SECYTA
2023

# XXII Meeting of the Spanish Society of Chromatography and Related Techniques

**S'Arenal, Mallorca, 16-18 OCTOBER 2023**

Organised by

SECYTA SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES

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# BOOK OF ABSTRACTS

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# WELCOME

Chromatography is a rugged analytical method that has undergone tremendous strides in recent years. Gas and liquid chromatographic methods coupled to mass spectrometry or other detectors are now capable of determining organic molecules over a wide range of volatility and polarity properties, even at trace levels. These analytical techniques provide the necessary tools to meet the societal challenges and demand for monitoring the more than 300,000 organic compounds in common use in our civilization, e.g. their occurrence in the environment, food intake, human exposure and other issues such as transformations by oxidation, photolysis, hydrolysis in the environment or metabolic processes in organisms, including humans.

However, human development is continuously increasing the number and diversity of chemical compounds to fulfill new societal requirements, which in turn increases the demand for better analytical methods with higher sensitivity, accuracy and precision for the understanding of the environmental and human threats posed by the new chemical species. Chromatography is at the cross-road of the analytical methods to meet these needs.

In this XXII Scientific Meeting of the SECyTA we provide an update on the latest advances in gas and liquid chromatography and related techniques. The topics that are welcome in this meeting include (i) developments on the theoretical fundamentals of column separation, (ii) hyphenated techniques and omics, (iii) miniaturization and automation, (iv) imaging, (v) green separation methods, (vi) chemometrics, and (vii) environmental, toxicological, forensic, food and nutritional analysis.

In collaboration with the Spanish Society of Mass Spectrometry there is a session specifically devoted to image applications of MALDI (Matrix-assisted laser desorption/ionization mass spectrometry) in which significant improvements have been reported over the past few years.

Furthermore, a special session is organized to critically discuss potential alternatives available in view of the progressive scarcity and increasing cost of helium, one of the most used gases in gas chromatography.

Last but not least, there are specific presentations devoted to the analysis and identification of micro/nanoplastics, and contaminants of emerging concern associated to them, in marine waters, freshwaters systems, biota and food commodities.

We welcome you all in Mallorca.

**Joan O. Grimalt and Manuel Miró**

## PHILIPPE SCHMITT-KOPPLIN

*Technical University of Munich*

**High resolution tailored metabolomics in the food-nutrition-health continuum**

Prof. Ph. Schmitt-Kopplin's team performs tailored and comprehensive metabolomics in the food-health continuum. He has a strong profile in analytical chemistry with integrated approaches combining (ultra) high resolution mass spectrometry, (µ)separation sciences, NMR-spectroscopy with (bio)informatics for the description of complex organic systems on a molecular level. A focus in the last decades was to implement ultrahigh resolution mass spectrometry into cross-Omics applications and for a rapid and robust tool for deep metabotyping and small molecules profiling. His focus is the chemical understanding of microbiomes in foods, health and environments and integrating these information with existing biological Omics data. His interdisciplinary studies are related to the interface of chemistry and biology. He is director of the research unit analytical BioGeoChemistry of the Helmholtz Munich and heads the Comprehensive Foodomics Platform at the Institute of analytical Food Chemistry of the Technische University Munich Germany.

## LARS WÖRMER

*MARUM – Center of Marine Environmental Sciences, University of Bremen*

**Reading the fine print: mass spectrometry imaging of molecular fossils in geological samples**

Lars Wörmer is a senior researcher at MARUM – Center of Marine Environmental Sciences, University of Bremen (Germany). He holds a MSc in Environmental Sciences and a PhD from Universidad Autónoma de Madrid (Spain). Lars is especially interested in chemical signatures archived in marine or lacustrine sediments, i.e., molecular fossils. These molecules can inform on past climate, but also on changes in terrestrial or marine ecosystems. In the last years, he has pioneered the implementation of Mass Spectrometry Imaging (MSI) in the Earth Sciences and is currently the scientific leader of the Geobiomolecular Imaging Laboratory at the University of Bremen. MSI of molecular fossils allows to map their distribution in the sedimentary archive with micrometer-resolution and thus to explore these records with unprecedented resolution and detail. As a result, climate and environmental reconstructions can now be performed at time scales (seasons to decades) extremely relevant from a human and ecosystem perspective.

## PAVEL KUBÁŇ

*Czech Academy of Science*

**Capillary electrophoresis as an all-in-one tool for the fully autonomous pretreatment and analysis of dried blood spots**

Pavel Kubáň graduated in Chemistry and Mathematics from Masaryk University, Brno, Czech Republic, obtained his Doctor of Philosophy degree from Mendel University, Brno, Czech Republic, Doctor of Science degree from Palacký University, Olomouc, Czech Republic, and Researcher Professor degree from the Czech Academy of Sciences. Currently, he is the Head and the Leading Scientist at the Department of Electromigration Methods at the Institute of Analytical Chemistry of the Czech Academy of Sciences.

His work is devoted mainly to capillary electrophoresis, liquid phase microextraction techniques and their coupling for direct analyses of complex samples. Recently, his research activities focused on the analysis of dried material spots, such as dried blood and dried urine spots. He is author of more than 110 scientific papers, reviews and book chapters and 70 contributions on scientific conferences. He is a member of the editorial board of the Separation Science Plus (Wiley), Talanta Open (Elsevier), and Journal of Pharmaceutical Analysis (Elsevier) journals. He is also the Head of the Science Council of the Institute of Analytical Chemistry, a member of the Science Council of the Czech Academy of Sciences and a member of the Czech Science Foundation committee for Analytical Chemistry.

**RAFAEL LUCENA**
*University of Cordoba*

**Biopolymer-based sorptive phases into stainless steel needles: microextraction and ambient mass spectrometry analysis in a single device**

Dr. Rafael Lucena Rodríguez is full professor in the Department of Analytical Chemistry at the University of Córdoba and member of the "Affordable and Sustainable Sample Preparation" research group. His research lines focus on analytical sample treatment (development of new extraction techniques and materials) and their direct coupling with instrumental techniques (vibrational spectroscopic techniques and mass spectrometry). He has participated in 16 research projects being Principal Investigator in four of them. He is co-author of 124 scientific papers and 22 book chapters. He has co-edited the book "Analytical Sample Preparation with Nano- and other High-performance Materials" (Elsevier, 2021). He is the author of three patents of invention and has participated in several R&D contracts. He has actively participated in the training of new researchers, directing 9 theses already presented and 4 in progress.

He has acted as evaluator of research projects and researcher contracts for the Spanish Research Agency. He has also acted as evaluator of projects for the National Commission for Scientific and Technological Research (Chile) and the Czech Science Foundation (Czech Republic).

He is currently secretary of the Chemical Institute for Energy and Environment (IQUEMA) of the University of Cordoba and associate editor of the journal "Advances in Sample Preparation" (Elsevier).

**ANA MARIA AGÜERA**
*University of Almeria*

**Target and suspect analysis of contaminants of emerging concern in water reuse practices: challenges and future perspectives**

Prof. Dr. Ana Agüera is full professor in Analytical Chemistry at the University of Almería and leader of the Environmental Analysis Unit at the Solar Energy Research Centre (CIESOL). Her research focuses on the application of low and high-resolution mass spectrometry to the identification and fate of emerging contaminants and their transformation products in the aquatic environment, as well as the analytical assessment of advanced wastewater treatment processes applied to complex effluents to get their regeneration and enable their reuse. She is especially interested in the study of the impact of reclaimed water reuse in the agricultural environment (water-soil-plant nexus). She is co-author of 2 patents and 175 scientific publications (h-index 66). For her research work, she has been included in the U. Stanford world ranking "The World Scientists: World's Top 2% Scientists (in 2020 and 2022)", "Analytical Chemistry" area.

# PROGRAM

## SUNDAY,15th October 2023

09:00-09:30 **Registration**

09:30-18:00 **Course on Advanced Mass Spectrometry**

## MONDAY, 16th October 2023

08:00-09:00 **Registration**

09:00-09:15 **Opening Ceremony**

09:15-09:55 **Opening Plenary Lecture**

*Chairs:*
*Esteban Abad, Institute of Environmental Assessment and Water Research (IDAEA-CSIC)*
*Juan Vicente Sancho, University Jaume I, Castelló*

**PL-1: Lars Wörmer,** *MARUM & Department of Geosciences. University of Bremen*
**Reading the fine print: mass spectrometry imaging of molecular fossils in geological samples**

10:00- 11:15 **Oral Communications (OC-Session 1)**

*Chairs: Esteban Abad, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) and Juan Vicente Sancho, University Jaume I, Castelló*

10:00-10:15 Beatriz Rocha
The growing power of MALDI Imaging to answer today's questions in Mass Spectrometry
*Especialista de Aplicaciones MALDI Imaging Bruker*

10:15-10:30 M. Garí, K. Polanska, A. Jankowska, D. Bury, H. Koch
Exposure and toxicological risk assessment of bisphenols, phthalates and non-phthalate plasticizers in children based on current HBM guidance values
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC)*

10:30-10:45 M. Hernández-Mesa, J. Marín-Sáez, J.A. Gallardo-Ramos, L. Gámiz-Gracia, A.M. García-Campaña
Unveiling the exposome: A LC-MS method for the determination of pesticides and mycotoxins in urine samples
*University of Granada – University of Lleida*

10:45-11:00 C. Bressan S. Coll, E. Alechaga, M. Carbó, N. Monfort, R. Ventura
Elimination profile of dexamethasone in dried blood spots (dbs) after oral administration
*Institut Municipal d'Investigació Mèdica, Barcelona*

11:00-11:15 O. J. Pozo, O. Khymenets, M. Montané, O. Vilarroya, S. Carmona
Determination of steroid phase II metabolites in urine: A chromatographic challenge for studying hormonal changes during pregnancy
*Hospital del Mar, Research Institute, Barcelona*

11:15-12:00 **Coffee Break & Exhibition**

12:00-13:15 **Oral Communications (OC-Session 2)**
*Chairs: Núria Fontanals, Rovira i Virgili University, Tarragona and Marinel·la Farré, Instituto de Diagnóstico Ambiental y Estudios del Agua (IDAEA-CSIC)*

12:00-12:15 A. Chana
Combinaciones de software y hardware: un paso más allá en la automatización en el desarrollo de métodos en cromatografía líquida. Aplicación a nanopartículas lipídicas
*Agilent Technologies*

12:15-12:30 E. Moyano, D. Rozúa, R. Wang, N.-I. Medina, O. Jáuregui, L. Puignou, F.J. Santos
Ion mobility-mass spectrometry in environmental and food analysis: Some case studies
*University of Barcelona*

12:30-12:45 G. Álvarez, J.D. Sánchez-Martínez, M. Karbalaei, M. Herrero, E. Ibáñez, A. Cifuentes
HRMS-based metabolomic profiling in natural products research: Bioactives wanted!
*Consejo Superior de Investigaciones Científicas (CSIC), Madrid*

12:45-13:00 L. Narduzzi, M. Hernández-Mesa, M.M. Delgado-Povedano, F.J. Lara, B. Le Bizec, A.M. García-Campaña, G. Dervilly
A comprehensive picture of PCB effects on pig metabolism using a multi-platform metabolomics and lipidomics approach
*University of Granada – LABERCA, Nantes*

13:00-13:15 X. Subirats, L. Redón, M. Rosés
Fast method for the characterization of liquid chromatographic systems based on Abraham's solvation parameter model
*University of Barcelona*

13:15-14:45 **Lunch**

14:50-15:30 **Oral Young Researchers (OY-Session 1)**
*Chairs: Francisco Javier Santos, University of Barcelona and Belén Gomara, Institute of General Organic Chemistry (IQOG-CSIC), Madrid-CSIC, Madrid. QOG-CSIC, Madrid*

14:50-15:00 S. Adámez
Study of the effect of inflammatory cytokines on human proximal tubular HK-2 cells through UHPLC-HRMS-based untargeted metabolomics
*University of Alcalá*

15:00-15:10 M.C. Mamani-Huanca
Application of a novel CE-MS metabolomics workflow in zebrafish larvae reveals novel effects of cortisol
*San Pablo-CEU University*

15:10-15:20 C.M. Bustamante
Simultaneous determination of pyrethroid, organophosphate, carbamate, triazole and neonicotinoid pesticides in urine by high-performance liquid chromatography coupled to tandem mass spectrometry
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

15:20-15:30 C. González Jiménez
Analysis of prostate specific antigen by capillary electrophoresis. Use of natural deep eutectic solvents in sample preparation and separation steps
*Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

15:30-16:10 **Plenary Lecture**
*Chairs:*
*Francisco Javier Santos, University of Barcelona*
*Belén Gomara, Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

**PL-2: Philippe Schmitt-Kopplin,** *Technical University of Munich*
**High resolution tailored metabolomics in the food-nutrition-health continuum**

16:10-17:10 **Poster Session 1 (P1-P16) & Coffee & Exhibition**

17:10-18:10 **Oral Communications (OC-Session 3)**

*Chairs: Begoña Jimenez, Institute of General Organic Chemistry (IQOG-CSIC), Madrid and José Antonio González, IRNAS-CSIC, Sevilla.*

17:10-17:25 J. Lluch
From Helium to Hydrogen, a cost-effective way to improve your lab´s green credentials
*LECO Instruments*

17:25-17:40 D.R. Riqueros, M.Garí, J.O. Grimalt
New rapid extraction methodology for the determination of organohalogen compounds in human serum
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

17:40-17:55 E. Antico, I. Quintanilla, C. Fontàs, A. Profumo, A. Speltini, F. Merlo
Modified polymeric films for the enrichment of steroid hormones and organophosphorous pesticides from water samples
*University of Girona*

17:55-18:10 B.L. van Drooge, J. Gili, C. Jaén, M. Viana
Firefighter's exposure to PAHs in prescribed forest fires analyzed by GC-Orbitrap-MS
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

18:10-19:00 **Oral Young Researchers (OY-Session 2)**

*Chairs: Begoña Jiménez, Institute of General Organic Chemistry (IQOG-CSIC), Madrid José Antonio González, IRNAS-CSIC, Sevilla*

18:10-18:20 A.J. Castillo-Zamora
Evaluation of a feed additive rich in polyphenolic compounds from white grape marc for fattening swine: untarget approach using MSPD-UHPLC-QTOF
*University of Santiago de Compostela*

18:20-18:30 C. Jaen Flo
Use of GC-MS/MS for the analysis of PAH, methyl-PAH, nitro-PAH and oxy-PAH in nanoparticles from the transport sector
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

18:30-18:40 P. Colomer-Vidal
Perfluoroalkyl substances in striped dolphins (Stenella coeruleoalba) from the NW Mediterranean Sea: Biomagnification and temporal trends (1990-2021)
*Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

18:40-18:50 P. Dominguez García
Development of a rapid direct-injection method for the determination of 80 pharmaceuticals in river water by liquid chromatography-tandem mass spectrometry
*Chemical Institute of Sarrià. University Ramon Llull*

18:50-19:00 C. Calero-Cañuelo, F.A. Casado-Carmona, R. Lucena, S. Cárdenas
Planar mixed-mode cationic exchange sorptive phases and ambient ionization mass spectrometry: A novel combination for the analysis of biofluids
*University of Córdoba*

19:15 **Welcome cocktail**

## TUESDAY, 17th October 2023

09:00-09:40 **Plenary Lecture**
*Chairs:*
*Ana M. García-Campaña, University of Granada*
*Francisco A. Casado, University of the Balearic Islands*

**PL-3: Rafael Lucena,** *University of Cordoba*
**Biopolymer-based sorptive phases into stainless steel needles: microextraction and ambient mass spectrometry analysis in a single device**

09:40-10:40 **Oral Communications (OC-Session 4)**
*Chairs: Ana M. García-Campaña, University of Granada and Francisco A. Casado, University of the Balearic Islands*

09:40-09:55 R.M. Prats, J.O. Grimalt, P. Fernández, B.L. van Drooge
Rotenone, a plant-derived piscicide for the eradication of allochthonous fish in Alpine lakes: Adaptation of water sampling strategies and analytical methodologies
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

9:55-10:10 I. Romero-Alfaro, E. Prats, D. Raldúa, J. Díaz, X. Ortiz, C. Gómez
Analysis of the neurotoxic effects of anatoxin-a and saxitoxin in zebrafish larvae
*Institut Químic de Sarrià (Universitat Ramón Llull), Barcelona*

10:10-10:25 M. Garcia-Torne, I. Flacó, E. Abad, M. Llorca, G. Sánchez, M. Farré
Assesment of antibiotics and emerging pathogens in the Ebro Delta, and the Albufera of Valencia
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

10:25-10:40 M. Llorca, A. Vega-Herrera, M. Farré
Human exposure to micro(nano)plastics and plastic additives
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona*

10:40-11:40 **Poster Session 2 (P17-P36) & Coffee Break & Exhibition**

11:40-12:25 **Poster flash discussion (PF-Session 1)**
*Chairs: Joan O. Grimalt, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona and Mario Fernández, Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

11:40-11:45 L. Mayo-Martínez
Chiralomics: A validated methodology for unveiling the role of D- and L- amino acids in health and disease
*University CEU San Pablo. Madrid*

11:45-11:50 L.M. San Emeterio
Thermal cracking and isotope fractionation of hydrocarbons: Evidence for limits to pyrolysis -compound-specific isotope analysis (Py-CSIA)
*Institute of Natural Resources and Agrobiology of Seville (IRNAS-CSIC)*

11:50-11:55 A.L. Ricci
Metabolomics and target trichothecene analysis to evaluate novel organic methods to prevent Fusarium Head Blight (FHB)
*University of Tuscia – University of Granada*

11:55-12:00 M. Granell
Development of a GC-(Q)Orbitrap method for the assessment of exposure to organic chemicals at trace levels by analysis of human plasma
*Universitat Jaume I, Castelló*

12:00-12:05 I. Jiménez Amezcúa
The effect of aging conditions on the changes of the aroma volatile profile and key odourants in garlic by SPME GC-MS and GC-O
Institute of General Organic Chemistry (IQOG-CSIC), Madrid

12:05-12:10 M. Soria Prieto
Determination of gadolinium contrast agents in urine samples using LC(IC) coupled to ICP MS
*Universitat Jaume I, Castelló*

12:10-12:15 A. Mena García
Development of a HPLC-DAD-ToF MS methodology for the authentication of Damiana (Turnera diffussa) extracts
*Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

12:15-12:20 M.V. Barbieri
Relevance of reductive photo- and bio-transformation processes in intermittent Mediterranean river
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

12:20-12:25 S. Gregorio
Development of a QuEChERS-based method coupled to LC-MS/MS for the determination of eight Persistent, Mobile, and Toxic substances in Cichorium endivia.
*Universitat Jaume I, Castelló*

12:25-13:25 **Round Table on helium shortage for GC/GC-MS**

13:30-15:00 **Lunch**

15:00-15:40 **Oral Young researchers (OY-Session 3)**

*Chairs: Juan Vicente Sancho, University Jaume I, Castelló and Enrique J. Carrasco-Correa, University of Valencia*

15:00-15:10 F. Soria Prieto
Method development to the determination of gadolinium contrast agents in soil samples
*University Jaume I. Castelló*

15:10-15:20 A. Moral
Application of homemade silica-based zwitterionic ion-exchange materials for the extraction of pharmaceuticals from environmental water samples
*University Rovira i Virgili. Tarragona*

