mode. For the sample preparation, firstly, a liquid-liquid extraction was carried out with different solvents (acetonitrile, water and methanol), being methanol that showed the best results. Secondly, 50 mg of each material (SBA-15-PE, SBA-15-PE-NH₂, SBA-15-PE-C8) were packaged in cartridges of 3 mL and different conditions were tested, in order to optimize the SPE procedure. Under optimized conditions, results revealed that SBA-15-PE-NH₂ was clearly more successful in the extraction of HMF. In addition, higher recovery values were obtained with this material (near 100%), compared with other analogous commercial sorbent (Discovery[®] DSC-NH₂) evaluated under similar conditions (41 ± 7%). The method developed was successfully applied to analyze different commercial cereals and insect bars.

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P100. DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COMPLEX DRY VEGETAL EXTRACTS USING QuEChERS AND MIP-SPE FOLLOWED BY GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Polycyclic aromatic hydrocarbons (PAHs) are a complex group of compounds which contain 2 or more aromatic fused rings. PAHs are formed mainly from human activities by incomplete combustion of organic matter. The interest for determination of this family of compound is based on the consideration of these substances as potentially carcinogenic and mutagenic [1,2] and additionally, the exposure to human population is very high due to its presence on dietary intake. A sensitive and selective method based in MIP technology has been developed for the determination of polycyclic aromatic hydrocarbons (PAHs) in complex dry plant extracts of *Panax ginseng*, *Salvia officinalis*, *Camellia sinensis*, *Zingiber officinale*, *Uncaria tomentosa*, *Humulus lupulus*, *Pinus maritima*, and the bee products, propolis and royal jelly. The method has been validated using gas chromatography coupled to tandem mass spectrometry. An additional sample treatment of the dry extracts is required because of the strong matrix effect observed in the analysis. This sample treatment is based on the use of MIP-SPE in addition to QuEChERS in order to eliminate interference substances that affect the adequate quantification of analytes. The method was accurately validated and the detection and quantification limits were established according to current EU Regulation (PAH4). The quantification limits range from 0.4 ng g⁻¹ for chrysene to 0.9 ng g⁻¹ for benzo(a)pyrene, with recoveries close to 100 % and % RSD lower than 12%. The method was satisfactorily applied to a large number of dry plant extracts and bee products from EU and non- EU manufacturers.

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P101. EVALUATION OF FUNCTIONALIZED ORDERED MESOPOROUS SILICAS FOR STRONG CATION-EXCHANGE SOLID-PHASE EXTRACTION OF TROPANE ALKALOIDS IN FLOURS

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Tropane alkaloids (TAs) are secondary metabolites produced by a wide variety of plants from the families of *Brassicaceae*, *Solanaceae*, etc. Atropine and scopolamine, the best-known representative TAs, are strong antimuscarinic agents. EFSA's Panel on Contaminants in the Food Chain delivered a scientific opinion on the risks to health related to the presence of TAs in foods. In this sense, the development and validation of analytical methods for the control of TAs in relevant matrices (such as flours and cereal-based food products) was recommended. Sample preparation is a key step for quantitative analysis of trace contaminants in complex matrices. Current trends in sample treatment are focused on the synthesis of new materials and their application as sorbent in solid phase extraction (SPE). In this sense, in the last years ordered mesoporous silicas have been presented as good alternatives to classical sorbents. In these work, an ordered mesoporous silica (SBA-15) was synthesized and functionalized with different amount of sulfonic acid groups (SO_3^-), in order to obtain materials with strong cationic-exchange retention mechanism. The resulting materials were comprehensively characterized and they showed high surface area (between 350 and 587 m^2/g), high pore volume (0.38 – 0.70 cm^3/g) and controlled porous size (36 – 56 Å). Elemental analysis indicated that the SO_3^- groups anchored to the silica surface of the pore walls were: 0.484 mmol/g for SBA-15-SO3-L, 0.944 mmol/g for SBA-15-SO3-M and 1.817 mmol/g for SBA-15-SO3-H. To verify the presence of SO_3^- moieties in the functionalized silicas, an acid-base titration was performed. The functionalized materials were evaluated as SPE sorbents for the extraction of atropine and scopolamine. Analyses were carried out by high performance liquid chromatography coupled to a triple quadrupole mass spectrometry detector with an ESI in positive ion mode. Best results were achieved with SBA-15-SO3-H, obtaining recoveries near to 100 % for both TAs in standard mixtures. The method developed was successfully applied to determine atropine and scopolamine in flours achieving good recoveries. Results obtained suggested that SBA-15-SO3-H is a promising sorbent for SPE and may be a good alternative to commercial sorbents, in order to determinate TAs in foods.

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P102. ISOLATION OF PROTEINS FROM SPENT COFFEE GROUND. IDENTIFICATION OF PEPTIDES IN THE PROTEIN HYDROLYSATES BY RP-HPLC-ESI-Q-TOF

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Coffee industry produces a large amount of waste that can be >50% of the fruit mass. Among them, the solid residue obtained during the brewing process, the so called spent coffee ground (SCG), is usually incinerated or disposed of in landfills with the subsequent air pollution and soil contamination, and therefore strategies for its management are needed. Extensive works have been developed for the extraction and application of polysaccharides, polyphenols and caffeine in food formulations. However, there are no works focused on peptide identification from SCG protein hydrolysates. In the present work, a protein extraction/clean up methodology has been developed to isolate coffee SCG proteins from samples obtained with different coffeemakers, to hydrolyze the proteins into peptides and to evaluate the roasting effect on the antioxidant or antihypertensive capacities of hydrolysates. In addition, the resulting peptides have been identified by RP-HPLC-ESI-Q-TOF technology. The combination of a urea-based extraction buffer together with the use of 3 kDa molecular cut-off filters allowed the extraction of a higher content of proteins as compared to conventional approaches, and demonstrated that this protein content was affected by the coffeemaker employed. In addition, the extracts obtained after the hydrolysis of espresso SCG proteins with thermolysin and alcalase enzymes demonstrated antioxidant and antihypertensive activities, the latter being higher when SCG samples were obtained from dark/medium roasted coffees. Finally, the characterization of these extracts by RP-HPLC-ESI-Q-TOF allowed the identification of more than 15 peptides in thermolysin hydrolysates, which might be responsible for the antihypertensive capacity observed.