15:20-15:30 L. Pont
Analysis of SARS-CoV-2 nucleocapsid protein by on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry
*University of Barcelona*

15:30:15:40 M. Mancera
Analysis of a utoantigenic peptides and lipids from hosphatidylserine liposomes for the treatment of autoimmune diseases
*University of Barcelona*

15:40-16:40 **Oral communications (OC-Session 5)**
*Chairs: Juan Vicente Sancho, University Jaume I, Castelló and Mario Fernández, Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

15:40-15:55 M. Lopez de Alda, C. Postigo, L. Moreno-Merino, E. López-García, J. López-Martínez
Anthropogenic chemicals of emerging concern in waters from the Northern Antarctic Peninsula Region
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

15:55-16:10 B. Jiménez, J. Muñoz-Arnanz, P. Colomer Vidal, M. Ros, A. Vicente, C. Salcedo, A. Bartalini, E. Nogales
Evolution of persistent organic pollutants (POPs) in air and water: over a decade of monitoring in Spain
*Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

16:10-16:25 E.M. Herrera-Hernández, A. Bartolomé Rodríguez, C. Flores Rubio, A. Puig Infante, P. Crespo Iniesta, J. Alández Rodríguez
Monitoring of the EU Watch List contaminants of emerging concern in the Spanish River basins
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC).* Barcelona

16:25-16:40 F.A. Casado-Carmona, A. Belhameid, A. Megriche, A.I. López-Lorente, R. Lucena, S. Cárdenas
Titanium dioxide nanoparticles@Nylon 6-coated magnetic adhesive tape for the extraction of benzophenones
*University of Cordoba*

16:40-17:40 **Poster Session 3 (P37-P56) & Coffee & Exhibition**

17:40- 18:20 **Poster flash discussion (PF-Session 2)**

*Chairs: Marta Lores, University of Santiago de Compostela and Ana M. García-Campaña, University of Granada*

17:40-17:45 M.V. Barbieri
Characterization of pesticide contamination and environmental impact in Doñana and Tablas de Daimiel National Parks
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

17:45-17:50 A. Zamora
Optimization of a solid phase extraction procedure for the simultaneous analysis of prioritary and emerging contaminants in Catalan seawater
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

17:50-17:55 I. Diez-Palet
Fingerprinting volatile organic carbon sources and assessment of their role in the formation of ozone and secondary organic aerosol in rural, sub-urban and industrial areas
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

17:55-18:00 C. Simarro-Gimeno
Study of catalytic ozonation as an advanced treatment for removal of pharmaceuticals in wastewater
*University Jaume I. Castelló*

18:00-18:05 O. Gomez-Navarro
Occurrence and spatial distribution of pharmaceuticals in Mediterranean intermittent river basins
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

18:05-18:10 M. Granell
Identification of volatile organic compounds responsible for odours in wastewater treatment plants by HS-SPME with GC-(Q)Orbitrap
*University Jaume I. Castelló*

18:10-18:15 D. González-Iglesias
Holm oak acorn bioactive extracts as antifungal therapies in forest pathology
*University of Santiago de Compostela*

18:15-18:20 I. Luque-Jurado
Advanced extraction techniques in combination with hydrophobic natural deep eutectic solvents for the improved extraction of birch (Betula sp.) bark bioactives
*Institute of General Organic Chemistry (IQOG-CSIC), Madrid*

18:30-19:30 **SECyTA General Assembly**

20:30 **Conference Dinner**

## WEDNESDAY, 18th October 2023

09:00-09:40 **Plenary lecture**
*Chairs:*
*Jordi Diaz, Chemical Institute of Sarrià. University Ramon Llull*
*M. Elena González-Peñas, University of Navarra*

**PL-4 Pavel Kubán**
**Capillary electrophoresis as an all-in-one tool for the fully autonomous pretreatment and analysis of dried blood spots**
*Czech Academy of Science*

9:40-10:40 **Oral communications (OC-Session 6)**
*Chairs:*
*Jordi Diaz, Chemical Institute of Sarrià. University Ramon Llull*
*M. Elena González-Peñas, University of Navarra*

9:40-9:55 M. Farré, A. Vega, E. Abad, M. Llorca
Environmental micro and nanoplastics composition analysis by liquid chromatography coupled to high- resolution mass spectrometry
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

9:55-10:10 E. Abad, J. de Boer, H. Fiedler
Worldwide study on POPs in ambient air using a comparative passive air sampling
*Institute of Environmental Assessment and Water Research (IDAEA-CSIC). Barcelona*

10:10-10:25 E.J. Carrasco-Correa, M. Beneito-Cambra, M.J. Lerma-García, E.F. Simó-Alfonso, J.M. Herrero Martínez, M. Miró
Exploring the potential of 3D printing in sample preparation devices
*University of Valencia –University of Balearic Island*

10:25-11:40 **Poster Session 4 (P57-P76) & Coffee & Exhibition**

11:40-12:25 **Oral communications (OC-Session 7)**
*Chairs: Manuel Miró, University of the Balearic Islands and*
*Marta Lores, University of Santiago de Compostela*

11:40-11:55 J.L. Benedé, V. Vállez-Gomis, E. Lara-Molina, M. López-Nogueroles, A. Chisvert
A miniaturized magnetic-based sample preparation approach for the determination of bisphenols in follicular fluid
*University of Valencia*

11:55-12:10 J.M. Herrero-Martinez, M. Vergara-Barberán, E.J. Carrasco-Correa, M. Beneito-Cambra, M.J. Lerma-García, E.F. Simó-Alfonso
Affinity sorbents in low-cost supports: A green and efficient path in microextraction
*University of Valencia*

12:10-12:25 A. Chisvert, L.M. Moreno-Calleja, J. Grau, J.L. Benedé
Determination of salivary biogenic amines by magnetic-based pipette tip microextraction as non-invasive cancer diagnosis
*University of Valencia*

12:25-13:10 **Closing Plenary Lecture**
*Chairs:*
*Manuel Miró, University of the Balearic Isla*
*Marta Lores, University of Santiago de Compostela*

**PL-5: Ana Agüera**
**Target and suspect analysis of contaminants of emerging concern in water reuse practices: challenges and future perspectives**
*University of Almeria*

13:10-14:00 **Closing Ceremony and Awards**

14:00 **Farewell Lunch**

# PLENARY LECTURES

## PLENARY LECTURE 1

## READING THE FINE PRINT: MASS SPECTROMETRY IMAGING OF MOLECULAR FOSSILS IN GEOLOGICAL SAMPLES

Lars Wörmer* and the Geobiomolecular Imaging team

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At the bottom of lakes and oceans, sediment layers slowly accumulate over hundreds to millions of years. Each layer captures characteristic chemical, physical and biological signatures of the time it was deposited. The sequence of these layers, i.e., the sedimentary archive, thus offers a unique collection of glimpses into the past. To interrogate the sedimentary archive for past climate and environmental conditions, as well as the ecosystem response to them, molecular biomarkers and proxies have become crucial tools. These molecular fossils are compounds that were once produced in the water column or on land, and then deposited and preserved in the sediment. Their value resides in the fact that they are highly diagnostic for a certain environmental parameter (molecular proxy) or a certain organisms or process (molecular biomarker).

Paleoclimate and paleoenvironmental reconstructions based on such molecular fossils are crucial in our effort to define the natural variability of the climate system, but also to better inform climate models and understand the extent and consequences of current and future warming. Conventional, extraction-based analysis however are strongly limited in the resolution at which these data can be generated. Typically, samples are cm-sized and thus can easily integrate hundreds of years in a single datapoint, even with high sedimentation rates. This is in stark contrast with our need to understand effects and changes at decadal, or even shorter, scales.

To overcome this issue, we here present the application of mass spectrometry imaging (MSI) for the analysis of molecular proxies and biomarkers in the sedimentary archive at ultra-high spatial (and thus temporal) resolution. First, we will provide methodological details on the pipeline we have developed for sample preparation, mass spectrometric analysis, and data evaluation [1]. Afterwards, we will show how previously inaccessible information can be uncoded and allows to demonstrate the effect of past global warming on the seasonal cycle in the tropical ocean [2], to compare current warming in the Mediterranean with the last interglacial, when temperatures were warmer than today [3], or to finely characterize the sequence of environmental changes preceding mass extinctions in deep time [4].

**Acknowledgements**
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# PLENARY LECTURE 2

# HIGH RESOLUTION TAILORED METABOLOMICS IN THE FOOD-NUTRITION-HEALTH CONTINUUM

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Metabolomics, as the comprehensive study of metabolic reactions in complex dynamic/ living systems is growing very rapidly and integrate analytical approaches (LC-MS, NMR and FTICR-MS) covering the description of only 10% of the experimental signals in databases. Important approaches thus are related to the description of the food metabolome with adapted strategies. Especially direct injection FTICR/MS enables a high throughput description of highly complex mixtures and food systems at the level of the elementary composition space. FTICR/MS will be presented is a strong tool to understand the chemical diversity in various study fields from food chemistry, biology, microbiomes towards the discovery of new bioactives.

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## PLENARY LECTURE 3

# BIOPOLYMER-BASED SORPTIVE PHASES IN STAINLESS STEEL NEEDLES: MICROEXTRACTION AND AMBIENT MASS SPECTROMETRY ANALYSIS IN A SINGLE DEVICE

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Mass spectrometry (MS) is a powerful tool for analyzing complex samples, as it can provide the required sensitivity and selectivity levels. Although a chromatographic step usually precedes it, this separation can be eliminated to reduce the analysis time. In this sense, a sample preparation procedure is mandatory to make the samples compatible with the mass spectrometer, fulfill the required threshold concentration values, and reduce the interferences. Substrate spray is an ambient (or direct) MS modality in which a sharp material (e.g., paper, wooden tips, coated blades, probes) is used as an electrospray (ESI) emitter when adding an organic solvent and applying a high voltage. Therefore, ESI is formed containing the analytes that are ionized and on-line entered into the MS inlet.

Hypodermic needles (HNs) can be used as ESI emitters because of their sharp end and conductive behavior. They can also be used as sorbent holders where different sorptive materials can be hosted. In this case, the whole analytical method can be carried out (including the conditioning, extraction, washing, and elution steps) in the same device. Thus, the extraction workflow is simplified using HNs as sorbent holders, extraction devices, and ESI emitters. Moreover, their low cost and worldwide availability make HNs affordable.

Two different types of sorptive phases have been used inside HNs. In the first approach, polydopamine (bioinspired polymer) was in situ grown and immobilized on the inner diameter of the HNs [1]. When the sample was pulled and pushed, the analytes interacted with the anchored polydopamine. However, the interaction was limited, and the extraction kinetics were affected. In a second approach, a fibered sorbent (a polyamide-cotton composite) was hosted inside the luer connection of the HN [2]. In this case, the sorbent-analyte interactions were favored. The different variables affecting the proposed ambient MS interface and associated with isolating the analytes from saliva samples were studied. Both proposed analytical methods were satisfactorily validated using matrix-matched calibration curves, and blind samples were analyzed to confirm the suitability of the proposed analytic methods for analyzing saliva samples.

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# PLENARY LECTURE 4

# CAPILLARY ELECTROPHORESIS AS AN ALL-IN-ONE TOOL FOR THE FULLY AUTONOMOUS PRETREATMENT AND ANALYSIS OF DRIED BLOOD SPOTS

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The golden standard in clinical testing is blood plasma obtained by centrifugation of venous blood. Micro-sampling of dried blood spots (DBSs) has become a viable alternative to venous blood collection already in the 1960s and has evidenced enormous interest during the recent COVID-19 pandemic. Despite numerous benefits, one of the major challenges in contemporary DBS analysis are the DBS processing and pretreatment, which require DBS transformation from a dry to a liquid sample and are usually performed manually. This involves punching out the DBS from a sampling card and the DBS elution, which is followed by centrifugation, evaporation, and reconstitution of the DBS eluate, and requires an additional manual transfer of the resulting eluate to an external analytical instrument. Semi-automated and automated systems for DBS processing were, thus, designed and commercialized recently. However, such systems have numerous limitations, have to be connected to another analytical instrument, are bulky and costly, and there has been a continuous quest for the development of simple and cheap solutions for unmanned DBS analyses.

One such solution was recently presented in our laboratory. This concept used a single off-the-shelf capillary electrophoresis (CE) instrument for executing all tasks of the analytical protocol. The actual lecture will summarize the general pitfalls of DBS analyses and will highlight the most recent developments of the autonomous CE protocol for quantitative DBS analyses achieved by our team. The proposed concept enabled high-throughput DBS analyses (up to several hundred DBS samples per 24 h) and its suitability for clinical assays was exemplified by the determination of endogenous markers (e.g. inorganic ions, amino acids, uric acid, creatinine) and exogenous species (e.g. blood anticoagulants, non-steroidal anti-inflammatory drugs, beta-blockers) in remotely collected DBS samples. The actual concept, therefore, represents a progressive clinical tool for personalized healthcare, screening populations at risk, and monitoring the effect of medical treatment on patients. It can be useful in critical (e.g. pandemic) situations as well as it might propel a shift from the actual sick-care to a prevention-based healthcare system. Moreover, the universal character of the elution and the analytical procedures, rapid DBS processing times, excellent separation efficiencies, great variability in CE separation and detection modes, and high sample throughputs imply that the presented concept can be extended to the determination of a wide range of analytes in various dried material spots and might play an important role in clinical, toxicological and forensic analyses in the future.

**Acknowledgements**
Financial support from the Czech Academy of Sciences (Institute Research Funding RVO:68081715) and the Grant Agency of the Czech Republic (Grant No. 23-05972S) is gratefully acknowledged.

## PLENARY LECTURE 5

# TARGET AND SUSPECT ANALYSIS OF CONTAMINANTS OF EMERGING CONCERN IN WATER REUSE PRACTICES: CHALLENGES AND FUTURE PERSPECTIVES

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The availability and quality of water is one of the most challenging problems worldwide, affecting human health, food production, and economic growth. Improving sustainable water management is mandatory to ensure quality water supply for human consumption and the adequate status of water bodies, and to increase resilience of agriculture to climatic changes. In this sense, the Sustainable Development Goal 6 (SDG 6: Clean Water and Sanitation, 2030 Agenda for Sustainable Development, United Nations), proposes to improve water quality by 2030, by reducing pollution, eliminating discharges, and minimizing the emission of chemicals and hazardous materials, halving the percentage of untreated wastewater and considerably increasing recycling and reuse globally.

Meeting these objectives involves carrying out actions in various areas, which include, among others: i) to control the pollution of water bodies by the performance of studies to identify and monitor contaminants and to evaluate their impact on the environment and human health; ii) to develop and implement new and more efficient wastewater treatment systems; and iii) to promote and ensure the safety of water reuse practices, thus contributing to the reduction of the overexploitation of current water resources and facing the more and more frequent drought periods. In accordance with these requirements, a new regulatory framework is being developed in the EU, which in recent years has involved the update and/or publication of new directives, and which reflects the growing interest of the EU in relation to the approach to chemical pollution.

In this context, analytical separation techniques, play an essential role. The development of simple and efficient monitoring strategies based on the use of advanced analytical instrumentation and screening workflows, the developments in non-target strategies for the identification not only of precursor pollutants but also of their transformation products, and the need for new sample pretreatment strategies applied to new and different matrices are challenges that must be faced. The Environmental Analysis Unit of CIESOL works on the characterization and treatment of pollution sources and on the analytical evaluation of reuse practices. Different examples of application of the developed strategies and ongoing projects will be presented.

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# ORAL COMMUNICATIONS

## OC-1

## The growing power of MALDI Imaging to answer today´s questions in Mass Spectrometry

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MALDI Mass Spectrometry Imaging (MALDI-MSI) emerging relevance is related to its powerful analytical capabilities to elucidate the spatial distribution of molecules on the surface of a huge variety of tissues or materials. This technology has been commonly implemented in medical research for disease diagnosis, prognostic biomarkers identification and therapeutic discovery, but is also becoming more popular for environmental and health risk assessment and food science applications.

Here we present the latest developments and advantages of Bruker´s technologies for MALDI-MSI experiments. We highlight the recently developed timsTOF Flex instrument which offers a state-of-the-art MALDI-time-of-flight (TOF)-MSI platform with the option to activate trapped ion mobility separation (TIMS), a new gas-phase ion-mobility separation. In brief, TIMS enables the acquisition of a collision cross-section (CCS) value as determined by the three-dimensional structural conformation of ionized analyte. This is particularly beneficial for the differentiation of isobaric and isomeric lipids and metabolites, which can now be resolved in the gas phase [1] providing at the same time, greater confidence in identification.

In the timsTOF Flex we combined MALDI source with laser-induced post-ionization, namely MALDI-2, to improve the ionization efficiency. This technique involves a second ionization event that is separated in space and time from the initial laser-desorption event to enhance the overall ion yield [2]. Therefore, here we also point out the benefits of applying MALDI-2 and microgrid to significantly improve the sensitivity and spatial resolution for mass spectrometry-based lipidomics approaches by the analysis of i.e. zebrafish embryos.

Finally, the integration of a native ESI/MALDI dual ion source for seamless switching between ionization modalities within seconds enabling powerful SpatialOMx® applications. By combining MALDI Imaging with classical lipid LC-MS/MS PASEF workflows, it is possible to identify biomolecules based on 4D-omics data and spatially locate specific biomolecules within a tissue section, providing a deeper understanding of the tissue microenvironment and its role in e.g., cancer progression.

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## OC-02

# Exposure and toxicological risk assessment of bisphenols, phthalates and non-phthalate plasticizers in children based on current HBM guidance values

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Bisphenols, phthalates and non-phthalate plasticizers are a group of chemical compounds widely used in the industry. They have been found to possess endocrine activity and thus may play a role in the development of adverse health outcomes. Online-SPE-LC-MS/MS was used for the determination of BPA and 21 phthalate metabolites (n=400) as well as their replacement alternatives BPF, BPS, three metabolites of diethylhexil-terephthalate (DEHTP) and three metabolites of Hexamoll-DINCH® (n=150) in urine samples of 7-year old children. The new replacement compounds included in this study were analyzed using a newly developed method that was validated according to the guidelines of the working group “Analyses in Biological Materials” of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the German Research Foundation [1]. This method was applied to determine the urinary concentrations of these compounds in children from Poland, estimate their daily intakes and cumulative risk assessment.

The main compounds found in urine were ΣDEHP, ΣDnBP, ΣMEP and ΣDEHTP, quantifiable in all children’s samples at median concentrations ranging between 35 µg/l and 70 µg/l. BPA accounted for the majority of bisphenols exposures (64%), although around 20% of children had quantifiable concentrations of the three bisphenols (median concentrations of 2.0 µg/l, 1.0 µg/l and 0.13 µg/l for BPA, BPF and BPS, respectively). Metabolites of the Hexamoll-DINCH® plasticizer were found at a lower concentration levels (median of 3.1 µg/l) in comparison with the other phthalate replacement compound analyzed (DEHTP, 35.4 µg/l).

BPA, BPS and Hexamoll-DINCH® concentrations observed in this study were well below the European human biomonitoring guidance values (HBM-GV) established for children populations [2-3]. However, for phthalates, daily intakes and hazard index calculations revealed that a small percentage of children (3-10%) exceeded the tolerable daily intakes established by EFSA, indicating that these children might be at risk of anti-androgenic effects from the individual and cumulative exposure to phthalates [4]. In any case, these calculations do not consider mixed exposures, such as towards different bisphenols, phthalates and non-phthalate plasticizers, simultaneously.