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P103. DEVELOPMENT AND OPTIMIZATION OF A METHOD FOR DETERMINATION OF ORGANOCHLORINE PESTICIDES IN MUSSEL BASED ON MINIATURIZED MATRIX SOLID-PHASE DISPERSION COMBINED WITH GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Elevated concentrations of organochlorine pesticides (OCPs) have been detected in marine environmental for decades [1]. Though many of these compounds are prohibited, they have actually been detected in a lot of ecosystems such as bivalve mollusc. Mussel has widely used as sentinel organism in monitoring of marine pollution besides being considered as appreciated seafood. They filter-feed by pumping and filtering large volumes of water, it makes them to accumulate chemical contaminants from the seawater giving an integrative measure of the bioavailability and levels in the environment [2]. A miniaturized methodology applied to the analysis of fourteen organochlorine pesticides (OCPs) in mussel samples was developed. This approach was based on matrix solid phase dispersion (MSPD) that enabled to perform extraction and clean up of sample at the same step. Subsequently, the extracts were readily analyzed by Gas Chromatography-Mass Spectrometry in tandem (GC-MS/MS). The extraction and clean up parameters, amounts of sample, drying agent (anhydrous sodium sulfate), dispersant agent (Florisil), extractant volume and solvent polarity were optimized by using a fractional factorial design 2^{5-1} . Four of five factors, sample and Florisil amounts, extractant volume and solvent polarity were statistically significant for some of OCPs. The analytical performance demonstrated a broad linear range ($R^2 < 0.998$), recoveries around 85% and relative standard deviation below 7%. The methodology was applied to the analysis of three mussel samples coming from Rías of Ferrol and A Coruña in Galicia coast (NW, Spain).

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P104. A NOVEL METHOD TO DETERMINE FIPRONIL AND FIPRONIL SULFONE IN EGGS BY CAPILLARY ELECTROPHORESIS AND SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION

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Fipronil is an insecticide that has been prohibited in food-producing animals. However, in 2017, it was involved in a European health alert due to its presence in fresh hen eggs because of an illegal use as a veterinary treatment. EU has set maximum residue limits in eggs as the sum of fipronil and its main metabolite, fipronil sulfone ($5 \mu\text{g}\cdot\text{kg}^{-1}$), so it is crucial to monitor them in these matrixes. Previous methods were restricted to liquid and gas chromatography. In the present study, we have developed the first methodology for the separation of fipronil and two metabolites, fipronil-sulfide and fipronil-sulfone by capillary electrophoresis (CE) with UV-detection. Different electrophoretic modes were evaluated. Micellar electrokinetic chromatography (MEKC) was eventually selected, using as background electrolyte a solution of 50 mM ammonium perfluorooctanoate pH 9.0 with 10% (v/v) methanol. The proposed method was combined with a simple sample treatment based on salting-out assisted liquid-liquid extraction (SALLE). Acetonitrile and ammonium sulfate were the extraction solvent and the salt selected for the determination of these compounds in eggs. However, an endogenous interferent peak did not allow fipronil-sulfide quantification using this extraction procedure but it was not a problem in the case of fipronil and fipronil sulfone. Validation parameters were investigated yielding satisfactory results. Precision, expressed as relative standard deviation, was below 14% and recoveries ranged from 83 to 88%. Limits of detection were $90 \mu\text{g}\cdot\text{kg}^{-1}$ for fipronil and $150 \mu\text{g}\cdot\text{kg}^{-1}$ for fipronil-sulfone. In terms of sensitivity, when intended for regulatory purposes, a sample treatment allowing extra preconcentration or more sensitive detectors, such as mass spectrometry (MS), would be needed in further works.

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P105. AUTHENTICATION OF *Garcinia cambogia* SUPPLEMENTS BY GC-MS

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Food supplements (FS) from *Garcinia cambogia* fruits have currently gained great attention as a natural alternative for weight loss due to their high content in bioactive hydroxycitric acid (HCA). Considering the high price of this fruit, *G. cambogia* based supplements are expected to be target of a variety of frauds such as discrepancies between the real content of bioactives and that stated in the label, adulteration with lower-priced species such as *G. indica*, addition of synthetic bioactives, etc [1].

The determination of HCA is usually carried out by HPLC-UV [2] but these methodologies has several drawbacks such as the use of long columns to overcome the low retention of this analyte, the possible coelution with ascorbic acid and the difficult hyphenation with mass spectrometry due to the use of non-compatible mobile phase additives. Therefore, the main objective of this work was to develop a method by GC-MS for the improved determination of HCA and other bioactives aimed to the authentication of *G. cambogia* FS. Sixteen FS based on *G. cambogia* extracts were bought in specialized shops, pharmacies and websites. Eight *G. cambogia* and *G. indica* rinds were also purchased online. Extracts from these rinds were obtained by solid-liquid extraction under different conditions and were considered as reference samples. Extracts were evaporated and derivatized prior to GC-MS analysis using a HT-5 capillary column. Temperature gradient and other experimental conditions were optimized for high throughput analysis (22 min/sample). The developed GC-MS method allowed the simultaneous detection of HCA without interferences and of other compounds declared in the label (vitamins and excipients). Moreover, detection of different compounds such as sugars, inositols and difructose anhydrides (markers of inappropriate processing or of fructose syrup addition) was achieved. The presence of inositols in some of FS samples under study (also detected in the reference extracts) might also be indicative of their authenticity. The optimized GC-MS method resulted to be a good alternative to conventional HPLC-UV for the determination of *G. cambogia* FS quality.

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P106. OLIVE RIPENESS AS A TOOL TO MODULATE PHENOLIC COMPOSITION OF OILS AND *IN VITRO* INHIBITION OF α -GLUCOSIDASE

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To adopt a healthy lifestyle is necessary to anticipate risk factors that favour the development of diseases, being the diet an important tool for this purpose. The intake of olive oil, as major fat in the Mediterranean diet, could be an alternative natural source of α -glucosidase enzyme inhibitors, which delay the digestion rate of carbohydrates decreasing, consequently, the impact of diabetes mellitus (DM). Galicia has gradually emerged as a new Spanish olive-growing zone with an increasing annual production of high quality extra-virgin olive oils (EVOOs) from old autochthonous varieties (the most popular known as 'Brava'). To this end, it is necessary to select the optimum ripening moment for olives. Therefore, the aim of the present work was to study the effect of the ripeness index of 'Brava' fruits i) on EVOO phenolic composition and ii) on *in vitro* α -glucosidase enzyme inhibition. 'Brava' EVOOs elaborated with olives selected at different degree of ripeness exhibited differences in their phenolic profile determined by LC-ESI-IT-MS [1]. In addition, phenolic rich extracts from such EVOOs were more active than acarbose [2]. 'Brava' EVOOs elaborated with olives classified with the highest degree of ripeness exhibited the strongest inhibitory activity (IC₅₀ value of 143 ± 5 μ g of dry extract/mL). However, our results suggest that the extracts obtained from 'Brava' EVOOs could represent natural valuable strategies to DM treatment, regardless of the 'Brava' olive ripeness.

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P107. EXTRACTION OF CHLOROGENIC ACID FROM BLUEBERRY POMACE USING NOVEL EXTRACTION TECHNOLOGIES

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Processing of blueberries into juice generates a considerable amount of a solid residue (pomace) generally treated as a waste. This by-product represents a good source for obtaining the valuable polyphenols since it contains high amounts of polyphenols among which highlights chlorogenic acid (CGA). The CGA is an important and biologically active dietary polyphenol, playing several important and therapeutic roles such as antioxidant activity, antibacterial, hepatoprotective, cardioprotective etc [1]. The extraction of CGA from blueberry pomace has been well studied, in terms of total polyphenol extraction, and numerous methods for polyphenols extraction are developed. However, there are new extraction technologies which give better yields and higher stability of polyphenols during extraction.