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# OC-3

# UNVEILING THE EXPOSOME: A LC-MS METHOD FOR THE DETERMINATION OF PESTICIDES AND MYCOTOXINS IN URINE SAMPLES

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The exposome paradigm has recently emerged as a new approach to link environmental exposures with their effects on human health [1]. The study of the chemical exposome involves the determination of xenobiotics and/or their metabolites (exposure biomarkers) in the biological fluids, as well as the biological response associated with exposure to chemicals (effect biomarkers). This is a real analytical challenge as exposure biomarkers are at very low concentrations compared to effect biomarkers [2]; thus, specific methods are applied to obtain complete information for an exposome study. Although non-targeted methods are considered the best approach to identify a wide range of exposure biomarkers, they can miss relevant chemical exposures due to the different physicochemical properties of xenobiotics. Targeted methods are required to quantify priority chemicals, but they must go beyond focusing on a small number of compounds with similar properties and structures [3].

This work proposes a targeted liquid chromatography-mass spectrometry (LC-MS) method for the determination of 30 pesticides (12 parent compounds and 18 metabolites) and 23 mycotoxins (14 parent compounds and 9 metabolites), which have been selected due to their extensive use in agriculture or their occurrence in food, respectively, and affect chemical exposure in humans. Urine samples were deconjugated with β-glucuronidase, subjected to salting-out liquid-liquid extraction, and analyzed by LC-MS. The method was applied to the analysis of urine samples from 22 farmers from Almería and Granada (Spain) and 23 samples from the general population. Higher pesticide exposure was observed in farmers due to occupational exposure, but similar mycotoxin exposure was observed in both groups. Farmers are more exposed to neonicotinoid pesticides and fungicides than the general population (≤ 86% and ≤ 35% detection rate, respectively), and at higher concentration levels (≤ 37.4 μg/g creatinine and ≤ 1.8 μg/g creatinine, respectively). Regarding mycotoxins, ochratoxin A and deoxynivalenol (or its metabolites) were the most frequently detected compounds (in 51% and 33% of the samples, respectively).

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# OC-4

# ELIMINATION PROFILE OF DEXAMETHASONE IN DRIED BLOOD SPOTS (DBS) AFTER ORAL ADMINISTRATION

Claudia Bressan(1),*, Sergi Coll(1), Élida Alechaga(1,2), Marcel·lí Carbó(2), Nuria Monfort(1), Rosa Ventura(1)

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Glucocorticoids, such as Dexamethasone (DEX), are widely used in sports medicine for their anti-inflammatory effects. Out of competition periods, there is no restriction for their use. In competition, their administration is allowed by routes considered of local action and prohibited by oral, injectable, venous and rectal routes as they can have positive effects on sport performances. Thus, the establishment of reporting levels able to discriminate between prohibited and allowed administrations is needed. The evaluation of the elimination profile of the substance is also required to avoid false positive results, especially when the drug is administrated by a forbidden route close to competition periods.

In the last years, the use of Dried Blood Spots (DBS) as alternative sampling technique for blood analysis is reaching an increasing interest in the anti-doping field. The collection of DBS is obtained from small volumes of capillary blood lied on filter paper and let to dry. Compared to the conventional blood extraction, this technique presents several advantages as the sampling is less invasive, rapid and does not required specialized personnel to be performed. It also facilitates transport and storage, reducing the costs. Conversely, the reduced sample volume available could represent a limitation and requires the use of highly sensitive instrumentation.

In this work, the quantitative determination of DEX in DBS samples was performed by liquid chromatography coupled to tandem mass spectrometry to assess the possibility to use DBS to detect the oral administration of the drug and to evaluate the elimination profile of the substance. The sample preparation consisted of a two-step extraction procedure with methanol:methyl tert-butyl ether (1:4)(v/v) and acetone. The method was validated for quantitative purposes, evaluating the limit of detection, limit of quantification, range of linearity, precision, accuracy, extraction recovery and matrix effect. The developed method was than applied to samples collected after the oral administration of DEX to healthy volunteers. Single dose (4 mg, n = 8 males) and multiple dose (2x2 mg/day, 5 days, n = 8 males) treatments were studied. DBS and plasma samples were collected at the same time points covering up to 3 days after administration and submitted to analysis. The elimination profiles of DEX in plasma and DBS were finally obtained and compared. For each volunteer, the decrease of cortisol levels was also evaluated in both matrices, as indicative of the systemic effect of the drug.

## OC-5

# 5DETERMINATION OF STEROID PHASE II METABOLITES IN URINE: A CHROMATOGRAPHIC CHALLENGE FOR STUDYING HORMONAL CHANGES DURING PREGNANCY

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Steroid hormones play a critical role in several physiological processes such as pregnancy. Thus, the determination of steroid hormones and metabolites, *aka steroid profile*, is a valuable tool to evaluate the variation of these hormones during pregnancy and their impact in health. The common analytical strategy for the determination of the steroid profile is based on the hydrolysis of the phase II conjugates, the extraction (either LLE or SPE) of the released steroids and their determination by GC-MS after derivatization. The occurrence of LC-MS/MS and its routine implementation in most laboratories opened the door for the direct detection of phase II metabolites. This direct detection not only simplifies the analytical approaches used for the detection of the steroid profile but also expands the knowledge about the steroidome since specific information on the balance between the different conjugates can be obtained. However, direct determination of phase II steroid metabolites presents several important analytical challenges which are delaying its common implementation in routine steroid analysis. Most of these challenges are related with the chromatography of the analytes. Thus, analytical methods aiming to directly determine phase II steroid metabolites should face up to (i) the wide number of species formed e.g. mono-sulfates, glucuronides, sulfo-glucuronides, bis-sulfates, bis-glucuronides, (ii) the high polarity of some metabolites such as bis-sulfates, (iii) the large number of isomeric species and (iv) the structural similarity of the steroid metabolites. In this presentation, we describe the impact of several chromatographic parameters, e.g. stationary phase, mobile phase, pH, temperature in the proper chromatographic behaviour of phase II steroid metabolites. We also present the application of the optimized conditions to evaluate the hormonal changes during preganacy.

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## OC-6

# COMBINACIONES DE SOFTWARE Y HARDWARE: UN PASO MÁS ALLÁ EN LA AUTOMATIZACIÓN EN EL DESARROLLO DE MÉTODOS EN CROMATOGRAFÍA LÍQUIDA. APLICACIÓN A NANOPARTÍCULAS LIPÍDICAS

## AGILENT TECHNOLOGIES

Antonio Chana, Agilent

La industria química, y en particular la industria farmacéutica, tiende cada vez más a la automatización. Y el análisis químico no es en absolute ajeno a esta tendencia. En esta presentación veremos cómo es posible automatizar el desarrollo demétodos analíticos hasta re ducir el tiempo de desarrollo y la intervención humana hasta un 90%, mientras se sigue los requerimientos regulatorios que postulan el uso del paradigma de calidad por diseño. Usaremos un ejemplo muy actual con el análisis del componente lipídico de nanopartículas nanolipídicas, muy utilizadas hoy en día en fármacos basados en RNA.

## OC-7

# ION MOBILITY-MASS SPECTROMETRY IN ENVIRONMENTAL AND FOOD ANALYSIS: SOME CASE STUDIES

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In the last decade, ion mobility spectrometry (IMS) has re-emerged as an analytical separation technique, especially in combination with mass spectrometry (IMS-MS), for small molecules. Interest in this technique is growing among researchers in different fields, including food science and environmental analysis. IMS enables the separation of isomers (base of charge, mass and shape) that are difficult to isolate and separate by both chromatography and mass spectrometry, despite the use of high resolution. The coupling chromatographic techniques to IMS-MS offers some significant advantages to overcome some limitations of traditional chromatography-high-resolution mass spectrometry. The selectivity of the method can be improved by providing an additional separation dimension that allows the separation of isobaric and isomeric compounds; the sensitivity of the method is increased by isolating the compounds of interest from background noise; and complementary information to mass spectra and retention time, the so-called collision cross section (CCS), can be obtained so that compounds can be identified with more confidence, either in targeted or non-targeted approaches. This presentation shows the potential of LC-IMS-HRMS for food and environmental analysis through some case studies: the determination of per- and polyfluoalkyl substances (PFAS) in river water samples, the analysis of marine biotoxins generated by microalgae, and the identification/characterization of polymeric procyanidins (low to high polymerization degree) in food extracts.

The three cases presented show different applications and advantages of LC-IMS-HRMS. The analysis of PFA in river water samples using drift tube ion mobility spectrometry (DTIMS) shows the separation of linear from branched isomers and the individual measurements of the corresponding CCS values, including short-chain PFCA, since a strategy is proposed to overcome the problems that have been observed so far. Regarding marine biotoxins, the 3D characterization of palytoxin and ovatoxins generated by the microalgae Ostreopsis c.f. ovata is performed by combining IMS-MS and molecular dynamics simulation. It is the first time that the 3D structure of these biotoxins is provided, and some surprising IMS results are justified by molecular dynamics simulation. Finally, polymeric procyanidins have been characterized in cranberry and grape extracts by IMS and all-ion-fragmentation (AIF-IMS). The UHPLC-IMS-HRMS/MS has been of great importance to separate and characterize the different degrees of polymerization of procyanidins, allowing sequencing bonds types (type A and B) and proposing a strategy to identify fraud in the manufacture of nutraceutical products.

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## OC-8

# HRMS-BASED METABOLOMIC PROFILING IN NATURAL PRODUCTS RESEARCH: BIOACTIVES WANTED!

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The hyphenation of HRMS instruments (working in the MS or MS/MS mode) with high throughput chromatographic techniques has become a powerful strategy for the sensitive screening analysis and rapid identification of secondary metabolites in complex natural matrices. In this regard, HRMS-based techniques, coupled via liquid chromatography (LC-HRMS) or gas chromatography (GC-HRMS), are the most popular options for comprehensive phytochemical profiling and metabolomic purposes in natural products research. These hyphenated approaches significantly increase the speed of analysis, improving selectivity, resolution and efficiency, and provide improved structural determination capabilities compared to other methods [1].

The huge amount of datasets generated by HRMS measurements pose a great challenge due to the need of processing thousands of MS features to extract useful information. This work addresses the enormous analytical challenge of characterizing bioactive molecules, frequently found in complex cocktails of phytochemicals, due to the limited coverage and diversity of MS databases for natural products annotation. In this regard, several data-mining strategies have emerged to facilitate the post-acquisition data processing [2]. The implementation of these filtering strategies is a powerful tool to increase the success rate in the discovery and annotation of new bioactive phytochemicals, overcoming the limitations of MS database search.

To uncover the complex relationship between the chemical composition and the observed bioactivity of natural products, untargeted and semi-targeted MS and MS/MS data-mining strategies were successfully implemented to characterize bioactive secondary metabolites from agri-food byproducts (e.g., procyanidins, terpenoids, crocins) as well as from valuable plants and algae (e.g., polyphenols, carotenoids and PUFAs). These natural matrices, were shown as promising sources of health promoting compounds with demonstrated anti-cancer, anti-inflammatory or anti-cholinergic properties under *in-vitro* bioactivity testing [3,4].

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## OC-9

# A comprehensive picture of PCB effects on pig metabolism using a multi-platform metabolomics and lipidomics approach.

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The "chemical space" of the serum metabolome is a very complex ecosystem that ranges from small (<100 Dalton) to large molecules (>1500 Dalton) and from very polar (logP <-5) to non-polar compounds (logP >5). Nowadays, none of the metabolomics methods is able to cover the whole metabolome, in terms of (chromatographic) separation and ionization of the metabolites. However, an extensive coverage of the metabolome is possible through the use of multiple analytical platforms.

In this study, multiple analytical platforms (CE-MS, HILIC-MS, RP-MS, GC-MS) have been deployed to determine the metabolic effect of low-dose chronic exposure to Polychlorinated Biphenyls (PCBs). Indeed, acute PCB exposures are known to have multiple deleterious effects on mammalian metabolism, and current chronic exposure levels are still a public health concern especially in specific parts of the general population. In this spirit, a metabolomics and lipidomics study on pigs exposed to environmental PCB levels has been carried out, aiming to identify early biomarkers-of-effect related to metabolic disorders generally induced by PCBs.

The results demonstrated that PCBs at low doses still affected the pigs' metabolism, and the effects are similar to higher exposure doses (dyslipidemia, disruption of the TCA cycle, alteration of several neurotransmitters and others), although lower in their magnitude [1]. The employment of multiple platforms acted like a multiple shotgun approach, highlighting different pathways/molecules of the pigs' metabolism. Each platform returned a list of different biomarkers-of-effect, which, taken all together, helped in the biological interpretation of the data and finally provided a complete picture about the aftermath of low-dose PCBs exposure. The multi-platform approach was clearly advantageous in terms of data interpretation, especially in the specific case of PCB exposure where the effects are not limited to individual metabolic pathways/tissues/organs.

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# OC-9B

# FAST METHOD FOR THE CHARACTERIZATION OF LIQUID CHROMATOGRAPHIC SYSTEMS BASED ON ABRAHAM'S SOLVATION PARAMETER MODEL

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The Abraham's solvation parameter model, based on linear solvation energy relationships (LSER), allows the accurate characterization of the selectivity of chromatographic systems according to solute-solvent interactions:

$$\log k = c + e{\cdot}E + s{\cdot}S + a{\cdot}A + b{\cdot}B + v{\cdot}V$$

where k is the retention factor and the v·V term accounts for the cavity formation in the solvent together with residual solute-solvent dispersion interactions, c is a system constant, and the rest of the terms are related to solute-solvent interactions. e·E term models the polarizability contributions from n- and π-electron pairs, s·S the dipole-type interactions (orientation and induction), a·A the hydrogen bond donation from solute to solvent, and b·B the hydrogen bond donation from solvent to solute. E, S, A, B, and V are solute descriptors available from commercial and open access databases. e, s, a, b, and v are the system coefficients, reflecting the differences in solute interactions between the solvated stationary phase and the mobile phase [1, 2].

However, this method, based on multilinear regression analysis, requires the measurement of the retention factors of a considerably high number of compounds, turning it into a time-consuming low throughput method. Simpler methods such as Tanaka's scheme are preferred. In the present work, the Abraham's model is revisited to develop a fast and reliable method, similar to the one proposed by Tanaka, for the characterization of columns employed in reversed-phase liquid chromatography and particularly in hydrophilic interaction liquid chromatography. For this purpose, pairs of compounds were carefully selected in order to have in common all molecular descriptors except for a specific one (for instance, similar molecular volume, dipolarity, polarizability, and hydrogen bonding basicity features, but different hydrogen bonding acidity). Thus, the selectivity factor of a single pair of test compounds can provide information regarding the extent of the dissimilar solute-solvent interactions and their influence on chromatographic retention. Therefore, five chromatographic runs in a reversed-phase column (four pairs of test solutes and a mixture of four homologues) are enough to characterize the selectivity of a chromatographic system.

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## OC-10

# From Helium to Hydrogen, a cost-effective way to improve your lab´s green credentials

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The use of hydrogen in analytical or industrial applications has always been controversial due to the problems derived from its handling. Likewise, the availability of helium, conceived as its safe substitute, has not always been nor will it be guaranteed in the future due to a variety of causes such as production, transport, or prices.

This presentation includes the workflow and the necessary requirements to transition from the classical GC methods developed with Helium as carrier gas towards their conversion into the corresponding fast methods with Hydrogen as carrier gas or FAST GC.,

As well as the determining technical factors in the development of new fast methods such as the design of ion sources are analyzed and its influence in the quality of identifications.

Likewise, experimental data from different types of samples obtained with the LECO´s Pegasus BT GC-TOFMS are shown in relation to analytical features such as analysis time reduction, chromatographic resolution, spectral similarity and sensitivity proving its suitability for FAST GC.

## OC-11

# NEW RAPID EXTRACTION METODOLOGY FOR THE DETERMINATION OF ORGANOHALOGEN COMPOUNDS IN HUMAN SERUM

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Persistent Organic Pollutants (POPs) are mhemicals that are present in all environments, including human tissues and fluids. Although the majority of these compounds were banned in the 1970s, they continue to be matter of public concern due to their toxicity and persistence in human fluids and the environment. Human Biomonitoring of both currently used and banned POPs is an extremely effective method for determining human exposures to these chemicals, mainly through the analysis of plasma or serum samples. There are several methodologies available for the analysis of POPs in serum. However, they are usually concerned on the analysis of polychlorobiphenyls (PCBs), DDTs, hexachlorocyclohexanes (HCHs), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB), e.g. the compounds that survive clean up by sulfuric acid oxidation. This study provides a new method that allows the determination of >40 POPs using small sample volumes and short sample handling times.

The new liquid-liquid extraction (LLE) methodology developed requires a low sample volume (~300-500 µl), which is half of the volume required by the previous methodology [1]. This reduction allows to work with lower solvent amounts. The new process, aside of using hexane for the LLE process, employs a mixture of water:methanol 75:25 v/v to precipitate proteins, but retaining all the compounds to be analyzed. Consequently, the number of compounds determined using the current methodology increases substantially, being able to analyze organochlorine pesticides such as DDTs, HCHs, PCBs, PeCB, HCB but also vinclozolin, polybromodiphenyl ethers, hexabromocyclododecane, octachlorostyrene, methoxychlor, endosulfans, heptachlors, chlordane, aldrin, endrin, dieldrin, mirex, technazene, quintozene and others.

Proficiency testing materials from two intercalibration programmes, e.g. AMAP Ring Test for POPs [2] and G-EQUAS [3], allowed us to compare the two methodologies, obtaining similar recovery rates and optimal results. In addition, for the analysis of PBDEs, the new methodology provided improved chromatographic separation. To evaluate the efficacy of the new methodology in terms of matrix effects and interferences, as well as experimental time, a total of 300 serum samples from the NFBC birth cohort study from Finland were analyzed [4]. The new methodology provided optimal results in samples from intercalibration programmes and concentrations among the range of similar population studies when analyzing real serum samples.

**Acknowledgements**

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## OC-12

# MODIFIED POLYMERIC FILMS FOR THE ENRICHMENT OF STEROID HORMONES AND ORGANOPHOSPHOROUS PESTICIDES FROM WATER SAMPLES

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The presence of organic compounds in waters is an issue of concern because they are not fully eliminated in conventional wastewater treatment plants, they tend to accumulate in living organisms and their toxicity and effects are not well known. Given this, it is important to develop new green methodologies for their enrichment, detection and monitoring. Polymer inclusion membranes [1] can be used as a material for this purpose. In this work, polymeric films without plasticizer and modified with either deep eutectic solvent (DES) or biochar, of natural origin, have been tested.

On the one hand, DES-modified films are prepared by the solving casting method, with cellulose triacetate (CTA) as the polymer, and as a deep eutectic solvent, several mixtures have been evaluated containing dodecanoic acid, camphor or menthol, and lidocaine, at different ratios. The targets pollutants in this study are organophosphorus pesticides and the film consisting of 70% CTA and 30% DES with dodecanoic acid:lidocaine (2:1) has demonstrated improved extraction results in preliminary test. Thin Film Microextraction (TFME) is performed by suspending a piece of the film (S-TFME) in the solution for one hour under stirring. After extraction, the ultrasound-assisted elution with ethyl acetate followed by GC-MS analysis has provided good performance in terms of reproducibility and recovery from different water matrices, and a working range from 1 to 130 ug $L^{-1}$.

On the other hand, Nuchar, a wood-derived biochar, has been entrapped obtaining carbon-based polymeric films, that have been tested for TFME of 16 steroid hormones. After a study about membranes' composition in terms of concentration of Nuchar and type of synthesis set up, the ceramic 3D-mold has been selected, achieving reproducible and ready-to-use membranes with composition fixed as 90% CTA and 10% Nuchar. After 8h microextraction, the best elution conditions for quantitative relative recovery are 2 mL MeOH: ACN 1:1, orbital shaker for 5 min, afore HPLC-MS/MS analysis. The final method has been satisfactory applied to river water and wastewater treatment plants effluent.

These analytical methods are valid tools in terms of analytical performance and promising in terms of greenness.

**Acknowledgements**

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## OC-13

# Firefighter’s exposure to PAHs in prescribed forest fires analazyed by GC-Orbitrap-MS

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Presence of polycyclic aromatic hydrocarbons (PAHs) in wildfire smoke is one of the reasons that occupational exposure as a firefighter is classified as “carcinogenic to humans” (group 1). Forest firefighters don’t wear respiratory equipment, which makes exposure to smoke very relevant.

Here we present a method for the for the analysis of PAHs in the 3 mm spot on filter strips from real-time Aethalometers AE51 (AETHLABS) equivalent black carbon (eBC) analyzers, used as personal exposure monitors for firefighters during a prescribed forest burns in Mediterranean forests (catalunya). The PM2.5 sample spot was taken from the filter strip, extracted and analyzed by Q-exactive GC Orbitrap mass spectrometry for particle-bound PAHs (benz[a]anthracene to benzo[ghi]perylene) with a LOQ of 0.5 pg/µL).

Peak eBC concentrations were around several 100s of µg/m3, and 5-h mean eBC concentrations were higher than 1 µg/m3. Benzo[a]pyrene concentration were also higher than 1 ng/m3 and related to extinction tasks during fires. Based on the eBC peak concentrations at 5-min monitoring resolution, the direct exposure to high concentrations of smoke particles and PAHs was probably even less than 1.5 h. Fullscan analyzis allows non-target analysis, and the detection and identification of several other organic compounds, related to biomass combustion.

The methodology allows the detection of PAH in portable Aethalometers and these results show that firefighters are exposed to significant PAH concentrations during prescribed burns, which is related to the activities around wildfires extinction, and forest types.

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B.L. van Drooge, R.M. Prats, C. Jaén, J.O. Grimalt. (2023). Determination of subpicogram levels of airborne polycyclic aromatic hydrocarbons for personal exposure monitoring assessment. Journal of Environmental Monitoring and Assessment, 195:368, https://doi.org/10.1007/s10661-023-10953-z

## OC-14

# ROTENONE, A PLANT-DERIVED PISCICIDE FOR THE ERADICATION OF ALLOCHTHONOUS FISH IN ALPINE LAKES: ADAPTATION OF WATER SAMPLING STRATEGIES AND ANALYTICAL METHODOLOGIES

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Rotenone is a naturally occurring isoflavonoid derived mainly from the roots of *Leguminoseae* plants. It disrupts mitochondrial respiratory chain processes in fish, so it has seen widespread use as an effective piscicide product since it enters the bloodstream of gill-breathing organisms particularly quickly. Rotenone has been applied for the large-scale management of water bodies (i.e., eradication of unwanted and allochthonous fish species in freshwaters) as its quick degradation potential theoretically limits its long-term impact on ecosystems. Rotenone concentrations in water are oftentimes determined on-site during fish control treatments using a simple and fast liquid chromatography (LC) separation with ultraviolet (UV) detection [1,2]. However, the limited accessibility and lack of energy sources in alpine lakes severely limit the water sampling and analytical strategies that can be applied. Moreover, LC-UV techniques have low selectivity and sensitivity, which hinders the detection of other rotenoid byproducts present in commercial rotenone formulations and their degradation products (e.g., rotenolone, deguelin, tephrosin). This limits the assessment of degradation profiles after the application of the piscicide.

We present a simple, optimized workflow adapted for the analysis of rotenone and five other rotenoids in alpine lakes. The analysis is performed by gas chromatography coupled to single-quadrupole mass spectrometry (GC-MS). This allows the analysis of reduced sample volumes (60 mL) that are collected at different points and depths in the lakes using an in-house built grab sampler device and that can be extracted on-site using easily transportable, manually-operated C18 solid phase extraction (SPE) columns. Analytical recoveries were 86 ± 7%, SPE breaktrough was lower than 1.3%, and quantification limits were 0.2–0.3 µg/L (typical application concentrations are around 300 µg/L). Repeatability tests showed variations in determined concentrations up to 17%. The application of this methodology after piscicide treatments in the lakes showed the quick decrease in concentrations of rotenone and its byproducts below quantification limits one to two weeks after the treatment, well below rotenone's no-effect concentration (~2 µg/L). Degradation products were still detectable after three weeks. Rotenone distribution in the lakes was potentially influenced by particulate matter and macrophyte presence, and possibly by defficient water mixing along the water column, but not by sampling position after a thorough application of the product.

### Acknowledgements

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## OC-15

# ANALYSIS OF THE NEUROTOXIC EFFECTS OF ANATOXIN-A AND SAXITOXIN IN ZEBRAFISH LARVAE

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The increasing global warming, result of the climate change, as well as the eutrophication are some of the factors responsible for the rising of harmful algal blooms (HABs) in aquatic systems. If optimal conditions take place, these phenomena can lead to the generation of cyanotoxins, secondary metabolites coming from cyanobacteria. When found in large amounts, these substances can produce adverse effects in living organisms and, in worst case scenarios, death. Due to the alarming climate situation and the risks that these substances represent against animal and human safety, this research aims to study the neurotoxic effects that two potent cyanotoxins, anatoxin-a and saxitoxin, have on living organisms using zebrafish larvae as an animal model.

To do so, first a chromatographic method was developed for the detection and quantification of saxitoxin using a HILIC column (150 x 2.1 mm, 2.6 µm, 100 Å by Phenomenex) in an ultra high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UPLC-MS/MS) system. Also, a previous chromatographic method for anatoxin-a was slightly modified, using a Lunar Omega Polar C18 (100 x 2.1 mm, 1.6 µm, 100 Å, Phenomenex) column in a liquid chromatography system coupled to a QTrap from SCIEX. Then, these two were used to analyse the stability samples of anatoxin-a and saxitoxin among 24-hour period.

Expositions of both cyanotoxins at four different levels were performed with zebrafish larvae, a commonly used animal model in ecotoxicology due to its genetic code resemblance with humans, among other advantages. The expositions were conducted with 7 days post-fertilization zebrafish larvae in an incubator at 28.5 oC with a 12:12 (light: dark) photoperiod. After a period of time of 24 hours, behaviour studies were performed to evaluate if the treated animals showed adverse effects when compared against the control group.

Finally, using a previously optimised method, the neurotransmitters from zebrafish larvae's heads were extracted and analysed using UPLC-MS/MS with a Premier Glycan BEH Amide (150 x 2.1 mm, 1.7 µm and 130 Å, Waters) column in order to study the variations in the metabolites' levels caused by each cyanotoxin at every concentration studied.

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## OC-16

# ASSESMENT OF ANTIBIOTICS AND EMERGING PATHOGENS IN THE EBRO DELTA, AND THE ALBUFERA OF VALENCIA

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Surface waters impacted by wastewater play an important role as reservoirs for antibiotic-resistant (AR) bacteria. [1] This work aimed to assess antibiotics, antibiotic-resistant genes (ARGs), and antibiotic-resistant bacteria (ARBs) in two sensitive areas, the Ebro Delta and the Albufera of Valencia.

The analysis of antibiotic residues in surface water and the influents and effluents of wastewater treatment plants (WWTPs) was performed by ultra-performance liquid chromatography coupled with high-resolution mass spectrometry tandem mass spectrometry (UPLC-HRMS/MS) with full-scan (FS) acquisition. A suspected screening approach was applied to identify the main antibiotic groups, followed by target analysis for confirmation and quantification. A total of 115 samples, corresponding to 33 WWTPs influent, 33 WWTPs effluent, and 49 surface waters, were analyzed. The most detected groups were quinolones such as ciprofloxacin and ofloxacin, the macrolides antibiotics such as clarithromycin, tetracyclines such as tetracycline, and sulfonamide antibiotics such as sulfamethoxazole, and trimethoprim, with concentrations ranging between the MLOQ and

17.9 µg/L. In the same samples, ARGs were studied by real-time polymerase chain reaction (qPCR), showing a general positive correlation between antibiotic residues and the ARGs. The presence of ARGs for the main antibiotic groups, macrolides (emB_1), tetracyclines (tetA_1 and tetPB_3), and sulfonamides (Sul1_1 and Sul2_1) was confirmed in most of the influents. Also, tetracyclines and sulfonamides ARGs were present at the effluents and in surface waters. In addition, ARGs for ß-lactams (blaCTX-M, pdp2b) were commonly found despite this class of antibiotics was seldom found. This fact agrees with previous works, and it is explained by the rapid ß-lactam antibiotics hydrolysis. In the case of bacteria, although the microbial load decreases in effluent compared to influent wastewater (2-3 log10 on average), the reductions between upstream and downstream wastewater do not comply with the latest European standards, Regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for water reuse. Finally, the correlation between antibiotics, ARGs, ARBs, other pathogens, physicochemical parameters and their correlations will be presented.

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## OC-17

# HUMAN EXPOSURE TO MICRO(NANO)PLASTICS AND PLASTIC ADDITIVES

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Humans are exposed to micro(nano)plastics (MNPLs) through inhalation, ingestion, and dermal contact, being food and drinking water considered as the primary sources of oral exposure [1]. In this work we present a compilation of investigations dealing with the assessment of the human exposure to MNPLs and plastic additives through water and fish.

In the case of water, we have evaluated the presence of MNPLs particles from 20 μm to 700 nm in bottled water commercialized in Spain [1] and in tap water from volunteers belonging to 42 postal codes from Barcelona metropolitan area [2]. In both cases, the analytical method consisted of water fractionated filtration as a pre-treatment, followed by toluene ultrasonic-assisted extraction and size-exclusion chromatography coupled to high resolution mass spectrometry (HRMS), equipped with an atmospheric pressure photoionization source, for the quantification of MNPLs. On the other side, reverse phase chromatography coupled to HRMS equipped with an electrospray ionization source was used for the detection of plastic additives. In both cases, the data acquisition was done in full scan mode at a resolution of 70,000 FWHM and, in parallel, data dependant scan of the 5 most intense ions. The tentative identification of MNPLs polymers was based on characterization of monomers by using Kendrick Mass Defect analysis, and confirmation and quantification using standards. In contrast, for plastic additives, the data was processed by CompoundDiscoverer3.0 software and further confirmation steps reaching level 2 by the application of different restriction parameters. For the evaluation of shellfish, mesocosms experiments where performed by exposing Mediterranean mussels to PE, PET and PS (size < 10 μm) through the diet, separately, at concentrations of 10 mg/L for 21 days. Then, the mussels were cryogenized, molturated and extracted by USAE with toluene for 1h. Then, the supernatant was reduced to 1 ml and analyzed according to MNPLs polymers methodology.

The results showed that PP, PE and PET were present in bottled waters, being PE the most frequently detected and quantified followed by PET [1]. In contrast, PE, PP, PI, PBD, PS, polyamide and polydimethylsiloxanes were identified in tap waters and PE, PP and PA were the most frequently detected [2]. Regarding plastic additives, stabilizers and plasticizers were the most detected ones among all the groups [1]. In the case of shellfish, humans can be more exposed to PE and PS through the ingestion of mussels according our results.

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# OC-18

# ANTHROPOGENIC CHEMICALS OF EMERGING CONCERN IN WATERS FROM THE NORTHERN ANTARCTIC PENINSULA REGION

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In this study, the occurrence of inorganic and selected organic anthropogenic chemicals of emerging concern (CECs) and their associated risks were evaluated in several inland freshwater and coastal seawater samples to assess the human footprint on the chemical pollution of Antarctic waters. Nicotine and tolytriazole, present in 74 and 89 % of the samples analyzed, respectively, were the most ubiquitous CECs in the investigated area. The most abundant CECs were citalopram, clarithromycin, and nicotine with concentrations reaching 292, 173, and 146 ng/L, respectively. The spatial distribution of CECs was not linked to any water characteristic or inorganic component. The contamination pattern by CECs in inland freshwater varied among locations, whereas it was very similar in coastal seawater. This suggests that concentrations in inland freshwater may be ruled by environmental processes (reemission from ice, atmospheric deposition, limited photo- and biodegradation processes, etc.) in addition to human activities. After risk assessment, citalopram, clarithromycin, nicotine, venlafaxine, and hydrochlorothiazide should be considered of concern in this area, and hence, included in future monitoring of Antarctic waters and biota. These results show that current measures taken to protect the pristine environment of Antarctica from human activities are not effective and to this end additional actions need to be considered [1].

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## OC-19

# EVOLUTION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN AIR AND WATER: OVER A DECADE OF MONITORING IN SPAIN

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Awareness of the risks that POPs pose worldwide led the international community to enact the Stockholm Convention (SC) in 2001 under the United Nations Environment Program. A continuous and long-term monitoring of POPs is essential in determining the effectiveness of the SC. In this study, framed within the Spanish Monitoring Program on POPs, which has been running since 2008, we evaluate data on POPs regulated under the SC in air from background and urban locations and water samples from the Duero River basin.

Air samples, obtained with passive sampling devices equipped with PUFs, were analyzed by GC-qMS or GC-QqQMS/MS or GC-HRMS depending on the target POPs considered in this study. UPLC-QqQ-MS/MS was used for the identification and quantification of the target POPs in water samples. All samples were spiked with $^{13}$C-labeled standards prior to their extraction, enabling the use of the isotopic dilution technique for quantitation purposes. Further details on the analytical processes are provided elsewhere [1,2,3,4].

Despite the wide range of concentrations measured for most contaminants, based on median concentrations, a common pattern of relative abundance in air could be observed: ∑PCBs > HCB > ∑HCHs > ∑DDTs > ∑PBDEs > ∑endosulfan > PeCB > PCDD/Fs. Median concentrations in background locations varied according to HCB > ∑HCHs > ∑PCBs > ∑DDTs > ∑endosulfan > ∑PBDEs > PeCB > ∑PCDD/Fs, while in urban locations ∑PCBs > ∑HCHs > HCB > ∑DDTs > ∑PBDEs > ∑endosulfan > PeCB > ∑PCDD/Fs. In general, statistically significant (Mann-Whitney, $p<0.05$) higher levels of total POPs were found in urban than in background locations, which underpinned the role of the former as main source for most target POPs (∑PBDEs, ∑PCBs, ∑PCDD/Fs, ∑DDTs, and ∑HCHs). Conversely, no significant differences between background and urban locations could be found for HCB, ∑endosulfan, and PeCB.

Regarding the regulated PFAS detected in the Duero basin, median concentrations showed a common pattern of relative abundance consisting of PFOS > PFOA > PFHxS. The highest levels of ∑PFAS and most of the individual compounds were detected near industrial and populated areas, suggesting these areas and related human activities are a source of PFAS pollution.

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## OC-20

# Monitoring of the EU Watch List contaminants of emerging concern in the Spanish River basins

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Within the framework of the Directive 200/60/CE, the analysis of emerging pollutants is considered in order to provide the Commission with high-quality monitoring information on concentration of these substances presents in water.

For this purpose, the Directive 2013/39/EC introduced the Watch List. This mechanism focuses on a limited number of substances temporarily included to collect data and to serve as a basis for future revisions of priority substances. Every two years, the European Commission revises the Watch List by replacing pollutants for which there is already sufficient data for risk assessment and including new ones of emerging concern. To date, four Watch List have been published: Decisions 2015/495/EU, 2018/840/EU, 2020/1161/EU and recently, 2022/1307/EU. In general, the Watch List includes substances such as pharmaceuticals, pesticides, hormones, UV filters and industrial additives.

In Spain, the General Directorate of Water of Ministerio para la Transición Ecológica y el Reto Demográfico is in charge of coordinating the work related to the Watch List. This monitoring program is carried out in 16 wastewater treatment plants (WWTPs) effluents and 20 receiving waters of the different Spanish River basins. Although the Water Framework Directive (WFD) does not apply directly to WWTP effluents, there is no doubt that its study provides very valuable information, to evaluate the impact of WWTPS on rivers and the efficiency of treatments used in them.

In the present work, the results of the different monitoring campaigns carried out are presented (2018, 2019, 2022 and current 2023 studies). The analysis of the compounds was performed by gas and liquid chromatography coupled to mass spectrometry (tandem and high resolution) [1,2]. The highest concentration was of ciprofloxacin [30-10,400 ng/L], venlafaxine [12-5,000 ng/L], the antibiotics macrolides (erythromycin, clarithromycin and azithromycin) [14-1,780 ng/L], sulfamethoxazole [70-820 ng/L] and amoxicillin [20-245 ng/L]. However, pesticides such as metaflumizone, thiamethoxam, clothianidin, miconazol, tetraconazol, penconazol were not detected. The substances included in the fourth list are currently under study.

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# OC-21

# TITANIUM DIOXIDE NANOPARTICLES@NYLON 6-COATED MAGNETIC ADHESIVE TAPE FOR THE EXTRACTION OF BENZOPHENONES

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In this communication a new planar sorptive phase for the extraction of benzophenones from water samples is presented. For the preparation of the sorptive phase, a piece of adhesive tape is used as a support, whose adhesive character attaches the particulate material. In this approach, titanium dioxide nanoparticles coated with nylon 6 are used as an extractant phase, which are deposited on the adhesive tape. On the other hand, magnetic nanoparticles are also supported on the adhesive tape to provide magnetism to the sorbent phase and allow its anchoring to the extraction device.

The sampler used for the extraction process consists of a small electric motor bound in a plug. A NdFeB countersunk pot magnet is attached to the motor using a screw. The magnetic character of this unit simplifies its anchoring, reducing its handling [1]. The sorbent phase is immersed in the sample and stirred by the motor to achieve the transfer of the analytes to it. The portable system integrates the sampling and extraction of the analytes in a single step. Thus, by allowing in-situ application, a large part of the problems associated with environmental analysis (i.e., sample heterogeneity, both spatial and temporal, high number of compounds at low concentration, as well as the high volume of sample to be stored) are avoided. In addition, all the problems related to sample transport are solved, since the analytes would be transported retained in the material, avoiding cross-reactivity [2].

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## OC-22

# Environmental micro and nanoplastics composition analysis by liquid chromatography coupled to high resolution mass spectrometry.

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The so-called marine litter, particularly microplastics (MPLs) and nanoplastics (NPLs) are ubiquitously distributed and recognised as an emerging environmental and human health risk. It is known that marine environments are one of the most impacted areas, and among them, coastal zones are the most contaminated ones. They are subjected to population pressure, tourism, harbours, desalination plants, marine traffic, and fish farms.