The present study aims to elucidate the effect of pulsed electric field (PEF), high voltage electrical discharges (HVED) and ultrasound (US) on the efficiency of the extraction of chlorogenic acid from blueberry pomace. All extractions were performed with methanol and ethanol based solvents (50%, v/v). LC-MS/MS and HPLC-DAD were employed for the determination of chlorogenic acid in obtained extracts. Ethanol based solvent was shown to be a better solvent for PEF and HVED extraction, and methanol based solvent for US assisted extraction. Considering treatment parameters, PEF assisted extraction was most effective at higher electric intensities (20 kV/cm), HVED at energy input of 22.05 kJ/kg and US assisted extraction at higher temperatures (80°C). The highest yields of CGA obtained by PEF, HVED and US were 568.86, 421.54, and 539.92 µg/g dw, respectively.

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**P108. ALTERATIONS IN MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)
COMPOSITION EXPOSED TO CYANOTOXINS AS REVEALED BY ANALYTICAL PYROLYSIS**

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Cylindrospermopsin (CYN) and Microcystin-LR (MC-LR) are biotoxins produced by cyanobacteria species that, as consequence of human-induced environmental changes i.e. increase of temperature due to climate change and the eutrophication of waters, proliferate together with an increase in the presence of the associated cyanotoxins. Both toxins are usually found in the aquatic environment, filter feeding organisms such as mussels are exposed continuously and at such quantity that may be the cause of metabolic alterations.

Analytical pyrolysis (Py-GC/MS) was used to evaluate compositional changes in *M. galloprovincialis* after exposure for 14 days to cyanotoxins of *Chrysochloris ovalisporum* (CYN) (0.785 µg mL⁻¹), *Microcystis aeruginosa* (MC-LR) (2.3 x 10⁻³ µg mL⁻¹) and a combination of both toxins (CYN + MC-LR). A group of mussels not exposed to the toxins was also included as control. Pyrolysis of lyophilized mussel flesh produced complex chromatograms (100 different compounds) but very similar between treatments. Major groups found were N compounds (pyridine, N-alkyl molecules) (18% ± 1.5) and peptide/protein derived (18% ± 2.4) that include alkyl indols and diketopiperazines (DKPs) as pyrolysis product from amino acid condensation, series of medium chain length (C₁₄-C₂₂) saturated, mono and polyunsaturated fatty acids (45% ± 5.0), sterols (8% ± 0.6) and aromatics with undetermined origin (9% ± 1.01). Chemometric treatment of the chromatographic data (variable reduction, PCA and hierarchical cluster analyses) allow us to discriminate between the different mussel populations exposed to cyanotoxins. The results are discussed in terms of the possible effects exerted by the biotoxins in mussel composition and possible metabolic pathways affected.

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P109. MULTI-MYCOTOXIN DETERMINATION IN UNIFEED BY (D)SPE-QUECHERS-HPLC-HRMS

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Mycotoxins are secondary metabolites generated by some fungi such as *Aspergillus*, *Penicillium* and *Fusarium*. Some of them may infect crops and produce mycotoxins during production and storage. Amongst the negative effects these toxins can represent to human and animal health, mutagenic, teratogenic, carcinogenic and immunotoxinogenic activities are reported, and they can also affect the productivity in dairy farms. The European Union (EU) has set some limits for mycotoxin maximum levels in feeding stuffs, particularly aflatoxin B₁, ochratoxin A, deoxynivalenol, zearalenone and fumonisins. In addition, since the co-occurrence of mycotoxins in feed is the most common scenario, the European Food Safety Authority (EFSA) recommends developing multi-mycotoxin analytical methods. In this work, 25 mycotoxins from different genera were then analysed in 100 unifeed samples collected in 20 dairy farms. A sample preparation methodology based on a combination of (d)SPE and QuEChERS extractions was used. A Liquid Chromatography-High Resolution Mass Spectrometry was employed by means of a SCIEX TripleTOF[®] 5600+ equipped with a DuoSpray[™] ion source, using Electrospray Ionization (ESI), both in positive and negative modes. A Shimadzu Nexera X2 HPLC system equipped with a Phenomenex Kinetex bioZen[™] Peptide XB-C18 column was used. A powerful workflow based on data-independent acquisition, (SWATH), was implemented. Analytical performance was evaluated in terms of linearity, repeatability and matrix effect. Relative recoveries were also measured, giving values between 60 and 120%. Matrix-matched calibrations was carried out and enabled reaching LOQs at the ng g⁻¹ level. The observed significant matrix effects, in most cases suppressive, varied amongst mycotoxins between -22 % and -89 %. Fumonisin B₁ and B₂, zearalenone, its metabolite β-zearalenol and enniatin B were the most frequently found mycotoxins in the unifeed samples.

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P110. DEVELOPMENT OF A HPLC-DAD-MS METHOD FOR THE QUALITY EVALUATION OF GUARANA (*Paullinia cupana*) SUPPLEMENTS

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Guarana (*Paullinia cupana*) is a very popular plant native to Brazilian Amazon, whose seeds are worldwide consumed as food supplements (FS), due to their beneficial stimulatory and antioxidant effects [1], among others. These activities are mainly related to different methylxanthines (caffeine, theophylline and theobromine) and phenolic compounds (catechin, epicatechin, epicatechin gallate, etc.) [2]. Despite guarana FS are generally perceived by consumers as natural and harmless antiobesity products, they had been target of different frauds and adulterations in the last years. Then, the development of improved analytical methodologies to evaluate their quality is of great interest.

In this work, a chromatographic HPLC-DAD-MS method for the simultaneous determination of methylxanthines and phenolic compounds in guarana FS was developed. Two kinds of commercial FS based on guarana extracts (GE) or ground seed powder (GSP), were purchased in specialized shops, pharmacies and web sites. Reference extracts from guarana seeds were laboratory-made by solid-liquid extraction under different conditions (time, temperature) to simulate FS industrial processes. A new HPLC-DAD-MS method was optimized by evaluating different columns and analytical conditions (mobile phases, additives, flows, MS acquisition mode, etc.). The best separation was achieved with a Poroshell 120 SB-C18 column (Agilent) using a gradient 0.1% acetic acid:acetonitrile and 0.4 mL min⁻¹ as flow rate. MS operating in positive mode was used for quantitative analysis. Bioactive caffeine was the most abundant compound detected in all analysed samples. Phenolic compounds and other methylxanthines evaluated as potential authentication markers, were also present in lower concentrations. Relationships of caffeine/theophylline and caffeine/theobromine concentrations determined in reference samples were used to establish an authenticity profile for evaluation of FS quality. The methodology here developed is a valuable contribution to the reliable and barely explored authentication of FS associated with the analysis of minor components.

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P111. CHEMICAL CHARACTERIZATION OF THE AROMA OF ARAGON NATIVE APPLES USING GC-OLFACTOMETRY AND GC-MS

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Apple cultivation is suffering a decline in its production and in its economic volume that makes it necessary to innovate and implement new products to invert that trend and at the same time, to preserve varietal diversity. For these reasons, in this work, the chemo-aromatic profiles of native apples of the region of Aragon (MA-257 and MA-144) were obtained and compared with those of the commercial variety Royal Gala. To this aim, aroma-representative extracts of each sample were prepared using a purge and trap system which involves passing a small stream of nitrogen through a vessel containing the apple sample cut in small regular pieces. Volatiles are trapped into a LiChrolut EN resin cartridge kept at 0°C. The extract is then eluted with dichloromethane containing 5% of methanol, it is further concentrated by solvent evaporation and analyzed by gas chromatography olfactometry (GC-O) and gas chromatography mass spectrometry (GC-MS). Overall 38 odorants were detected in the three varieties, of which 34 could be correctly identified by odor, retention indexes in two phases and Mass Spectrometry. Analysis of variance (ANOVA) was performed on the olfactometry scores to determine whether observed differences between varieties were significant. Results reveal the presence of some aroma compounds common to all varieties such as ethyl esters (ethyl butyrate, ethyl 2 and 3-methyl butyrate) and also some other aroma compounds which seem to be specific for a single variety. For example, diacetyl and ethyl isobutyrate were exclusively found in MA-144; β -damascenone and E-whiskylactone were found in MA-257, exclusively. These differences could make these varieties more attractive for the consumer and could represent an increase in quality, production and distinction in the market.

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P112. BODY-DECORATING PRODUCTS: CURRENT ANALYTICAL PERSPECTIVE OF PERMANENT AND TEMPORARY TATTOOS INGREDIENTS

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Tattoos, both permanent and temporary, can be included in the group of new format cosmetics or those considered as *borderline* products [1]. Their regulation and the absence of systematic surveillance on their safety are recently important issues [2], in particular with regard to their main ingredients, the pigments. These ingredients are synthetic and organic hydro- or liposoluble compounds, with a wide range of solubilities. Most of them are photochemical unstable, but little is known about potential degradation products.

This work compares the analytical approaches developed so far to control the safety of tattoo products: inks, permanent make-up, sticker tattoos and henna products. The ingredients included were the pigments along with their impurities and degradation products [3]. They can contain hazardous chemicals such as PAH mainly from black inks, primary aromatic amines from azo-colorants or metals from inorganic and organometallic pigments. Other undesirable additives, such as plasticizers or *para*-phenylenediamine, are also considered.

A pre-treatment step is recommended prior to the analysis, to avoid possible matrix interferences. In most cases a simple approach is used, an easy extraction normally assisted by ultrasound followed by centrifugation. Analytical procedures are quite varied: from spectroscopic to chromatography techniques, depending on the type of analysis required: qualitative or quantitative; and it is common to find combinations of complementary methods.

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P113. NON-TARGETED UHPLC-HRMS (ORBITRAP) FINGERPRINTING IN THE AUTHENTICATION OF CRANBERRY NATURAL PRODUCTS AND PHARMACEUTICALS

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Recently cranberries have attracted much attention due to their high content of proanthocyanidins (PACs) and the capacity of some of them (A-type PACs) to prevent urinary tract infections (UTIs). The fact that only A-type PACs have the required bioactive capacity and that pharmaceutical laboratories frequently assess the total content of PACs by non-selective colorimetric methods, unable to differentiate between A- and B-type PACs (the last ones more present in other fruits such as grape, blueberry, raspberry), demonstrates the importance of developing analytical methods to authenticate the fruit of origin employed and to prevent frauds.

Non-targeted UHPLC-HRMS (Orbitrap) fingerprinting was employed to characterize, classify and authenticate natural and pharmaceutical cranberry-based products [1]. Different fruits (cranberry, blueberry, raspberry, grape) as well as cranberry pharmaceuticals were analyzed after a simple sample extraction procedure. UHPLC-HRMS fingerprinting data, obtained in both positive and negative ESI modes, exploring also the possibility of data-fusion, were evaluated as a source of potential chemical descriptors. Unsupervised principal component analysis (PCA) and supervised partial least squares-discriminant analysis (PLS-DA) were exploited for authentication purposes, showing good results with a 100% sample classification rate. Data were further treated by partial least squares (PLS) regression to quantify the percentages of fruit extracts (grape, blueberry and raspberry) used for adulteration in cranberry extracts, showing that adulterant levels below 2.5% can be successfully quantified with low enough calibration and validation errors.

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P114. DETERMINATION OF BIOACTIVE COMPOUNDS IN SERIAL EXTRACTS OF CHIA LEAF (*SALVIA HISPANICA* L.) BY UHPLC-HRMS

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Consumers' interest in foods that are nutritionally balanced and with health benefits has increased. Food industry is paying attention to the use of the ancestral seed *Salvia hispanica* L., commonly known as chia. At present, only the chia seeds, which are a natural source of omega-3 and omega-6, fiber, proteins and natural antioxidants, are commercialized; while the chia plant is commonly used as fertilizer to soils or treated as a waste after harvest. However, there are some studies revealing the presence of several bioactive compounds such as flavonoids (e.g., vitexin and orientin) and some hydroxycinnamic acids in chia plant ethanolic extracts [1]. Therefore, it can represent a by-product that could be considered a great source of bioactive compounds with unexplored potential applications to the food industry.

In this work, UHPLC-HRMS (Orbitrap) was employed to tentatively identify and determine bioactive compounds present in different leaf extracts of chia plants of black and white origin obtained with solvents of different polarity (ethanol, ethyl acetate, dichloromethane and hexane) to address chia plant by-product revalorization. Caffeic, rosmarinic, protocatechuic and *p*-coumaric acids, coumaric acid-O-hexose, kaempferol and genistein were mainly found in polar solvent (ethanol and ethyl acetate) extracts; salvianolic acid F isomer and dimethyl quercetin in ethyl acetate and dichloromethane extracts, while ferulic acid, luteolin-O-glucuronide, acetyl orientin, vitexin and orientin compounds were found in all the extracts. Correlation of bioactive compounds with the antioxidant capacity of the obtained extracts, which was higher on ethanolic extracts and on black chia against white chia, was also studied.

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**P115. PHYTOCHEMICAL CHARACTERIZATION OF *RAMALINA STRIATULA* EXTRACTS
OBTAINED BY GREEN COMPRESSED FLUID TECHNOLOGIES IN A BIOREFINERY
APPROACH**

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The *Ramalina* genus is a greenish lichen with approximately 246 species distributed around the world, of which 118 species have been chemically or biologically studied [1]. However, to the best of our knowledge, no studies have investigated the *Ramalina Striatula* species. Lichens contain different lichen secondary metabolites, such as usnic acid which has been incorporated in cosmetics, perfumery and traditional medicines for years due to their proven spectra of biological activities, including antimicrobial, antiviral, anticancer, anti-inflammatory and analgesic properties [2].