In this talk, the composition (polymers and plastic additives) analysis of MPLs and NPLs by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) will be introduced, and different examples of application to marine samples will be summarised. Examples of the analysis and the levels of polymers and plastic additives in surface waters [1], sediments [2], the marine atmosphere [3], and fish [4-5] will be shown. Special focus will be done on our recent Mediterranean studies because of the plastic marine litter generating activities in this region and its characteristic morphology of semi-enclosed sea is currently considered one hot spot of MPLs pollution in the world.

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# OC-23

# WORLDWIDE STUDY ON POPs IN AMBIENT AIR USINGA COMPARATIVE PASSIVE AIR SAMPLING

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From 2008 to 2011, UNEP coordinated the GMP1 and analyzed PAS/PUF samples from 32 countries in Africa, GRULAC and the Pacific Islands to lay down the basis for the GMP2 project to further support developing countries [1-3]. The GMP2 was implemented from 2016 until 2021 whereby samples were collected mainly in 2017 and 2018; some countries did expose their polyurethane foam disks (PUFs) in 2019. For GMP1 and GMP2, the guidance document recommendations were strictly applied to all POPs analyzed. Each sampling site had 12 PAS/PUF exposed at the same time and the PUFs were distributed to different laboratories for the analysis of the various types of POPs. The occurrences, relative abundances, and distribution of the different compounds, their seasonal fluctuations, and regions, were to be addressed.PUFs were exposed for two years and changed every three months between 2017 and 2019. The projects addressed 26 of the 34 POPs listed in either Annex A, B, or C of the Convention, whereby polychlorinated biphenyls (PCB) were include twice, namely as the sum of six indicator PCB (PCB6) and as dioxin-like PCB (dl-PCB). [4][5][6][4, 6] Not included were chlordecone, PCNs, PCP, SCCP, and dicofol as well as methoxychlor, dechlorane plus and UV-328. Here we assess the amounts of 28 POPs measured in 381 ambient air samples on a comparative basis for national and regional occurrence, i.e., within a UN region but also for the geographic location such as latitude or altitude.

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## OC-24

# EXPLORING THE POTENTIAL OF 3D PRINTING IN SAMPLE PREPARATION DEVICES

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Over the past years, the integration of 3D printing has become widespread across various industries, particularly within scientific fields like Analytical Chemistry. In this field, 3D printing has emerged as a nascent technology with applications ranging from the creation of detectors and electrodes to sample preparation devices and chromatographic separations. Several prevalent 3D printing technologies, namely Fused Deposition Modelling (FDM), vat polymerization, photopolymer inkjet printing, and selective laser sintering, offer distinct advantages and disadvantages. These technologies enable the fabrication of novel 3D printed structures that are either difficult or costly to produce using alternative methods.

Therefore, 3D printing presents an intriguing prospect, especially within the field of sample preparation and separation techniques. Notably, FDM and vat polymerization have emerged as the most widely employed techniques in sample preparation and separation techniques due to their affordability, ease of use, and other noteworthy merits. FDM provides a wide range of materials, while vat polymerization offers high chemical stability. These advancements open up new avenues for incorporating functional and selective materials like metal organic frameworks, nanoparticles, and selective ligands into 3D printed extraction and separation systems. Consequently, 3D printing serves as a captivating alternative to traditional supports in this context.

This communication aims to explore the current trends in 3D printing for the development of novel devices in both sample preparation, separation and detection. To illustrate the wide range of possibilities in sample preparation, noteworthy examples will be selected. These examples showcase the extensive potential of 3D printing in revolutionizing sample preparation methodologies.

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### OC-25

# A MINIATURIZED MAGNETIC-BASED SAMPLE PREPARATION APPROACH FOR THE DETERMINATION OF BISPHENOLS IN FOLLICULAR FLUID

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Bisphenols, particularly bisphenol A (BPA), are the primary monomers used as additives in the manufacturing of resins or polyacrylates found in consumer products. However, it has been reported that the exposure to these compounds is related to endocrine-disrupting and reproductive effects, among others. For this reason, the development of analytical methods for their determination in biological matrixes are needed to monitor the population exposure to these compounds, and thus to draw conclusions about infertility problems. On the other hand, the analysis of biological samples is sometimes a challenge due to their scarcity. Therefore, sufficiently miniaturized approaches are required to carry out their treatment. This is the case of miniaturized stir bar sorptive dispersive microextraction (mSBSDME), recently presented by our group as efficient tool for the analysis of low-availability samples [1].

In this communication, a mSBSDME approach followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis has been developed for the determination of nine bisphenols in follicular fluid (FF). Prior the preconcentration and determination of the target bisphenols, the FF was pretreated with acidic acetonitrile and percolated through a solid-phase extraction zirconia cartridge to remove both proteins and phospholipids. Then, the protein- and phospholipid-free supernatant was evaporated and reconstituted in phosphate buffered saline solution (*i.e.,* donor phase). For the extraction of the target analytes from the donor phase, a magnetic-modified covalent organic framework (COF) was used as sorbent. Under the optimized conditions, the method was properly validated, showing good analytical features in terms of linearity, limits of detection, and precision. Finally, the proposed method was applied to five FF samples provided by IVIRMA Barcelona, obtaining quantitative relative recoveries in all the cases by internal standard calibration.

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### OC-26

# AFFINITY SORBENTS IN LOW-COST SUPPORTS: A GREEN AND EFFICIENT PATH IN MICROEXTRACTION

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Sample preparation remains a crucial stage to extract and/or preconcentrate the analyte/s of interest from different types of complex samples such as biological fluids, foodstuffs or environmental samples [1]. Currently, several microextraction techniques are established as reliable and environmentally friendly sample preparation procedures. However, one of their main disadvantages of the sorbents used in these techniques is their limited selectivity for trace analysis in many complex samples. To overcome this problem, several strategies that promote the use of affinity-based materials employing selective ligands such as antibodies or other biomimetic recognition elements (such MIPs and aptamers) have been proposed [2]. In particular, aptamers are single-stranded RNA or DNA molecules, able of developing well-defined 3D structures, that exhibit high affinity and specificity for specific targets, ranging from individual molecules to entire organisms. They have many advantages such as high reproducibility and ease synthesis, higher stability, no need of animals, relatively low cost, and tailorable chemical modification [3,4]. However, at present, their combination with low-cost traditional extraction formats and emerging supports (such as paper-based devices and 3D-printed objects), are still in its infancy stage. In this communication, representative analytical applications that use aptamer-based materials in different low-cost designed devices will be described. Additionally, the future developments offered by this green and efficient pairing in the field of sample treatment will be outlined.

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## OC-27

# MAGNETIC-BASED PIPETTE-TIP MICROEXTRACTION AS A HIGH-THROUGHPUT, PORTABLE AND AFFORDABLE APPROACH FOR THE ANALYSIS OF LOW-AVAILABILITY SAMPLES

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This communication presents, in a summarized way, different methods developed by our research group for the analysis of low-availability samples, as is the case of some biological fluids. More specifically, they cover the determination of different biomarkers, such as testosterone in saliva to detect hormonal disorders, like early puberty in children or unethical practices from athletes; the determination of biogenic amines (putrescine and related ones) to diagnose cancer; and the determination of cortisol in serum from preterm newborns to early detect sepsis and overcome undesired side-effects. All these analytical methods have the common denominator of using the so-called magnetic-based pipette-tip microextraction (M-PTME), a high-throughput, portable and affordable approach recently presented by our research group [1], showing excellent features from both the sample preparation and clinical points of view.

This sample preparation approach consists on quickly disperse a suitable magnetic sorbent for the target analytes in a low volume of sample by means of a disperser solvent, and the resulting dispersion is immediately aspirated through a pipette tip containing a small neodymium magnet inside, which entraps the magnetic sorbent containing the target analytes. The sample is then discarded, and the subsequent cleaning and desorption steps are conducted by aspirating/dispensing the suitable solvents.

The superior performance of this lab-made approach over other well-stablished and commercially-available pipette tip extraction strategies, such as pipette-tip solid-phase extraction (PT-SPE) and dispersive pipette-tip extraction (DPX), is demonstrated in terms of operability and affordability.

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# YOUNG RESEARCHER ORAL COMMUNICATIONS

## OY-1

# STUDY OF THE EFFECT OF INFLAMMATORY CYTOKINES ON HUMAN PROXIMAL TUBULAR HK-2 CELLS THROUGH UHPLC-HRMS-BASED UNTARGETED METABOLOMICS

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Acute kidney injury (AKI) is a clinical state related to different renal disturbances that has an elevated mortality rate and affects about a half of care unit patients [1]. Irrespective of AKI's aetiology, proximal tubular epithelial cells (PTEC) have been recently proposed as the pathogenic and therapeutic target in AKI [2]. Inflammatory cytokines contribute to PTEC injury leading to the deterioration of renal function and AKI development [3], but the biomolecular mechanism is barely unknown. Taking this into account, metabolomics analysis could play a relevant role in characterizing and establishing the metabolic profile of PTEC surrounded by an environment rich in inflammatory cytokines, thereby helping to understand better the renal alterations leading to AKI. Accordingly, in this study we have developed an *in vitro* simulation of this pathological scenario by exposing human kidney-2 cells (HK-2), an immortalized human PTEC-derived cell line, to inflammatory cytokines in order to evaluate the metabolic cell changes through a non-targeted metabolomics approach. First, different metabolic extraction protocols were tested to obtain the maximum number of molecular features from cells and extracellular fluids of two experimental groups: control and HK-2 treated with cytokines. Then, the different samples were analyzed by using an untargeted metabolomics platform based on Ultra High-Performance Liquid Chromatography (UHPLC)-Mass Spectrometry. Data obtained were analyzed by non-supervised (PCA) and supervised (PLS-DA) multivariate statistical tools. Considering the PLS-DA results, variable importance on projection values (VIP), metabolites revealing significant differences between experimental groups were selected for further identification because they could be considered as potential inflammatory biomarkers.

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# OY-2
# APPLICATION OF A NOVEL CE-MS METABOLOMICS WORKFLOW IN ZEBRAFISH LARVAE REVEALS NEW EFFECTS OF CORTISOL

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In response to stress, a cascade of hormones can be triggered, resulting in physiological changes through a variety of metabolic pathways. Cortisol is the primary stress hormone in humans and zebrafish and is responsible for several post-stress metabolic changes aimed at restoring homeostasis. Cortisol is mediated by two nuclear receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). These receptors have different affinities for cortisol, with the GR activated at elevated cortisol levels and the MR activated at basal cortisol levels. While the role of cortisol-GR signaling during stress is well characterized [1], the role of the MR in mediating the effects of cortisol is less well-known.

The zebrafish (*Danio rerio*) is increasingly used as an animal model for metabolic disorders in biomedical research [2]. Therefore, we developed a workflow for profiling polar ionogenic metabolites in zebrafish larvae by capillary electrophoresis coupled to mass spectrometry (CE-MS). During method development, special attention was paid to the selection of a suitable homogenization and an efficient metabolite extraction strategy. The workflow was applied in an untargeted metabolomics study in extracts from individual zebrafish larvae and small pooled groups of wild-type and ubiquitous MR knockout zebrafish larvae exposed to vehicle (WT-V/MRKO-V) or exogenous cortisol treatment (WT-F/MRKO-F).

As a result, we observed that the area of the signals obtained from extracts of pools containing 1, 2, 4, 8, 12, 16, 20, and 40 zebrafish larvae showed a linearity with $R^2>0.98$. We performed three biological replicates for six selected endogenous metabolites during three days and obtained RSD values of peak areas between 9.4-17.7%. Furthermore, the method allowed the analysis of 61 endogenous metabolites in a pool of 12 zebrafish larvae and 29 endogenous metabolites in an extract from a single zebrafish larva. In addition, the present study observed the ability of the GR to modulate metabolism independently of the MR, as well as the cooperation of both receptors in maintaining the metabolomic balance. Likewise, we identified differential regulation of cystathionine by MR. All in all, this technique has the potential to identify novel pathways and mechanisms of action in zebrafish larvae.

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## OY-3

# SIMULTANEOUS DETERMINATION OF PYRETHROID, ORGANOPHOSPHATE, CARBAMATE, TRIAZOLE AND NEONICOTINOID PESTICIDES IN URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Pesticides are non-biogenic chemicals designed to kill pests. These compounds are commonly used in agriculture as well as in domestic and gardening applications. Their activity is not highly selective being also toxic for other non-target species, including humans [1]. After exposure, the human body typically metabolize and excrete pesticides via urine within 4-48 hours, depending on the compound. Pesticide exposure levels can be assessed and monitored over time by quantifying specific or general metabolites present in urine. While organophosphate and pyrethroid pesticides have been generally monitored [2], other groups such as those of the carbamate, triazole and neonicotinoid families have received lower attention.

In the present study, an analytical methodology for the quantification of organophosphate and pyrethroid pesticide metabolites and these new fungicides and insecticides in human urine has been developed. The new compounds encompass carbofuran, tebuconazole, acetamiprid, imidacloprid, clothianidin, thiamethoxam, dinotefuran and some of their metabolites, such as 3-hydroxy-2,2-dimethyl-3H-1-benzofuran-7-yl, 5-(4-chlorophenyl)-2,2-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)-1,3-pentanediol, N-(6-chloro-3-pyridylmethyl)-N'-cyano-acetamidine, 5-hydroxy-imidacloprid, clothianidin-desmethyl, thiamethoxam-desmethyl, and dinotefuran-desmethyl.

The chromatographic separation of the compounds was performed on a Betasil C18 column (100 mm × 2.1 mm, 3 µm particle size). Acetonitrile and a mixture of HPLC H2O with 5% methanol and 1% acetic acid were used as a mobile-phases. The instrumental determination is performed by liquid chromatography coupled to tandem mass spectrometry.

The optimized methodology has been tested in the analysis of urine samples from 7-year-old children of the REPRO_PL birth cohort. Preliminary analyses showed that hydroxytebuconazole and acetamiprid-N-desmethyl were detected in 100% and 89% of the samples, respectively, at median concentrations of 0.39 ng/ml and 0.58 ng/ml, respectively. Carbofuran, a pesticide currently forbidden in Europe, has been detected in around 20% of the samples analyzed. Further results are expected to be completed in order to estimate the overall exposure and risk assessment of these neurotoxic compounds in children.

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## OY-4

# ANALYSIS OF PROSTATE SPECIFIC ANTIGEN BY CAPILLARY ELECTROPHORESIS. USE OF NATURAL DEEP EUTECTIC SOLVENTS IN SAMPLE PREPARATION AND SEPARATION STEPS

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In order to search for specific biomarkers of prostate cancer (PCa), the use of proportions of proteoforms of prostate-specific antigen (PSA) in urine analyzed by capillary electrophoresis (CE) is being investigated by several groups, including ours. On the other hand, we are also interested in promoting Green Analytical Chemistry with approaches such as the use of natural deep eutectic solvents (NADES).

In this study analysis of PSA by capillary electrophoresis (CE) in the free zone (CZE) [1] and in the SDS (CE-SDS) modes was performed. CE-SDS methods were developed and employed for the analysis of PSA under reducing and non-reducing conditions. For both, CZE and CE-SDS, modes, compatibility of these analyses with the presence of synthetic urine and of a NADES used as a sample preservative in the PSA sample was studied. In addition, the use of the NADES as background electrolyte additive or as capillary wall coating to increase the resolution of the CZE analysis was evaluated. On the other hand, extraction of PSA from either water or synthetic urine employing an aqueous biphasic system (ABS) with the NADES and dipotassium hydrogenphosphate as salt was evaluated.

Results indicated that the presence of urine or NADES precluded CZE separation of PSA isoforms, and that NADES, either used as a capillary modifier or as an additive to the CZE separation buffer, did not improve the separation of isoforms. The CE-SDS analysis were compatible with the presence of synthetic urine or NADES in the PSA sample and showed size variants that could correspond to “nicked” forms of PSA, potential markers of PCa [2]. The synthetic urine matrix influenced the recovery yield (RY%), evaluated by CE-SDS, of PSA in the NADES-rich phase of the ABS; RY% was three times lower in synthetic urine than in water. The percentage of extraction efficiency (EE%) in the NADES-rich phase of the ABS was 100% for both matrixes [3].

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# OY-5

# EVALUATION OF A FEED ADDITIVE RICH IN POLYPHENOLIC COMPOUNDS FROM WHITE GRAPE MARC FOR FATTENING SWINE: UNTARGET APPROACH USING MSPD-UHPLC-QTOF

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To meet the growing food demand of more than 26 billion animals for human consumption[1], approximately 6 MT of feed is required, representing 32% of the world's cereal production and occupying two-thirds of the global agricultural area [2]. To address this issue, this study focuses on the improved integration of livestock into the circular bioeconomy by increasing the proportion of by-products or residues in animal diets. In response to this need, the effect of adding a polyphenol-rich bioactive extract derived from white grape marc, "e-Vitis", to the feed of fattening swine was evaluated. A 0.1% of e-Vitis was included in the feed and the main bioactive compounds were monitored along the digestive tract of the pigs. For this purpose, an innovative application of the matrix solid phase dispersion technique (MSPD) was used on samples of gastric contents, duodenum, jejunum, ileum, caecum, colon, faeces, and urine, which allowed a rapid and selective methodology for the identification of the main bioactive compounds. Using high-resolution QTOF (Quadrupole Time-of-Flight) spectrometry and metabolomics tools, two groups of swine (with and without enriched feed) were distinguished, which showed a differentiating effect of the extract. It was observed that the extract-enriched feed had a bioamplifying effect, due to the capacity of the main polyphenols characteristic of Albariño grape marc (gallic acid, catechin, epicatechin), to encapsulate the bile acids present in the gastric contents, such as taurodeoxycholic acid (TDCA) and glycochenodeoxycholic acid (GCDCA). This polyphenolic envelope prevented the degradation and complete digestion of these acids, which are linked to the reduction of cellular oxidative stress, prevention of cardiovascular disease, liver protection and antiviral effect, thus allowing their transport along the digestive tract. These results highlight the potential of using agricultural by-products to improve feed efficiency and animal health in livestock production.

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# OY-6

# USE OF GC-MS/MS FOR THE ANALYSIS OF PAH, METHYL-PAH, NITRO-PAH AND OXY-PAH IN NANOPARTICLES FROM THE TRANSPORT SECTOR

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Although the EU directives have been sharpened over the last decades, air pollution in European cities is still a threat to its population. With the applied regulations, many studies have described changes in airborne particles sizes and chemical composition over the years [1]. These two factors are strongly associated with the health effects of the atmospheric particulate material causing different impacts on cardiovascular and respiratory systems [2]. The ultrafine particles (<100 nm) are of particular concern as they can translocate from lungs into the blood circulation leading to more acute effects [3]. In urban environments, a major fraction of these ultrafine particles is generated by the transport sector either from road, rail, air, or sea transportation.

Many organic compounds originate from transport sources, being the polycyclic aromatic hydrocarbons (PAH) the most studied due to their known toxicity. The reaction of these compounds with the radicals present in the atmosphere can give rise to even more toxic compounds, since these have direct effects on the organism, while the original compounds (PAHs) require enzyme activation. These formed compounds, such as nitrated (NPAH) and oxygenated (OPAH) PAHs, are usually analysed by GC-NICI-MS but require additional analysis and not much data on them is available in the literature.