Considering the bioactive potential of the different *Ramalina* species, a biorefinery approach for the valorization of this lichen is proposed in this work. For this purpose, a pressurized liquid extraction (PLE) procedure was applied to the residue of *Ramalina Striatula* after supercritical CO₂ extraction (SFE-CO₂). SFE was carried out at 300 bar and 40 °C for 30 min. *Ramalina* extracts obtained by SFE were chemically characterized by GC-MS.

PLE was carried out at 100 bar for 20 min. Two different temperatures were studied (40 °C and 180 °C) and two green solvents (water and ethanol) were used. The extracts with highest and lowest antioxidant activities were characterized by HPLC-MS/MS in order to correlate chemical composition to antioxidant activity. Our results will discuss this new evidences based on the presence of fatty acids, depsides, depsidones and dibenzofurans.

Acknowledgements

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h ANALYSIS AND QUANTIFICATION OF *SAMBUCUS NIGRA* EXTRACTS FOR POTENTIAL SKIN APPLICATION

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Natural products are valuable and precious resources and thus they come with their fair share of challenges concerning the provision of enough amounts for cosmetic or food supplement development. *Sambucus nigra* L. is a great example of this success, it is recognised as an herbal product with a long-standing use, particularly because the use of its flowers [1]. The aim of present work was to biologically characterize and to identify the major compounds present in methanol extract of flowers obtained by ultrasound method. Identification of compounds was performed using HPLC–ESI–MS/MS method operating in negative and positive modes. Several flavonoids were identified such as rutin (major compound) and isoquercetin, among others. We verified that this extract presents some interesting biological activities to skin application. Therefore, it was encapsulated into different nanocarriers (based on polymers - PLGA and PCL, and lipidic-based carriers - ethosomes) as final dosage form, to stabilize this extract. The preliminary encapsulation efficiency studies, was carried out by HPLC–ESI–MS/MS operating in MRM mode, and was based in rutin quantification. Results revealed that PLGA and PCL nanoparticles and ethosomes encapsulated 52.1±28.6%; 100.0±0.0% and 99.9±0.0% of the extract, respectively. Further research will focus on the re-assessment of the biological activities into nanocarriers using the same models.

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P117. EXTRACTION OF BIOACTIVE COMPOUNDS FROM POMEGRANATE SEEDS USING HIGH INTENSITY FOCUSED ULTRASOUNDS AND PRESSURIZED LIQUIDS

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The growing world population and, consequently, the increasing demand for foods results in the generation of tons of food wastes, which constitutes a priority environmental issue for the United Nations Organization and the European Commission. Moreover, the aging population has raised the incidence of serious diseases, which seem to be strongly influenced by diet habits [1]. The development of strategies for the valorization of food by-products by the extraction of bioactive compounds can give an answer to both demands, the reduction of waste generation and the development of smart foods to reduce the incidence of certain diseases. Processing of pomegranate generates by-products such as seeds, with high content in proteins and lipids. Pomegranate seeds lipid fraction is exploited by the cosmetic industry, but no effort has still been made to use their protein fraction (20% of the seed). The aim of this work was to develop green methodologies for the extraction of proteins and bioactive compounds from pomegranate seeds using both ultrasounds and pressurized liquids. Different *in vitro* assays were used to assess extracts bioactivity and RP-HPLC-ESI-MS/MS was employed to identify peptides and polyphenols present in the extracts. The optimization of both pressurized liquid extraction and ultrasound assisted extraction allowed to obtain the maximum protein yield under alkaline conditions. Protein extracts were subjected to *in vitro* gastrointestinal digestion. The hydrolysis of proteins resulted in an increase of the bioactivity in both extracts. HPLC-MS/MS was used to identify polyphenols and peptides in most active extracts.

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P118. ANALYTICAL PLATFORM FOR THE ANALYSIS OF VARIABILITY INTRODUCED BY

10 S. *CEREVISIAE* STRAINS IN TEMPRANILLO WINES

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This work consists in the development of an analytical platform to characterize volatile compounds produced by *Saccharomyces cerevisiae* strains during wine elaboration using 3 extraction methods and chromatographic quantifications. Micro-fermentations were realized with ten *S. cerevisiae* commercial wine strains (Lallemand Bio SL, Spain) in synthetic must supplemented in polyphenols and aroma precursors extracted from Tempranillo grapes. After fermentation, wines were submitted to accelerated anoxic ageing. To capture volatile compounds produced and evaporated during fermentation, a LiChrolut EN resin cartridge was placed into the headspace of fermenters. Retained compounds were eluted with dichloromethane:methanol. Extracts were characterized by gas chromatography olfactometry (GC-O) and fermentative volatile compounds were quantified by gas chromatography mass spectrometry (GC-MS). In young wines, major fermentative compounds were extracted using liquid/liquid micro-extraction. Extracts were quantified by gas chromatography coupled to flame ionization detector (GC-FID). In young and aged wines, trace varietal compounds and polyfunctional mercaptans were extracted by solid phase extraction (SPE) with LiChrolut EN resin enriched in mercaptan trapper. Trace compounds were eluted, mercaptan trapper were deactivated and polyfunctional mercaptans were eluted. Both extracts were analyzed in GC-MS. This analytical platform aims to quantificate 80 volatile compounds commonly found in young and aged wines fermented by *Saccharomyces cerevisiae*.

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P119. NATURAL PIGMENTS-BASED TATTOOS: ANALYTICAL APPROACHES FOR THEIR SAFETY CONTROL

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Tattoos can be included in the group of new format cosmetics or those considered as *borderline* products [1]. Temporary tattoos are a popular alternative to permanent ones. The use of natural origin pigments as henna tattoos, stand out. But recently, another type also based on plant pigments emerges as an alternative, namely jagua tattoos. Henna contains lawsone or 2-hydroxy-1,4-naphthoquinone (HNQ) as the active ingredient, but nowadays natural henna is usually mixed with additives, mainly *para*-phenylenediamine (PPD), which is widely recognized as a potent sensitizer [2]. Jagua contains geniposide and its bioactive compound, genipin. Lately, it has been suggested that jagua may constitute a potential new allergen in temporary tattoos [3]. Regulations on plant pigments-based temporary tattoos are still unformed and most of the commercial products are unlabeled.

Therefore, it is necessary to develop appropriate methodologies for the analysis of these complex samples that can be purchased on a well-known website and available to anyone. This work shows the first results obtained to identify these types of inks according to their markers (HNQ and PPD in henna, genipin and geniposide in jagua). Easy extraction methods have been tested based on vortex or ultrasound-assisted techniques. Chromatography with MS detectors allows the simultaneous analysis in the samples, taking into account that some of them are mixtures. This could allow a rapid quality control analysis of these products.

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P120. CHROMATOGRAPHIC INSIGHTS FOR TRACKING BIOACTIVE COMPOUNDS IN WOODY BY-PRODUCTS

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The manufacture of wooden hardboards and fibreboards is distinguished in some companies using water without the addition of other chemical substances. This aspect converts the different by-products of this industrial approach into a highly attractive source of bioactive compounds that are originally in the raw materials, which on the other hand, come from sustainable forests.

These by-products could be exploited to obtain natural extracts with added value, which could be reused in the food, cosmetic or pharmaceutical industry, reducing the environmental impact of the industrial activity and obtaining, in parallel, an economical profit.

The by-products characterized in this work imply different steps of the industrial process. Besides, different types of wood were considered. In this way, wooden chips (from pine, walnut and cherry) come from the first industrial step, and screw waters (oak, chestnut and eucalyptus) from the washing of the chips. Finally, the concentrate of eucalyptus (liquor) is the main industrial by-product, obtained by pressing the fibres.

The raw materials, and their derived extracts (aqueous extracts and green-organic solvents based extracts) were analysed by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (GC-MS, LC-MS/MS) to evaluate the main extractable organic wood compounds, including both volatile and non-volatile organic compounds.

Significant differences have been observed in the obtained chromatographic profiles for the different studied by-products and for the different wood types. A high number of compounds from different chemical nature have been identified, highlighting the presence of terpenes, phenolic compounds, omega-3 fatty acids, and precursors of fragrance synthesis. Most of these compounds present antioxidant and antibacterial properties.

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P121. EFFECT OF EXPERIMENTAL PARAMETERS IN THE MATRIX SOLID-PHASE DISPERSION EXTRACTION ON THE INDIVIDUAL POLYPHENOLS FROM APPLE PEEL

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Matrix solid-phase dispersion (MSPD) extraction was invented in 1989 by Barker [1] and since then it is used for the extraction and isolation of a wide range of compounds including polyphenols from various plant samples. However, to the best of our knowledge the use of MSPD for the extraction of polyphenols from apple peel has not been considered yet. In this study, the extract volume (1-5 mL), amount of methanol in eluting solvent mixture (0-100 %) and matrix/dispersant ratio 1:1-1:5 (m/m) were optimized by response surface methodology in order to attain the maximum yields of catechin, epicatechin, procyanidine B1 and B2, quercetin-3-glucoside, quercetin-3-rutinoside and chlorogenic acid from freeze-dried apple peel. A high-performance liquid chromatography (HPLC) with diode array detection (DAD) was used for the identification and quantification of polyphenols. The selected experimental design included seventeen experiments, which allowed evaluating the second order interactions between factors. The ANOVA of the obtained data showed that extraction volume was significant ($p < 0.05$) for all investigated polyphenols except procyanidine B1. For methanol in eluting solvent mixture, the linear positive (catechin, procyanidine B1, quercetin-3-glucoside, quercetin-3-rutinoside and chlorogenic acid) and negative (procyanidine B2) effects were found to be statistically significant ($p < 0.05$). Additionally, a significant negative quadratic effect of this factor was found for all investigated polyphenols except for quercetin-3-rutinoside and chlorogenic acid. The optimal conditions for the extraction were calculated by multiple response optimization that led to the following values: extraction volume of 5 mL, 61 % of methanol in eluting solvent mixture and matrix/dispersant ratio 1:4 (m/m).

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P122. EVOLUTION OF AROMA AND POLYPHENOLS OF MENCÍA WINE DURING WINEMAKING

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Most published studies on winemaking processes are conducted under controlled conditions and on a laboratory scale. This is mainly due to the lack of accessibility to industrial samples, so that only the initial and final points of the process are usually characterized, i.e. the resulting musts and wines. However, the dynamics and evolution of volatile compounds responsible for aromas and polyphenols can change enormously when the process is developed on an industrial scale. A deeper understanding of the intermediate points throughout the winemaking process can facilitate decision making for wineries to reorient the process towards producing higher quality products. In order to understand the dynamics of aromas and polyphenols in industrial winemaking, the evolution of Mencía wine has been studied using chromatographic techniques. Aromatic markers have been selected and monitored by solid phase microextraction and gas chromatography with mass spectrometer. On the other hand, the polyphenolic profiles were obtained with liquid chromatography both with diode array detector and with mass spectrometer.

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P123. DRIVING PROTEOMIC NANOLC-MS TO NEW CROSSROADS, ARE WE HEADING TOWARDS EXTRA SENSITIVE OR INCREASED THROUGHPUT APPLICATIONS?

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Since the late 1990s, NanoLC-MS has established itself as a technique of choice for the analysis of highly complex proteomic samples. Especially in shotgun proteomics, the high separation resolution and peak capacities that can be achieved on a well packed nanocolumn have proven their worth, in protein identification and biomarker discovery research. In combination with high resolution mass spectrometry, large steps have been made in knowledge and understanding of proteins, protein complexes and protein interactions. With the emergence of Ultra High Pressure Liquid Chromatography (UHPLC) in NanoLC, smaller particles 2 μ m and longer columns have been utilized to further improve the analysis results. Drawback of these smaller particles is the increased back pressures they require, ranging towards 1200 bars. These high back pressure therefore limit the length of the packed bed to typically 50cm. A recent and highly attractive alternative for packed bed nanocolumns are the micro-Pillar Array Column technology columns, or μ PAC™. These micromachined NanoLC chips have an perfectly ordered separation bed of freestanding μ -pillars, resulting in much lower back pressures, allowing for a wide flow rate flexibility(1). The high permeability and low on-column dispersion of the μ -pillar separation bed result in reduced peak dispersion, maintaining much more concentrated sample components during the separation, resulting in unprecedented separation performance(2). This allows column lengths of up to 200cm, with typical back pressures of only 120 bars. Such 200cm long μ PAC columns are a new tool for deep diving proteomics, using gradient times of 4 to 6 hours, providing an increase peak capacities and maintaining excellent resolution. As we will demonstrate, outstanding results in both DDA and DIA analysis are readily achieved, independent of the NanoLC system used. However, NanoLC-MS proteomics is, in the eyes of many, at a crossroad. Are we to pursue more sensitive workflows for single cell analysis, or are we leaving the nanoliter per minute flow rates behind in favour of higher throughput in the field of clinical proteomics? In this presentation, we will show the initial results for both approaches, utilizing the unique separation features of the μ PAC column, especially in the 50cm length format. Applications moving towards single cell analysis will be shown, demonstrating the sensitivity that can be achieved, as well as the reproducibility and robustness of the μ PAC columns (3). For more throughput or clinical proteomics workflows, the use of 1 μ L/min and higher flow rates is demonstrated, utilizing gradients of 30 minutes and less.