In the present study we are describing a method for the simultaneous analysis of PAH, NPAH, OPAH and methyl-PAH from ultrafine particles of the transport sector by GC–MS/MS using an Agilent 7000 Series Triple Quad equipped with a HP-5MS 60 m capillary column. The instrument operated under electron impact ionization and multiple reaction monitoring (MRM) mode was used for acquisition. The method was applied to 58 targeted compounds with instrumental limits of detection ranging between 0.04 and 1.74 pg/μL injected. Other polar compounds (saccharides and secondary organic acids and polyols) were also analysed by GC-MS to perform a source apportionment study.

In the present study, sub 100 nm particles were collected with state-of-the-art instruments near the airport, harbour, and arterial roads in the city of Barcelona in different seasons to analyse the organic composition of nanoparticles emitted by these transport modes.

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## OY-7

# Perfluoroalkyl substances in striped dolphins (Stenella coeruleoalba) from the NW Mediterranean Sea: Biomagnification and temporal trends (1990-2021)

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Poly- and Perfluoroalkyl Substances (PFAS) are widely recognized as a class of pollutants known for their ability to bioaccumulate and biomagnify in the environment. In this study, the objective is to determine the biomagnification rates of PFAS in sexually mature striped dolphins and assess the temporal trends of PFAS concentrations over the past three decades (1990-2021) in the North-Western Mediterranean Sea. Thirteen out of the 19 targeted PFAS compounds were detected in the digestive content of the dolphins, while all 17 PFAS compounds were detected in the liver samples. Concentrations of PFAS in the digestive content ranged from 50 to 1611 ng/g, while the liver samples showed concentrations ranging from 268 to 7014 ng/g. The most prevalent compounds found in both types of samples were linear perfluorooctanesulfonic acid (n-PFOS) and perfluorooctanesulfonamide (FOSA), which were present in all samples. Additionally, perfluoroundecanoic acid (PFUnDA), perfluorotridecanoic acid (PFTrDA), and perfluorononanoic acid (PFNA) were also detected in significant quantities. Interestingly, the study found that long-chain PFAS compounds exhibited greater biomagnification rates compared to short-chain PFAS compounds in striped dolphins. This suggests a potential impact on the health of these marine mammals due to the accumulation of long-chain PFAS in their systems. The analysis of the samples indicated that half of the digestive content samples exceeded the Environmental Quality Standards (EQS) concentrations, indicating that the consumption of polluted prey may pose health risks for striped dolphins. When examining the temporal trends, the concentrations of most long-chain PFAS compounds showed an increase from 1990 to 2004-2009. However, concentrations appeared to stabilize during the period of 2014-2021, potentially reflecting the impact of country regulations and industry initiatives aimed at reducing PFAS pollution. These findings underscore the persistent presence of banned PFAS compounds in the marine ecosystem and highlight the need for ecological risk assessments and the development of management strategies to mitigate PFAS pollution in marine environments. In conclusion, this study provides valuable insights into the biomagnification rates of PFAS in striped dolphins and highlights the persistent nature of these pollutants in the North-Western Mediterranean Sea. The identification of specific PFAS compounds and their temporal trends contribute to the understanding of PFAS pollution and support future efforts in assessing ecological risks and implementing effective management strategies in marine ecosystems.

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# OY-8

# Development of a Rapid Direct-Injection Method for the Determination of 80 Pharmaceuticals in River Water by Liquid Chromatography-Tandem Mass Spectrometry

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Pharmaceuticals residues are classified as Emerging contaminants (ECs) becoming a huge threat for the population and the environment [1]. Pharmaceuticals administrated at home or in pharmacies are excreted by faeces and urine after consumption, and wastewater treatment plants (WWTPs) are not able to remove all pharmaceuticals residues that eventually will end up in the aquatic media (rivers and sea) [2]. For this reason, analytical techniques must be developed and implemented for the monitoring of these ECs.

For the last decade, extraction techniques such as solid phase extraction (SPE) have become very popular among scientific community and the injection to standard LC-MS chromatography has been giving good results for the identification and quantification of pharmaceuticals [3]. However, analytical instruments are becoming more and more sensitive and sometimes extraction procedures can be avoided. For these reasons, direct injection methods are prominent which simply consist of injecting directly the water matrix into LC instruments [4]. Direct injection shows great advantages. It eliminates the extraction step which is time and cost consuming. Also, no solvents are needed, the total waste is reduced, and human errors are less likely to occur.

In this work, a direct injection method was developed for the monitoring of 80 pharmaceuticals in river water using Liquid Chromatography-Tandem Mass Spectrometry (SCIEX Q-TRAP 7500 ®), working in MRM mode and ESI+/- ionization respectively. Quality assurance was performed for all compounds and high sensitivity of the instrument allowed to arrive at very low concentrations (1 ng L-1 for most of the compounds). Furthermore, recoveries and matrix effect were very consistent, and results were obtained with just the injection of 100 µL of river water previously filtered.

Finally, samples were analysed from river water, wastewater and reclaimed water from two main Catalan rivers (Llobregat and Besòs).

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## OY-9

# PLANAR MIXED-MODE CATIONIC EXCHANGE SORPTIVE PHASES AND AMBIENT IONIZATION MASS SPECTROMETRY: A NOVEL COMBINATION FOR THE ANALYSIS OF BIOFLUIDS

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A planar sorptive tape (ST) based on mixed-mode cationic exchange (MCX) polymeric particles is proposed as a sorbent in thin film microextraction [1]. These particles have been widely reported in solid phase extraction (SPE) and they involve a double interaction mechanism by hydrophobic and cation exchange interactions [2,3]. The preparation of the materials is simple and consists of immersing a segment of aluminum foil covered with a double-side adhesive tape in a vial containing the microparticles. The preparation of materials is affordable, rapid, and the extraction unit is disposable and cheap. Moreover, the analytical workflow allows the simultaneous extraction of the samples, which is crucial when rapid decision-making is needed in clinical/toxicological analysis.

In a first approach, these sorptive phases have been used to isolate and quantify two basic compounds (methadone and tramadol) in saliva samples by direct infusion mass spectrometry (DI-MS). The method has demonstrated its applicability in biofluids collected from patients under opioids treatment [4]. To extend the application field, its potential for the extraction of four basic drugs of abuse (cocaine, codeine, methadone, and methamphetamine) from saliva samples was attempted. Different variables related to the extraction procedure have been evaluated (ionic strength, agitation rate, extraction time, and dilution factor), some of them using a multivariate approach. Two different analysis workflows have been studied. Firstly, the tapes followed the conventional solvent elution, and the resulting eluates were analyzed by DI-MS. Secondly, the direct ambient analysis of the tapes by aluminum foil electrospray-ionization mass spectrometry (Al-foil ESI-MS) was tested with satisfactory results.

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## OY-10

# METHOD DEVELOPMENT TO THE DETERMINATION OF GADOLINIUM CONTRAST AGENTS IN SOIL SAMPLES

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Gadolinium-based contrast agents (GBCA's) are frequently used in magnetic resonance imaging (MRI) to enhance the contrast of the images obtained. These compounds reach the wastewater treatment plants, where they are not eliminated, to be finally released into the environment. These compounds are nowadays of environmental concern and are usually analyzed using LC-ICP-MS coupling techniques [1,2].

In the framework of a research project to reuse wastewater, samples of irrigated fields where used to develop a method to the determination of several GBCAs in soils. The separation method uses an anion exchange column with a mobile phase based on $NH_4NO_3$ at pH 9.2. The selected agents, Gadoteridol, Gadobutrol and Dotarem, were separated in less than 2 minutes at a flow rate of 1 mL min$^{-1}$.

Preliminary experiments were conducted with aqueous standards, the fortified extract from a blank soil and the fortified extraction solution. The results showed the absence of matrix effect in both signal and retention time, although a small portion of the compounds was retained in the soil.

Afterwards, recovery assays were performed using fortified soil. To this end, a blank soil sample was suitably spiked at 2.5 ng g$^{-1}$. The grinded and sieved (fraction below 0,5 mm) soil were completely wetted with the spiking solution, homogenized and left to dry during 48 hours. 1 g of fortified dry soil was mixed with 10 mL of extractant solution and stirred for different times. The present study shows the results obtained with the different extraction solution assayed. All of them consisted of a 5 mM $NH_4NO_3$ at different pH values, namely 0.45, 5.5, 9.2 and 12.

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## OY-11

# Application of homemade silica-based zwitterionic ion-exchange materials for the extraction of pharmaceuticals from environmental water samples

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The use of sorptive techniques is the preferred way to extract analytes from liquid samples, being the solid-phase extraction the common technique. The development of new materials that can be applied to solid-phase extraction is one important research area [1]. One problem is the extraction of ionic and ionizable compounds, there are commercial solutions that allow the extraction of cationic or anionic analytes (Oasis MCX, Strata X-AW...), however, the simultaneous extraction of both type of analytes is not possible with commercial sorbents.

In this sense, in the present study, three homemade silica-based zwitterionic ion-exchange materials were synthesized through sol-gel reactions. After the functionalization, the sorbents had quaternary amines and sulfonic groups, allowing them to perform strong anion and cation-exchange interactions.

The three sorbents were evaluated for the SPE of acidic and basic pharmaceuticals at different pH. The best performing sorbent was the one functionalized with 2-(methacryloxy) ethyl dimethyl-3 (sulfopropyl) ammonium hydroxide. This sorbent was selected and its SPE method was optimized in terms of pH, loading volume and elution conditions, being the optimal conditions pH 5, a variable volume depending on the matrix and 5 mL of 1% $NH_4OH$ in MeOH.

The optimized method was applied for the extraction of the pharmaceuticals from river, effluent wastewater and influent wastewater samples. The method was validated in terms of apparent recovery, matrix effect, intra-day and inter-day precision and detection and quantification limits.

The pharmaceuticals were quantified in several samples of each matrix, ranging the concentration of the compounds from <MDL to 401 ng/L in river samples, from <MDL to 2938 ng/L in effluent wastewater samples and from <MQL to 9542 ng/L in influent wastewater samples.

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## OY-12

# ANALYSIS OF SARS-CoV-2 NUCLEOCAPSID PROTEIN BY ON-LINE APTAMER AFFINITY SOLID-PHASE EXTRACTION CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus that causes an infectious respiratory disease called coronavirus disease 2019 (COVID-19), which has originated a major health crisis on a global scale in recent years [1]. The nucleocapsid protein (N protein, relative molecular mass ~51,000) is one of the most abundant structural proteins in SARS-CoV-2. Despite it is an immunodominant antigen in host immune responses that can be used as a good diagnostic biomarker [2], more information on this protein is needed to better understand the mechanisms of the disease, as well as for designing novel vaccines and drugs for COVID-19 prevention and treatment. In this work, an aptamer affinity sorbent was prepared for clean-up, preconcentration, separation, and characterization of N protein by on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry (AA-SPE-CE-MS) [3]. AA-SPE microcartridges were packed with a sorbent based on magnetic bead particles modified with an aptamer against the N protein. After a very challenging optimization of several parameters of the AA-SPE-CE-MS method, which needed the use of lab-made hydroxypropyl cellulose (HPC) coated capillaries to prevent protein adsorption on the inner capillary wall, the sample was loaded in separation background electrolyte (BGE, ammonium acetate 10 mM, pH 7.0), while the retained protein was eluted with acetic acid 1 M, pH 2.3. The developed method with N protein standards was repeatable in terms of migration times and peak areas, satisfactorily linear between 2.5 and 25 $mg \cdot L^{-1}$, and the limit of detection (LOD) was 0.5 $mg \cdot L^{-1}$, leading to a sensitivity enhancement of 500 times compared to CE-MS. The AA-SPE-CE-MS method was applied to the analysis of N protein in human saliva, pointing out its great potential for the development of accurate and reliable SARS-CoV-2 complementary analytical methods.

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## OY-13

# ANALYSIS OF AUTOANTIGENIC PEPTIDES AND LIPIDS FROM PHOSPHATIDYLSERINE-LIPOSOMES FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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In patients suffering from autoimmune diseases there is a failure in the peripherical tolerance recognition that results in an undesirable damage of healthy cells and tissues. Currently, only palliative treatments based on anti-inflammatories and immunosuppressors are available. Ahead Therapeutics is developing a new approach based on the encapsulation of the autoantigen responsible for generating the autoimmunity in phosphatidylserine-liposomes (PS-liposomes) to generate peripheric tolerance to this antigen. This is plausible taking advantage of the physiologic mechanism of tolerance generation by bio-mimicking apoptotic cells with PS-liposomes [1]. Using this strategy, the immune system stops attacking its own cells and tissues. Only by changing the autoantigen encapsulated in the liposomes, different autoimmune diseases can be treated. The efficacy of this approach has been demonstrated in six different autoimmune diseases: type I diabetes, multiple sclerosis, celiac disease, rheumatoid arthritis, myasthenia gravis, and optical neuromyelitis ([2] and unpublished data).

In order to progress in the pre-clinical phase and move towards the commercialization of this novel product, developing analytical methodologies to characterize its main quality attributes is mandatory. In this regard, liquid chromatography becomes an essential tool for the identification and quantification of both autoantigenic peptides and lipids from PS-liposomes [3]. Two different HPLC methods were developed in this study, one by HPLC-UV and another by HPLC-ELSD. A liquid-liquid extraction procedure was first applied to the PS-liposomes formulation to extract and separate the autoantigenic peptide and lipids into two different phases. The organic phase, which contains the lipids was analyzed by HPLC-ELSD, while the aqueous phase, containing the encapsulated peptide, was analyzed by HPLC-UV. Both methodologies allowed a satisfactory quantification without interferences between them. Finally, methods were validated in terms of accuracy, precision, linearity, LOD and LOQ.

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# POSTER COMMUNICATIONS

# P1

# USING UNCOMMON UHPLC CONDITIONS FOR INCREASING CHROMATOGRAPHIC SEPARATION OF ISOMERIC BILE ACIDS

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In the last years, and especially when working with mass spectrometry instruments, including tandem (MS/MS) and high-resolution (HRMS), ultra-high performance liquid chromatography (UHPLC) systems have become the most usual liquid chromatographic separations. UHPLC is referred to chromatographic separations performed using analytical columns with polymeric particles with <2 µm size. When using these columns, and based on Van Deemter equation, the chromatographic resolution is not decreased in spite of increasing mobile phase flow, obtaining faster separations and reducing analysis time. Thus, common UHPLC-MS/MS set-ups include high mobile phase flow and high temperatures for reducing system backpressure. Shorter chromatographic runs are obtained by both the higher mobile phase flow and higher column temperature, as at high temperatures the partition of the compounds is increased to the mobile phase, reducing the interaction with stationary phase and reducing retention times.

Nevertheless, in some specific cases, these common UHPLC conditions could reduce chromatographic performance compromising the adequate separation of isomeric compounds. In this study we optimized the chromatographic separation of 10 bile acids (Cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, ursodeoxycholic acid, α-muricholic acid, β-muricholic acid, hyocholic acid, hyodeoxycholic acid, and murideoxycholic acid) for their determination in mice faeces by UHPLC-MS/MS. Among the studied compounds, there were different isomeric bile acids with shared MS/MS transitions, that must be then chromatographically resolved. The aim was to develop a robust method with isomer separation using UHPLC a standard mobile phase consisting on water and methanol (both with 0.01% formic acid and 1 mM ammonium formate), and an C18 analytical column (Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm). In spite of the used gradient, α-muricholic acid and β-muricholic acid were not resolved at 10% of baseline using the UHPLC standard conditions of 0.3 mL min$^{-1}$ of flow rate, and maintaining the column at 55 ºC. For increasing the affinity of compounds in stationary phase, and thus their retention, column temperature was decreased to 30 ºC. This change produces that the partition of the compounds is favoured to the stationary phase, increasing the different interaction with stationary phase between α-muricholic acid and β-muricholic acid, and obtaining a complete chromatographic separation. On this way, the method was then optimized for the remaining bile acids, and it was applied for the quantification of these compounds in mice faeces.

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## P2

# SINGLE AND DUAL SYSTEMS OF CYCLODEXTRINS/AMINO ACID-BASED IONIC LIQUIDS FOR THE ENANTIOMERIC SEPARATION OF KETOROLAC BY ELECTROKINETIC CHROMATOGRAPHY

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The determination of chiral drugs is of vital importance in the bioanalytical and pharmaceutical fields due to the different activity that the enantiomers of a chiral compound can show in chiral environments. This fact makes imperative the development of methodologies for the enantiomeric separation of these drugs. In Electrokinetic Chromatography (EKC), the main mode of Capillary Electrophoresis to carry out chiral separations, the use of versatile and low-cost chiral selectors is gaining attention. In this context, amino acids-based ionic liquids (AAILs) could be excellent chiral selectors or additives in dual systems with cyclodextrins (CDs).

The present research work deals with the development of new analytical methodologies enabling the enantiomeric separation of ketorolac (an anti-inflammatory drug) by EKC. With this aim, the discrimination power of chiral single systems (at pH 3.0 and 7.0), based on the use of six neutral CDs or six AAILs (made up of different protein (L-Arginine) or non-protein (L-Citrulline, L-Homoarginine) amino acids as anionic counterparts, and tetramethylammonium ($TMA^+$) or tetrabutylammonium ($TBA^+$) as cationic counterparts), was studied. Among all these systems, only β-CD, γ-CD, and heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD) allowed to obtain the enantiomeric separation of ketorolac. Then, the combination of these three CDs with the AAILs in dual systems was also evaluated. Since changes in the electroosmotic flow (EOF) direction were observed in these dual systems, the effect of the addition of increasing concentrations of TMA-OH or TBA-OH on the EOF migration was investigated. Then, the reversal in the EOF migration could be attributed to the adsorption of these cations onto the inner capillary wall. From all the results obtained, two different methodologies based on a single (TM-β-CD at pH 7.0) and a dual system (TM-β-CD plus TMA-OH at pH 3.0) were optimized by using two designs of experiments to obtain the most appropriate experimental conditions to achieve the chiral separation of ketorolac. To demonstrate the suitability of the developed methodologies, their analytical characteristics were evaluated, and they were subsequently applied to the determination of ketorolac in a pharmaceutical formulation.

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## P3

# HILIC-MS and CE-MS-based characterization of the metabolic profile of human plasma: a comparative study

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Metabolomics systematically measures metabolites in a biological sample at a defined state and time point. The metabolome covers a wide range of different metabolites, many of which are still unknown [1]. Until now, most metabolomics analyses have been performed by reverse-phase liquid chromatography coupled to mass spectrometry (RPLC-MS), which omits much of the polar fraction of the metabolome [2]. Hydrophilic interaction chromatography (HILIC) is a type of LC used in recent years for separating polar compounds, and it could even improve the coverage of polar compounds in metabolomic studies compared to capillary electrophoresis coupled to MS (CE-MS).

Human plasma is one of the least invasive biological samples with valuable metabolic information since it contains a wide variety of low molecular weight compounds, which can provide information in a metabolic study of the individual's health status, disease evolution, and effect of the different treatments.

In this study, human plasma was characterized using both HILIC-MS and CE-MS to compare the capabilities of both techniques. For HILIC, the identification of the metabolites was performed using standard mixtures and MS/MS analysis, allowing the generation of a database of polar metabolites in plasma. Additionally, CE-MS was applied in normal and reverse polarity, using standard mixtures, in-source fragmentation, and the CE-MS database "CEU Mass Mediator" for metabolite identification [3].

We obtained in HILIC-MS a metabolic profile of 258 metabolites in positive and 345 metabolites in negative ionization modes. HILIC exhibited a different selectivity in the separation of compounds compared to CE-MS, providing complementary information for the analysis of polar compounds. HILIC extracted more metabolites from different classes, such as alcohols, ketones, carbohydrates, fatty acids, and organic acids. At the same time, more amino acids, peptides, purines, pyrimidines, and pyridines were detected by CE-MS.

In conclusion, our comparison allowed us to specify the strengths of each technique and evaluate which one is better to use according to the objective of the study. Both provide valuable and unique information and may become complementary techniques in the future.

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## P4

# CHIRALOMICS: A VALIDATED METHODOLOGY FOR UNVEILING THE ROLE OF D- AND L-AMINO ACIDS IN HEALTH AND DISEASE

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Chirality, the property of being non superposable in its mirror image, is a key feature in molecular recognition, enzyme catalysis, and signal transduction. Amino acids (AAs), the structural elements of proteins, are chiral biomolecules. However, the chiral analysis of AAs in complex biological matrices poses numerous analytical challenges, and chiral analysis has been scarcely performed in faeces. Therefore, we propose a reliable methodology that combines two analytical methods for the absolute quantification of 17 pairs of D and L AAs in lyophilised faeces using RP-LC/QqQ-MS in positive ionization mode after chemical derivatization with (S)-NIFE (N-(4-Nitrophenoxycarbonyl)-L-phenylalanine 2-methoxyethyl ester) based on a previously validated method [1]. The methods were optimised and validated according to the EMEA guidelines for selectivity, sensitivity, intra- and interday precision and accuracy, matrix effect, and stability [2].

To our knowledge, it is the first time that absolute concentrations of D-glutamic and D-aspartic acids have been determined in faeces. Furthermore, the method met all the specifications, reporting limits of 2 ppb for all the species studied. The methodology was later applied to the study of Anorexia nervosa and matching healthy controls as a proof of concept. Our findings indicate a decrease in total AA in patients, possibly due to the metabolic shift in AN toward protein catabolism during starvation. Plasma AAs may be used as energy substrates, leading to reduced concentrations in faeces, potentially inducing dysbiosis. This is supported by the negative correlation found between plasma/feces AAs in our patients. Furthermore, major disturbances were found in absolute and relative concentrations of D/L-AAs. Given the role of D-serine, D-alanine, D-aspartate, or D/L-glutamate in glutamatergic transmission through NMDA receptors, modified abundances in patients suggest alterations in neuromodulation, as previously reported in other psychiatric disorders.

In general, this method allows for accurate quantification of D and L AAs, improving the understanding of their impact on health and disease.

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## P5

# INTERACTIVE WEB APPLICATIONS AS A TOOL FOR ROUTINE QUANTIFICATION OF CHEMICAL POLLUTANTS IN HUMAN AND ENVIRONMENTAL SAMPLES

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Interactive web applications are useful, flexible and functional tools that allow the user to explore data while manipulating inputs and a diversity of parameters. Web applications have been used in a diversity of research topics, including analytical chemistry. We propose the use of interactive web applications for the automatization of the quantification process in the analysis of large number of human and environmental samples. Quantification after liquid or gas chromatography and/or mass spectrometry techniques are usually performed using company softwares (*e.g.* Agilent®, Waters®, etc.), although users are, in many occasions, manipulating manually the data using spreadsheets (*e.g.* Excel®), hence increasing calculation errors while handling the data.

We have developed two interactive web applications using shinyapp, a package for the R programming environment that makes possible to develop and run web-apps within an active R session or remotely [1]. The first shinyapp, created for the analysis of pesticide metabolites in urine after Liquid Chromatography coupled to Tandem Mass Spectrometry (HPLC-MS/MS), allowed the aggregation and quantification of 14 compounds into a single database, after automatizing the intermediate steps (*e.g*. blank and QCs determinations, Ion Ratios and posterior calculations after the optimization of the calibration curve). The second shinyapp has been created for the analysis of >35 persistent organic pollutants in serum samples after Gas Chromatography with Electron Capture Detector (GC-ECD). This shinyapp incorporated also the calculations for recovery percentages and detection and quantification limits.

The two shinyapps have been used for the analysis of >700 human samples from European Birth Cohorts: the REPRO_PL birth cohort in Poland (n=400 urine samples) and the NFBC birth cohort in Finland (n=300 serum samples) [2-3]. The use of these shinyapps have allowed to automatize the quantification process reducing considerably the timelines for this kind of human biomonitoring studies. The shinyapps are currently available in GitHub Gist [4].

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# P6

# Determination of plastic additives and other micropollutants in laundry greywater facilities intended for water recycling

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Laundry greywater may contain a wide variety of contaminants, such as softeners, plasticizers, emulsifiers, preservatives, surfactants, phthalates, pharmaceuticals and microplastics, along with many other emerging and priority contaminants [1]. However, due to the complexity of the laundry greywater matrix and the lack of analytical methodologies, there is a very limited knowledge on plastic additives and other emerging micropollutants. In this work, plastic additives and other micropollutants, such as pharmaceuticals and endocrine-disrupting compounds (EDCs) were analyzed in greywater of laundries of different size, clients, and services, located in Spain. Selected plastic additives, pharmaceuticals and EDCs were extracted from laundry greywater using solid-phase extraction and were analyzed using UHPLC-MS/MS [2,3]. Additionally, the phthalate DEHP was analyzed by a novel analytical methodology based on on-line headspace solid-phase microextraction coupled to gas chromatography tandem mass spectrometry (HS-SPME-GC-MS/MS).

Acceptable recovery results (40-120%) were observed for all the selected contaminants. Method detection limits (MDLs) of pharmaceuticals ranged from 0.9 to 350 ng/L, while MDLs of EDCs varied from 0.3 to 141 ng/L. In the case of DEHP, MDL was established at 15.5 ng/L.

This study is anticipated to pave the way for the development of rapid screening methods for emerging organic contaminants in laundry greywater, thereby creating new opportunities for efficient monitoring and analysis.

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## P7

# Application robustness of the 6495 triple quadrupole LC/MS system for non-stop Pesticide analysis in black tea matrix

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System robustness is of utmost importance especially when analyzing samples for routine, in-production, type of analysis. Additionally, when evaluating samples for meaningful scientific results, analysis of a large population of samples is necessary for good population statistics. The new 6495 triple quadrupole LC/MS system (G6495D) is equipped with VacShield and iFunnel technology that aims to provide high sensitivity and high-performance analysis while being robust and rugged enough to withstand the effects of deposition from a complex and dirty matrix.

- VacShield –ion injector capillary removal mechanism that enables quick routine-maintenance, reduces downtime, and preserves system stability.
- iFunnel Technology – a dual-staged stacked ring ion funnel used to compress and concentrate the ion beam. Innovations within the iFunnel evacuate matrix components while maintaining injection-to-injection MRM precision.
- Instrument Intelligence – built into the overall system architecture to monitor and ensure that the instrument is in good operating condition.

Compared to non-iFunnel systems, the 6495 LC/TQ provides about 10x improvement in signal while providing superior injection-to-injection measurement robustness and precision at sub-millisecond dwell times.

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# P8

# MULTI INSTRUMENTAL APPROACH FOR THE ANALYSIS OF PESTICIDES AND THEIR METABOLITES IN LIVER CELLS TO ASSESS CYTOTOXICITY AND TRIGLYCERIDE INDUCTION

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Pesticides are man-made chemicals destined to kill insects and other plagues. Humans are exposed to pesticides in their daily life, and those exposures might have multiple detrimental effects on their health [1], including the liver [2], the organ where pesticides are metabolized in order to be excreted via urine [3].

An analytical study was conducted on a set of 105 samples encompassing 21 compounds: 9 pesticides, one herbicide (atrazine) and their metabolites as well as bisphenol A. The pesticides included pyrethroids (cypermethrin and cyfluthrin, together with their metabolites, 3-phenoxybenzoic acid and 4-fluoro-3-phenoxybenzoic acid), organophosphates (diazinon, pirimiphos-methyl, coumaphos and chlorpyriphos, and their metabolites, 2-isopropyl-6-methyl-4-pyrimidol –IMPY-, 2-diethylamino-6-methyl pyrimidin-4-ol, 3-chloro-4-methyl-7-hydroxycoumarin and 3,5,6-trichloro-2-pyridinol), as well as vinclozolin (and its metabolite, 3,5-dichloroaniline), atrazine (atrazine-desethyl), carbofuran (3-hydroxy-carbofuran and 3-ketocarbofuran) and propiconazole. Hepatic cellular cultures using HepaRG cells were exposed to parent pesticides and their metabolites and incubated up to 48 hours. Supernatant samples were collected at different incubation times: 1h, 5h, 10h, 24h, and 48 h.

The studied compounds were analyzed using different instrumental techniques. Most of the compounds were firstly analyzed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), and afterwards confirmed by LC-MS/MS. Depending on their physical-chemical properties, some compounds such as chlorpyrifos, cypermethrin, cyfluthrin and vinclozolin were liquid-liquid extracted and analyzed by gas chromatography with an electron capture detector (GC-ECD). A comparison between the different analytical techniques for some compounds has been carried out by calculating the 25% uncertainty of each analysis.

Except in the case of propiconazole, atrazine, atrazine-desethyl and IMPY, the analyses showed that concentrations decreased over time. For some parent pesticides such as cypermethrin, cyfluthrin and diazinon, cells produced specific metabolites and their concentrations increased until 48h. These trends show a progressive absorption and pesticide metabolization by Hepa RG cells as incubation time increases. The methodological comparison denoted a good concordance between the obtained results by the employed chromatographic techniques.

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## P9

# DETERMINATION OF SARS-CoV-2 RNA IN EXHALED BREATH, INDOOR AND OUTDOOR AIR

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A method to identify and quantify SARS-CoV-2 genetic material in ambient air and human exhaled breath using the ORF1ab, E and N genes of the SARS-CoV-2 genome has been developed. The RNasaP human gene was also analysed for internal control of the extraction process. Air was pumped through PTFE membrane filters of 0.3 µm pore size (flow 4.5 L min$^{-1}$; 4 hours). Bio-VoC devices were used for collection of exhaled breath, both total and alveolar air. After sampling, the filters were introduced into vials containing KaiBiL Viral Transport Medium. RNA was extracted from these solutions using the KingFisher purification system. Once extracted, real-time reverse transcription polymerase chain reaction (PCR) was performed using the LightMix Modular SARS-CoV-2 E-gene. The extraction and PCR replication recoveries were nearly 100%. The viral genetic amounts in the samples were also determined by reverse transcription-PCR quantification (RT-qPCR). These methods were used to analyse diverse general environments and indoor air and exhaled breath in a hospital in which COVID patients were treated.

The indoor and outdoor air not related with hospital installations showed negative results for the three SARS-CoV-2 genes, but positive for RNasaP, indicating that this method successfully detected genetic material. According to these results, the COVID-19 regulations of ventilation and people dispersion in the studied environments were effective.

In the hospital, the highest occurrence of RNA was found in the rooms with COVID-19 patients (mean 2600 c/m$^3$) and the adjacent corridor (mean 4000 c/m$^3$), the latter containing more RNA with statistically significance ($p < 0.01$). The airborne SARS-CoV-2 RNA in the corridors with rooms without COVID-19 patients or care rooms of uninfected patients were ten times lower, 190 c/m3 and 180 c/m3, respectively, without significant differences. The exhaled breath of patients showed positive indicators of the SARS-COV-2 ORF1ab, E and N genes when sampled on the day or one day after hospital admission. Some patients with hospital stay after several days still showed SARS-COV-2 genes in the exhaled breath despite negative results in the nasofaringeal tests.

## P10

# THERMAL CRACKING AND ISOTOPE FRACTIONATION OF HYDROCARBONS: EVIDENCE FOR LIMITS TO PYROLYSIS -COMPOUND-SPECIFIC ISOTOPE ANALYSIS (Py-CSIA)

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Pyrolysis compound specific isotope analysis (Py-CSIA) is a relatively novel hyphenated technique for which no standard materials have been developed. For conventional CSIA (GC-C-IRMS), standard isotopically characterized materials are available and used to cast calibration curves and to validate and correct analytical data. In this work, the suitability of CSIA standard type C-4 (Indiana University, Stable Isotope Laboratory) [1] as standard for Py-CSIA $\delta^{13}$C isotope analysis is tested. The C-4 standard is a mixture of *n*-alkanes containing 5 odd-chain homologous ($C_{17}$ to $C_{25}$) with decreasing concentrations dissolved in hexane.

The C-4 standard (5 µl) was added to an Auto-Rx (F-LAB, Fukushima, Japan) glass fibre disc placed in Eco-Cup SF deactivated steel capsules (F-LAB) and allow the solvent to evaporate for 10 min at 25 °C. Samples were introduced into a micro-furnace pyrolizer (model 2030D; F-LAB) at different temperatures (250, 300, 400, 500 and 600 °C). A C-4 direct injection (DI) was also analysed. The pyrolyzer is attached to a Trace GC Ultra system (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a 30 m capillary column (Agilent J&W HP-5msUI). The chromatographic flux is directed -via a ConFlo IV universal interface- to a GC-Isolink with a combustion micro-reactor set at 1020 °C and to the Delta V Advantage IRMS (Thermo Fisher). Each sample was analysed 10 times. Details of the chromatographic conditions in [2].

At low temperatures (250 °C), $\delta^{13}$C depleted values were obtained for the C-4 components, attributed to incomplete thermal evaporation of the hydrocarbons withj low temperatures favouring evaporation of lighter molecules [3]. At higher temperatures (>500 °C), a small cracking of standard's $C_{23}$ and $C_{25}$ occurs, coincident with small $\delta^{13}$C shifts of lower chain length ($C_{17}$ and $C_{19}$), probably due to additions from the long chain as previously observed in conventional CSIA [4]. However, these changes are assumable and we conclude that the C-4 standard can be used confidently for Py-CSIA at temperatures between 300 and 600 °C.

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## P11

# REMOVAL OF BISPHENOLS FROM ENVIRONMENTAL WATER USING CARVONE AS A SUSTAINABLE SOLVENT

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Water quality is one of the main concerns for the preservation of natural ecosystems and, by extension, human health. However, environmental water is affected by many human and industrial activities that can produce the contamination of water bodies at several levels. Among anthropogenic pollutants of special concern, those presenting endocrine-disrupting properties are of particular interest due to their potential to cause wildlife and human alterations, being bisphenol A (BPA) one of the most relevant, since its concentrations have increased worldwide in environmental waters [1]. On the other hand, nowadays society demands procedures for organic contaminants removal from water bodies to be more environmentally friendly and sustainable, following the principles of Green Chemistry [2]. In this context, the use of sustainable solvents, such as terpenoids, appears as a promising alternative to conventional methodologies for water purifications.

In this work, a method based on liquid-liquid extraction (LLE) of BPA and its main substitutes (i.e., BPB, BPF, BPS, BPZ, BPAF, BHPF, and TMBPF) from environmental waters using carvone as sustainable extraction solvent was developed using a Box-Wilson Central Composite Experimental Design. The most relevant variables in the LLE procedure (carvone-to-water ratio, vortex speed, and time of extraction) were optimized, with extraction efficiency as the response to be maximized. Optimum conditions were found at 1.18 for the carvone-to-water ratio and vortex at 1800 rpm for 5.35 min. This methodology was successfully applied to a real environmental water artificially contaminated at 20 ppm of each bisphenol, with extraction efficiencies always higher than 99.3%. Finally, the reusability of carvone was studied, and even after 10 extraction cycles, there was no loss of extraction efficiency.

In conclusion, due to the good characteristics of carvone as a sustainable solvent, its low environmental impact, high extraction efficiency, and reusability, the developed extraction method can be a good option for the remediation of water contaminated with bisphenols.

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## P12

# COSMO-RS APPROACH TO A GREENER REMEDIATION OF BISPHENOLS POLLUTED WATER

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Bisphenols are compounds that can mimic hormones in the body, leading to endocrine disruption and potential long-term health consequences. They are widely used in the manufacturing industry and can leach into the environment through various sources. Although several processes are being applied to remove bisphenols from water sources (biodegradation, sorption, etc.), the use of eco-friendly liquid-liquid extractions has not yet been adequately explored, due to the large number of potential solvents and the extensive experimental work required. Computational simulation methods, such as those based on the Conductor-like Screening Model for Real Solvents (COSMO-RS), could greatly reduce costs and speed up the selection process of the best solvents for a specific purpose.

Thus, the aim of this study was to apply the COSMO-RS theory to perform an *in silico* screening of the most suitable solvents (among more than 200 terpenoids) for the purification of environmental waters contaminated with bisphenols (BHPF, BPA, BPAF, BPB, BPF, BPS, BPZ, and TMBPF). Factors such as solvent capacity, toxicity, biodegradability, and physicochemical properties were considered in the selection.

The calculated $\sigma$-potentials of these bisphenols showed that they can only act as Hbond donors, therefore those terpenoids capable of acting as Hbond acceptors should be good solvents. This was confirmed computationally, as all compounds with higher solvent capacity values had carbonyl or hydroxyl functional groups. Among these terpenoids, carvone was the best option due to its low melting point (-25 °C), good biodegradability (100% in 4 days), and low ecotoxicity. COSMO-RS was also used to simulate the behavior of the carvone-water interface. The low values obtained for the interfacial tension should promote a fast and efficient bisphenol mass transfer. In fact, the estimated extraction efficiencies in a carvone:water system (1:1) reached nearly 100% for all studied bisphenols.

The obtained results indicate that carvone may be an excellent extractant, capable of remediation of bisphenol-contaminated waters in a rapid, efficient, and environmentally friendly manner. Besides, the COSMO-RS theory has proven to be an effective tool for an *in silico* approach to select the most suitable solvent for a given application.

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## P13

# DEVELOPMENT OF A CHEMOMETRICS-ASSISTED MALDI-TOF-MS METHOD FOR PROTEIN PROFILING AND CLASSIFICATION OF COMMERCIAL QUINOA GRAINS

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Quinoa (Chenopodium quinoa Willd.) is a native grain from the Andes mountains of South America that is gaining a great popularity worldwide because it is a rich source of nutrients, bioactive compounds, complete essential amino acids, and high-quality proteins [1-3]. Demand for quinoa grain products is rapidly increasing; hence they are prone to adulteration with cheaper cereals [4].

In this study, we describe a novel approach for protein profiling, peak detection, and classification of four different commercial quinoa grains from Peru and Bolivia based on the combination of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and chemometrics [2]. After a rapid and simple extraction of proteins, MALDI-TOF-MS was applied to obtain characteristic mass spectra profiles from the soluble protein extracts. Data preprocessing and peak detection with MALDIquant [2], an open-source proteomics software that offers a collection of procedures for MALDI-TOF-MS, allowed the detection of the most relevant protein peaks in the mass spectra profiles. Finally, unsupervised and supervised multivariate data analysis methods (i.e., principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), respectively) were applied to efficiently classify and differentiate the commercial quinoa grains while tentatively identifying the most important proteins for the discrimination. The proposed approach could find application in quality control, authentication, and food fraud prevention programs. Furthermore, it could also be applied to protein profiling and classification of other food products, especially those presenting complex spectra with highly overlapped peaks.