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P124. MAXIMIZE THE OUTPUT OF ROUTINE PROTEOME ANALYSES BY USING MICRO PILLAR ARRAY COLUMN TECHNOLOGY

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As an alternative to the conventional packed bed nano LC columns that are frequently used in bottom-up proteomics research, PharmaFluidics offers micromachined nano LC chip columns known as micro pillar array columns (μ PAC™). The inherent high permeability and low 'on-column' dispersion obtained by the perfect order of the separation bed makes μ PAC™ based chromatography unique in its kind. The peak dispersion originating from heterogeneous flow paths in the separation bed is eliminated (no A-term contributions) and therefore components remain much more concentrated during separation resulting in unprecedented separation performance. The freestanding nature of the pillars also leads to much lower backpressure allowing an high operational flow rate flexibility with exceptional peak capacities. Complementary to its landmark 200cm long column suited to perform comprehensive proteome research, a 50cm long μ PAC™ column is presented in a more routine research setting. With an internal volume of 3 μ L, the column is suited to perform high throughput analyses with shorter gradient solvent times (30 to 90 minute gradients) and over a wide range of flow rates (100 to 2000 nL/min). Recently performed experiments with 500ng of HeLa cell digest indicate that an increase in protein identifications up to 50% and a gain of 70% in peptide identifications can be achieved when comparing the 50cm μ PAC™ column to the current state-of-the-art in packed bed columns. The conventional packed bed columns (2 different vendors) used for this benchmarking experiment were 15cm in length and were packed with sub 2 μ m porous silica particles. LC pump pressures needed to operate these classical columns at a flow rate of 300 nL/min range between 200 and 300 bar, whereas only 40 bar was need to operate the 50cm μ PAC™ column at the same conditions.

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P125. TRANSLATING ANALYTICAL PYROLYSIS FINGERPRINTS OF SOIL ORGANIC MATTER TO CLIMATIC VARIABLES

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Environmental research about causes and effects of progressive soil desertification is attracting the attention of the scientific community. Factors that accelerate soil organic matter (SOM) mineralization may also induce structural changes in its molecular composition, but the specific impact of different climatic factors (annual precipitation, number of days with precipitations, temperature...) has not yet been the subject of systematic research. This study attempts to identify potential climatic molecular proxies in SOM, i.e., pyrolytic compounds correlated with specific climatic indices. A set of 16 Spanish soils under differing climatic conditions were selected. Molecular characterization of SOM was done by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) applied to whole soil samples. Up to 193 compounds were identified corresponding to alkanes, alkylbenzenes, *N*-compounds, olefins, phenols and carbohydrate derivatives. Simple and multiple correlations between pyrolytic and climatic variables showed that the concentration of several compounds was significantly correlated ($P < 0.05$) with particular climatic variables. In a second stage, using van Krevelen [1, 2] diagrams, it was possible to display different patterns of pyrolytic compounds responsive to specific climatic characteristics. Finally, extrapolation functions were applied to simulate SOM molecular composition under future climatic scenarios, e.g., changes in temperature, evapotranspiration, rainfall, etc. This study demonstrates the potential of Py-GC/MS in identifying specific molecules and compound families useful for predicting climate impact on SOM, as well as for monitoring soil quality in terms of the progress of desertification.

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P126. ASSESSMENT OF A NEW DERIVATIZATION REAGENT FOR THE ANALYSIS OF GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID

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Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide widely used as a crop desiccant. It is one of the most used herbicides in agriculture and is also widely applied for urban and residential weed control. Aminomethylphosphonic acid (AMPA) is its main metabolite and both compounds have to be analyzed together because they accumulate in agricultural soils and environmental waters. The physicochemical properties of these compounds result in liquid chromatography being considered as the main suitable analysis methodology, but a derivatization reaction is usually required to reduce the polar characteristics of both compounds. The derivatization reagent most commonly used is 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) [1], which requires derivatization times between 3-24 h and the application of a clean-up step to extract the excess of FMOC-Cl and other organic impurities [2].

In this study, a new derivatization reagent, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC), has been evaluated, which is also used in the derivatization and analysis of amino acids [3]. The effect of different parameters that could affect the derivatization process (i.e. derivatization time and temperature) have been assessed applying a two level factorial design and it has been observed that the reaction is achieved in few seconds at room temperature, which simplifies the derivatization conditions required with other reagents. Moreover, the excess of reagent can be easily separated chromatographically adjusting the pH of the mobile phase and it is not required a clean-up step to extract this excess. It has been observed that the derivatized compounds are stable for at least 7 days at 4 °C.

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P127. A HPLC-FLD METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF MYCOTOXINS IN ANIMAL FEED USING SOLID PHASE COMBINED WITH DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

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Mycotoxins are secondary toxic metabolites produced by different species of filamentous fungi such as *Aspergillus*, *Fusarium* and *Penicillium*. It is proven that they can produce serious adverse effects on the health of animals which have been fed with contaminated food. Aflatoxins, especially aflatoxin B₁, are considered as human carcinogens (group I) by the International Agency for Research in Cancer. They are known to have a strong activity on the liver, causing both structural and functional damage. Others, such as ochratoxin A classified as a possible human carcinogen (Group 2B) develops nephrotoxic, hepatotoxic and immunotoxic effects in addition to poor weight gain, and decreased egg production. On the other hand, zearalenone has estrogenic activity and causes reproductive problems in animals such as hyperestrogenism, sterility and abortions. The contamination of feed and raw materials by this group of toxins is a global problem. Furthermore, climate change and globalization of the markets produce unpredictable changes in toxin distribution. For these reasons, the study and development of reliable, robust, fast and economic analytical methods is indispensable for the control of animal feed contamination with mycotoxins.

In the present study, a SPE-DLLME-HPLC-FLD method has been developed and validated for the simultaneous determination of 7 mycotoxins in animal feed: aflatoxin B₁, aflatoxin B₂, aflatoxin G₁, aflatoxin G₂, zearalenone, ochratoxin A and ochratoxin B. Sample treatment is simple, rapid and cost effective. It is based on extraction with ACN/H₂O/formic acid (79/20/1) followed by a clean-up step with SPE cartridges OASIS PRIME HLB. Afterwards, a second clean-up process is based on dispersive liquid-liquid microextraction using chloroform. Validation of the method has been based on the study of limits of detection and quantification, linearity, precision, accuracy and recovery. The limits of quantification were between 1.26 µg/kg for aflatoxins B₂ and G₂, 4 µg/kg for aflatoxins B₁ and G₁ and 84 µg/kg for zearalenone.



P128. LC-MS ANALYSIS OF ISRIB IN PLASMA AND BRAIN SAMPLES

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The Integrated Stress Response (ISR) is a homeostatic process activated upon cellular stress due to extrinsic factors (i.e., hypoxia, amino acid and/or glucose deprivation and viral infection) or intrinsic factors, such as endoplasmic reticulum (ER) stress due to accumulation of unfolded proteins in the ER or oncogenes [1]. The activation of the ISR due to ER stress has been extensively documented in neurodegenerative diseases [2]. Compounds acting downstream the ISR pathway could represent new therapeutic treatments for neurodegenerative diseases. In this study, an ISR Inhibitor (ISRIB, [3]) was administered intraperitoneally to mice. To determine the quantity of ISRIB that penetrates the blood-brain barrier (BBB), a method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated enabling the quantitation of ISRIB in plasma and brain samples. Briefly, the method consisted of a protein precipitation technique for both matrices, followed by LC-MS/MS analysis using a triple quadrupole instrument and electrospray ionization in positive mode. Chromatographic separation was carried out using an Acquity UPLC[®] BEH C18 column (2.1 mm x 50 mm i.d., 1.7 µm particle size). Mobile phases contained water and acetonitrile with 0.1% of formic acid. Elution was performed in gradient mode. A selected reaction monitoring method was used to follow four specific transitions for ISRIB and two for Tolbutamide, used as internal standard. ISRIB was intraperitoneally administered to twelve mice (5mg/kg) during 7 days. After that, concentration of ISRIB in plasma and brain samples collected after 0min, 15min and 8hours was quantified.

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P129. LIPID ANALYSIS OF BEEF MEAT: CAN MALDI-TOF MASS SPECTROMETRY BE USED TO DEFINE DIFFERENTIAL PROFILES FOR BOVINE BREEDS FED UNDER DISTINCT FAT CONTENT DIET?

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Lipid constitution of bovine muscle tissue is affected by several factors, including genetics and diet (1). We have previously characterized the intramuscular fatty acid composition of Nellore and Brangus bovine breeds fed with contrasting fat levels (2). MALDI-TOF mass spectrometry (MS) has been used to characterize intact lipid components in bovine muscle (3). The aim of this study is to use MALDI-TOF MS to differentiate bovine muscle from different breeds under distinct fat diets. Nellore (n = 11) and Brangus (n = 12) bulls were randomly assigned to a low (LFD) or a high fat diet (HFD): 3.2% versus 6.4% ether extract. The diets had similar energy and protein levels and were composed by sorghum silage (30% dry matter), soybean hulls, ground corn, soybean meal, urea and a mineral mixture. HFD additional fat derived from cottonseed (18% dry matter), in substitution to ground corn (31% versus 52%, on HFD and LFD, respectively). The experiment lasted 71 days and *Longissimus dorsi* muscle samples were processed for lipid analysis by MALDI-TOF MS. Folch method was used to lipid extraction from 15 g of muscle tissue and lipid concentration within samples ranged from 8.7 to 44.14 mg/mL. Total lipids (1 μ L) were directly spotted in MALDI plate with dihydroxybenzoic acid (DHB) and 9-aminoacridine (9-AA) as matrices (4,5). Mass spectra were acquired from m/z 680 to 1580 in the negative-ion reflectron mode with an Autoflex mass spectrometer (Bruker Daltonics). As a rule, 9-AA ionization yielded more peaks and with higher intensity than DHB. Several m/z could be assigned as glycerophospholipids in LIPID MAPS[®] Lipidomics Gateway search, however the resolving power of MALDI-TOF is not enough for lipid identification, despite good resolution (~0.2 FWHM) of our peaks. We then processed Nellore LFD, Nellore HFD, Brangus LFD and Brangus HFD mass spectra profiles with ClinProTools[™] software (Bruker Daltonics) for fishing biomarkers but no differences could be assigned. More analysis shall be performed but so far the answer to the aforementioned title question is no. To further explore our samples next step will be the use of Solid Phase Extraction (SPE) and Thin Layer Chromatography (TLC) hyphenated to MALDI-MS to improve our mass spectra profiles regarding phospholipid moiety analysis.

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P130. EXTRACTION OF MYCOTOXINS FROM EDIBLE INSECTS USING NATURAL DEEP EUTECTIC SOLVENTS: A GREEN ALTERNATIVE TO CONVENTIONAL METHODS

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Although analytical methodologies for food analysis have significantly improved in recent years, organic solvents such as acetonitrile, methanol, isopropanol and hexane continue to be broadly applied in extraction and purification processes. Many of them present risks to human health and the environment due to their volatility and toxicity. Therefore, their replacement by non-hazardous and biodegradable solvents has become a top priority in Green Chemistry applications. In particular, the extraction of mycotoxins from foodstuff avoiding the use of traditional solvents requires a significant effort, as these contaminants are found in very complex matrices and at low concentration levels. In addition, their structures and physicochemical properties are very heterogeneous. Therefore, the development of environmentally friendly methodologies that allows the simultaneous and efficient extraction of several mycotoxins is a challenge. In this work, a simple, rapid, green and sustainable solid-liquid microextraction method, based on natural deep eutectic solvents (DES) [1] and liquid chromatography with triple quadrupole mass spectrometry (UPLC-MS/MS) has been developed, for the simultaneous determination of six mycotoxins (fumonisin B1, fumonisin B2, ochratoxin A, T-2 toxin, HT-2 toxin and mycophenolic acid) in cricket flour. Different combinations of DES, consisting of choline chloride (ChCl) in various mixing molar ratios with glucose (sugar), ethylene glycol (alcohol), malonic acid (acid) and urea (amide) were prepared. Experimental design methodologies were applied to obtain the optimal extraction conditions. A fractional factorial design (FFD) was used to screen the significance of the water content (%), the volume of extractant (μL), sample size (mg), temperature ($^{\circ}\text{C}$) and extraction time (min). The significant factors identified by ANOVA analysis were further optimized with the aid of the desirability function. Under optimal conditions, recoveries of the target mycotoxins varied between 70% and 104% (except T-2 toxin with 50%) and limits of quantification were lower than those usually permitted by legislation in food matrices, with precisions (intra and inter day) lower than 13%. The method was finally validated according to the requirement of the European Commission for cricket flour.

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P131. PHOTO-TRANSFORMATION PRODUCTS FROM HALOGENATED PHARMACEUTICALS

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Since many pharmaceutical active compounds (PhACs) present recalcitrance through conventional biological wastewater treatments, other natural degradation mechanisms like photolysis arises as relevant. Effluents from the wastewater treatment plants contain several contaminants of emerging concern that can be partially or totally degraded under solar irradiation. As result, photo-transformation products (photo-TPs) are produced, which have different properties as solubility, partition coefficient (Log Kow), and ecotoxicity in comparison with their respective precursors. Halogenated PhACs represent around 30% of the commercialized drugs and their photo-TPs normally have an ecotoxicity higher than the original molecules. In addition, many studies establish a positive relationship between the presence of these TPs and the formation of toxic disinfection byproducts. Because commercial analytical standards for these substances are very rare, approaches as suspect screening and non-target analysis have been used instead of target analysis. In order to achieve the lowest limits of quantification and the best accuracy as possible, pre concentration of sample through solid phase extraction and analysis through ultra-performance liquid chromatography coupled to high resolution mass spectrometry (SPE-UPLC-HRMS) are the most recommended technique. In this work, some photo-TPs obtained after photolysis carried out in solar simulator are identified by using an Orbitrap mass spectrometer, acquiring the MS/MS spectra in both data dependent analysis (ddMS2) and data independent analysis (DIA) in order to characterize the analytes. The presence of the precursors atorvastatin, sitagliptin, iohexol, sertraline, and losartan and their photo-TPs are also investigated in a real sample of surface water

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