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## P14

# POLYPHENOLIC FINGERPRINTS OBTAINED BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY TO CLASSIFY HONEYS ACCORDING TO THEIR FLORAL ORIGIN

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Honey is a natural product, a saturated carbohydrate-rich solution obtained through the diligent work and effort of the honeybees (*Apis Mellifera*) [1,2]. It is widely used in medicine and as functional food due to their beneficial nutritional and therapeutic properties [3]. An important parameter of honeys is the antioxidant capacity, which is a factor closely associated with the content of phenolic compounds present in the samples. In addition, the phenolic profile is related to the type of flowers from where bees collect the pollen, and therefore, this information can be used to detect adulterations or to develop novel methodologies to determine the floral origin.

In this study, the honey polyphenolic fingerprint of 101 Spanish honeys coming from three guaranteed floral origins (chestnut, heather and thyme) has been obtained by LC×LC. The optimal column combination was selected according to the number of detected peaks and the highest peak capacity. The most effective column combination was found when PFP and C18 columns were used in the first and the second dimensions, respectively.

Under optimal conditions, more than 103 peaks were observed for each honey sample, although only 26 peaks were common to all the samples. Then, a linear discriminant analysis model was constructed using the normalized volume of the common peaks as predictor variables, in order to classify honeys according to their floral origin. All samples were correctly classified in their corresponding floral origin with a 95% probability of assignment. Therefore, the results obtained demonstrated that polyphenolic fingerprints are strongly correlated with honey floral origin and can be effectively used for identification purposes.

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# P15

# DIFFERENTIATION OF MONOFLORAL HONEYS BASED ON THE PROTEIN PROFILE USING LINEAR DISCRIMINANT ANALYSIS

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The classification of honeys according to botanical origin is commonly done using pollen analysis techniques, which involve the identification and quantification of pollen grains in honey. This process is tedious, since the pollens are manually counted in each sample using optical microscopy. For this reason, it is necessary to develop new techniques that provides easy and low-cost methodologies to carry out the classification of the botanical origin. An alternative to pollen analysis techniques is the analysis of honey proteins, since it offers information about the botanical origin, possible adulterations, honey quality and even the type of bee that prepares the honey. Nevertheless, the amount of this component in honey is only around 0.5% since it comes from bees and from plants that collect pollen from bees. Therefore, it is necessary to use sample preparation procedures to extract and preconcentrate the proteins in honey. This technique can be done by using new materials with specific interactions with proteins.

In this work, solid phase extraction is proposed to extract and preconcentrate proteins from honey prior to their analysis by liquid chromatography. For this purpose, a specific material based on an organic polymer modified with gold nanoparticles was used. These nanomaterials present specific interactions with proteins, which will allow to eliminate the possible interferents from the sample and pre-concentrated the proteins above the detection limits. Thanks to this system, the protein profile of the honeys can be obtained and related to the botanical origin using classification techniques such as linear discriminant analysis. Concretely, this methodology has been applied to the extraction and analysis by chromatography of 71 honeys from 3 different botanical origins (Heather, Chestnut and Thyme). Subsequently, the data obtained from the chromatographic profile were used to obtain predictive models of the botanical variety of honey (certified through its pollen composition). The results showed that the use of the protein profile is a simple and fast method to predict the botanical variety of the honeys studied with a classification success of 100% using the leave-one-out technique.

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## P16

# PRECONCENTRATION AND DERIVATIZATION OF MINOR CARBOHYDRATES IN HONEY PRIOR HPLC ANALYSIS

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Carbohydrates (CHs) are essential components of honey, constituting approximately 70- 80% of its composition and contributing to its distinctive taste. The primary CHs found in honey are glucose and fructose, accounting for 95-99% of the total carbohydrate content. However, there are also minor carbohydrates present in honey that can significantly influence its flavor and properties. The composition of these minority sugars is influenced by factors such as botanical and geographical origin, bee species, and environmental conditions. Therefore, analyzing the profile of CHs in honey can serve as a valuable parameter for developing novel chemometric models to classify honey based on various characteristics mentioned earlier.

Currently, commonly employed methods for determining the total amount of CHs in honey include polarimetry, refractometry, the Lane and Eynon method, or near-infrared spectroscopy. However, to obtain a comprehensive profile and identify different types of CHs, separation techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) need to be employed. HPLC is commonly used to analyze the two primary sugars (glucose and fructose) as well as the main disaccharide (sucrose). On the other hand, GC can be used to determine the minority CHs; however, it often requires time-consuming derivatization protocols as a prerequisite.

This study aims to develop novel, efficient, and cost-effective derivatization methods for CHs analysis. In addition, a novel isolation and preconcentration for CHs using functional materials has been developed based on porous organic polymers modified with polyvinyl alcohol. Moreover, the proposed methodology will be employed to characterize honeys sourced from various geographical locations and the obtained data will be used to build a chemometric model based on linear discriminant analysis, enabling the classification of honey based on its geographical origin.

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# P17

# ANALYTICAL DEVELOPMENTS FOR THE STUDY OF ORGANOPHOSPHATE AND PYRETHROID PESTICIDE METABOLITES IN URINE OF CHILDREN AND ADOLESENTS FROM RURAL AND URBAN AREAS

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Analytical procedures for the analysis of organophosphate (OP) and pyrethroid (PYR) pesticide metabolites in urine have been developed. These methods have been applied to the study of children and adolescents (n=410) in Slovenia. The results were adjusted for specific gravity to normalize for daily dilution. The participants included 246 individuals from three rural areas (Prekmurje) and 164 individuals from Ljubljana [1], representing an urban setting. Among the six OP metabolites and two PYR metabolites analyzed, 4-nitrophenol (PNP), a parathion metabolite, was found in the highest concentrations in both urban and rural areas, followed by 3-phenoxybenzoic acid (3-PBA), which is a generic metabolite of several pyrethroids,. Notably, 3,5,6-trichloro-2-pyridinol (TCPY), a chlorpyrifos metabolite, exhibited higher concentrations in rural areas but was in low levels in Ljubljana.

Regarding potential sources of exposure among rural participants, the study investigated diet and living with pets. Positive associations were found between sauerkraut consumption and 3-PBA levels, while negative associations were observed between legume consumption and 2-(diethylamino)-6-methyl-4pyrimidinol (DEAMPY) and 3-PBA levels. Living with pets during winter was positively associated with 3-PBA levels.

No significant differences were found in the concentrations of pesticide metabolites between the three rural areas studied. However, children exhibited higher concentrations compared to adolescents, particularly for TCPY. Concentrations of PNP and TCPY differed significantly between winter and spring, with higher PNP levels in spring and higher TCPY levels in winter. The average estimated daily intakes based on urinary metabolite measurements did not indicate a significant risk of adverse health effects from exposure to these pesticides, both in rural and urban regions.

Comparing the distributions of pesticide metabolites between children from Prekmurje (rural) and Ljubljana (urban) within Slovenia, greater variability in concentrations was observed among children from the Ljubljana region. In contrast, metabolite concentrations in the rural area exhibited a narrower range. It is important to note that exposure to pesticides cannot be simply predicted based on rural or urban residence. Various factors influence exposure levels, and it is inaccurate to classify individuals only based on their residence.

**Acknowledgements**
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## P18

# Analysis of Intentionally Added Sexual Enhancers in Dietary Supplements by LC-MS/MS

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The lack of regulation for dietary supplements labelled as "natural" has led to a public health concern as many of these products contain active ingredients like those found in prescription medications, without being declared on the label. This is particularly relevant for natural libido boosters, which are marketed as dietary supplements that improve male sexual function. In recent years, serious side effects related to the use of natural sexual enhancers have been reported, leading health authorities in several countries to issue alerts and recommendations regarding their use. Despite these warnings, many natural sexual enhancers still contain medicinal compounds without being declared on the label, partly due to the lack of regulation in many countries. To analyze the presence of adulterants in natural sexual enhancer samples, liquid chromatography coupled with mass spectrometry (LC-MS) methods are usually used. These methods aim to quantify PDE5 inhibitor drugs and their analogues in the enhancer products. In the present work, an MRM method was used for quantification using a UHPLC-QQQ instrument. The developed quantification method was validated and applied to determine sildenafil, tadalafil, vardenafil, yohimbine and desmethyl carbodenafil concentration in several dietary supplements. The developed method showed a linear range from 50 to 1000 mg/kg for all five evaluated compounds. Moreover, the method presents an instrumental detection limit below 35 mg/kg and an instrumental quantification limit below 40 mg/kg for all the studied compounds. The fundamental analytical parameters were studied using commercial aphrodisiac dietary supplementary samples. The obtained results proved sufficient precision (RSD d 15%), accuracy (80  120% recovery), and robustness (RSD d 15%) and the method has been accredited under ISO17025. The outstanding results lead to simple sample preparation and a robust analytical method.

# P19

# Development and application of an analytical method to determine bile acid in pig serum

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Bile acids are primarily produced in the liver and actively transported to the gut. Here, they act as potent "digestive surfactants" to promote absorption of lipids (including fat-soluble vitamins), acting as emulsifiers. They can undergo secondary biotransformation by gut microbiota, promoting or limiting their bioactivity. Bile acids account for about 50% of the cholesterol turnover; therefore, changes in their concentration can be dangerous for health. However, several drugs, toxins and environmental contaminant have been related to disturbance of bile acid homeostasis [1], but their role is still underestimated.

In this study, an analytical method for the determination of bile acids in pig serum has been developed by using liquid chromatography coupled to triple quadrupole mass spectrometry via electrospray ionization (LC-ESI-QqQ-MS). Fourteen pure bile acids standards were injected at 5 mL/L in the QqQ-MS instrument to determine the optimal source conditions and transitions parameters to maximize the analytical signal. Three different reverse-phase (RP) chromatographic columns have been tested for the separation of the bile acids, namely 1) EVO-C18 (1.7 µm, 2.1 * 100 mm, Phenomenex), 2) C18-PFP (1.7 µm, 2.1 * 150 mm, Fortis) and 3) hypersylil gold C18 (1.9 µm, 2.1 mm * 150, Thermo). The C18-PFP column was selected, as it was the most suitable to separate bile acids, including positional isomers. The mobile phase consisted of A: 5 mM of ammonium formiate and 0.01% formic acid in water and B: Methanol. The gradient started from 50% of B, hold for two min and the risen to 95% of B at 15 min, hold for 3 min and then brought back to 50/50 of A/B, until minute 21 with a flow of 0.2 mL/min for the first 10 minutes and 0.3 for the rest. The syringe washing solvent was isopropanol/water (50/50, v/v) and with a washing step of 20 s.

The developed method was able to detect bile acids in real serum samples, with detection limits below 1 µL/L and quantification limits below 2 µL/L. The standard and matrix-matched calibration curves were lineal across the range of 1 µL/L to 1 mL/L and no matrix effect could be noticed. Therefore, the method has been applied to analyze bile acid concentrations in serum samples from pigs exposed to 20 ng/kg by weight of Aroclor1260 (a Polychlorinated Biphenyls (PCBs) mixture), to study whether contamination with low doses of PCBs could determine changes in bile acids concentration. The results demonstrated that the method is able to detect most of the bile acids in the pig serum and to highlight changes in their metabolism.

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## P20

# Biomimetic dispersive solid-phase microextraction: A novel concept for high-throughput estimation of human oral absorption of organic compounds

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There is a need for novel in vitro analytical methodologies that are properly validated to predict the oral absorption and bioaccumulation of organic compounds in humans, without relying on animal models [1]. The conventional log P parameter may not accurately predict bioparameters, as it solely considers the compound's hydrophobicity while neglecting its actual interaction with eukaryotic cell components. This contribution introduces a groundbreaking approach, employing biomimetic microextraction based on immobilized phosphatidylcholine, which acts as a surrogate for the plasma membrane, on organic polymeric sorptive phases. This method enables the estimation of human intestinal effective permeability for various pharmaceuticals, which are also recognized as contaminants of emerging concern.

An HPLC-UV method was used to obtain the experimentally biomimetic extraction data which proves to be a superior parameter compared to other molecular descriptors in developing reliable prediction models for human jejunum permeability, with an $R^2$ value of 0.82. Furthermore, the incorporation of log D and the number of aromatic rings in multiple linear regression equations enhances the correlations significantly, yielding an improved $R^2$ value of 0.98.

This research is expected to pave the way for rapid in vitro screening methods for assessing the oral absorption of organic contaminants of emerging concern in human exposomics.

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## P21

# FEED SUPLEMENTED WITH POLYPHENOLS FROM GRAPE MARC. CHROMATOGRAPHIC FINGERPRINTING IN CHICKENS

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The World Health Organization (WHO) lists antimicrobial resistance (AMR) among top 10 hazards for global health. To fight against this threat, the development of alternative antimicrobial products for the control and prevention of the most relevant diseases in animal production is mandatory. In this way, the use of natural extracts derived from agrifood byproducts as raw materials, such as grape marc, is a very suitable and still little explored option. On the other hand, of all terrestrial meat producing animals, broilers have the highest relative daily weight gain and the lowest feed conversion. Therefore, broiler meat can be considered as a relatively sustainable source of animal protein.

This work has a double objective: to evaluate the differences between conventional feed and feed supplemented with extracts rich in bioactive compounds derived from grape marc; and to follow such bioactive compounds through the digestive system of broilers fed with different doses of the extract.

Liquid extracts were obtained by medium scale ambient temperature (MSAT) system, using an optimized procedure [1] and they were converted to solid by spray-drying. Both feed samples and biological samples (crop and caecum) were extracted by matrix solid-phase dispersion (MSPD) and 60 target polyphenols were monitored by liquid chromatography tandem mass spectrometry (LC-MS/MS). The use of high resolution mass spectrometry employing QTOF was also considered to perform a deep characterization of the feed and biological samples and to elucidate metabolites. Preliminary results revealed several differences between the animals fed with feed containing the grape marc extracts in different dosage and those fed with the control feed.

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# P22

# CAPILLARY ELECTROPHORESIS ANALYSIS OF CHARGE AND SIZE VARIANTS OF THERAPEUTIC MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAbs) are immunoglobulin molecules which mainly belong to the IgG class. Their knowledge is acquiring enormous interest due to their use as therapeutic drugs to treat a (wide) variety of diseases, ranging from cancer to rare diseases. The mAb therapeutics market was estimated to be valued at $205 billion in 2022 and it is estimated to be worth $534 billion by 2030 [1].

Different molecules (proteoforms) can be present in each given mAb. These differences can arise from variations in the peptidic chain, in glycosylation or in other post-translational modifications (PTMs). As a result, charge and size variants of the immunoglobulin are formed. Moreover, the structure of the mAbs, affecting their therapeutic properties, can be modified due to different factors along the production and storage.

Therefore, strict controls are required to ensure the quality of these therapeutic drugs.

Among the different modes of capillary electrophoresis (CE), capillary zone electrophoresis (CZE) is adequate to separate molecules based on their differences in charge/size ratio while CE with sodium dodecyl sulfate (CE-SDS) allows separating analytes differing in size.

In this work variants of two therapeutic mAbs, namely Bevacizumab and Denosumab, were analyzed by CE. The influence of several factors on the separation of charge and size variants was studied. The simultaneous separation of the main peak and the acidic and basic variants of the drugs in a single CZE analysis was achieved. CE-SDS under reducing and under non-reducing conditions made possible the analysis of size variants of the mAbs. For both CE modes the short-end injection procedure permits performing fast screening, while longer effective separation lengths using the long-end injection procedure provides higher resolution.

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# P23

# Metabolomics and target trichothecene analysis to evaluate novel organic methods to prevent Fusarium Head Blight (FHB)

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Wheat holds its place among the most consumed cereals due to its beneficial nutritional values. Alongside the wheat genetic improvement programs, it is necessary to formulate revolutionary approaches able to control major diseases such as *Fusarium* head blight (FHB). To date, the FHB pest control through synthetic fungicides resulted troublesome in terms of soil and environmental pollution, and efficiency and costs of the treatment. Recently, the application of nanotechnologies-based strategies is showing the concrete possibility to minimize the use of pesticides in agriculture, by controlling the release of active ingredients, resulting in better coverage, enhancing its anti-pest properties while reducing the chemical dispersion in soil and water. Among others, cellulose nanocrystals (CNC) provide an economical and effective solution holding all these qualities.

In this study, the role of amylose-enriched starch in FHB resistance is investigated from a chemical point-of-view in three different bread wheat genotypes with different amylose content (Sumai3, Cadenza and Cadenza High Amylose) treated with a nanotechnology bio- based agrochemical (composed of CNC as carrier, high amylose starch as excipient, chitosan hydrochloride and gallic acid as antifungal active compounds) and a reference fungicide (tebuconazole). The presence of trichothecenes in the samples is being evaluated using a targeted approach developed on a triple quadrupole instrument coupled to liquidchromatography through an electrospray ionization source (LC-ESI-QqQ-MS/MS). Furthermore, two metabolomic methods developed on a hybrid quadrupole time-of-flight mass spectrometer coupled to liquid chromatography through ESI ionization (LC-ESI-Q-ToF- MS) have been deployed to investigate the effect of the treatments on the different varieties. The results show that each treatment induces specific metabolic responses in the different varieties, some of which might be biomarkers of FHB resistance.

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## P24

# Development of a GC-(Q)Orbitrap method for the assessment of exposure to organic chemicals at trace levels by analysis of human plasma

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Air pollution, a major component of the exposome, is a prevalent and important risk factor for human health. While the general population is often exposed to low concentrations of persistent and non-persistent chemical pollutants, more recent studies are already exploringa wider range of exposures [1]. Thus, it is crucial to advance the development of analytical methods by combining non-targeted analytical strategies with traditional targeted quantification methods. For this purpose, high resolution mass spectrometry techniques in combination with both, liquid chromatography and/or gas chromatography have become thebest choice as they provide full spectrum information at accurate mass together with enough sensitivity in order to achieve both strategies in a unique analysis [2].

In this scenario, a method that allows the simultaneous quantification and confirmation of 214 compounds in plasma samples from women living in industrial areas surroundings has been developed and validated using GC-(Q)Orbitrap technology. Compounds belong to several chemical families, such as pesticides, OPEs, PCBs, PAHs and alkyl-PAHs. To improve the quantification accuracy, 15 isotopically labelled standards were used. Samples were treated using a freeze liquid-liquid extraction (LLE-freezing) method with hexane:dichloromethane 80:20. The accuracy and precision of the method were evaluatedby recovery experiments (n=6) on human plasma samples spiked at two concentration levels (5 and 25 ng $mL^{-1}$). In most cases, recoveries between 70% and 120% were obtained, with an RSD less than 20%. The limits of quantification for most compounds were 2.5 ng $mL^{-1}$, which corresponds to the lowest point at which sensitivity is optimal on the calibration curve.

In addition, an untargeted analysis of the samples was performed, focusing on compounds containing chlorine (Cl), bromine (Br) or fluorine (F) in their molecular formula. As a result, 5 compounds (1 PAH and 4 alkyl-PAH) were found in a concentration range of 1-3 ng $mL^{-1}$ in bythe targeted analysis, and 7 compounds were tentatively identified by the untargeted analysis, such as bumetrizole and AM2201 benzimidazole analog.

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## P25

# DETERMINATION OF GADOLINIUM CONTRAST AGENTS IN URINE SAMPLES USING LC(IC) COUPLED TO ICP MS

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Gadolinium is a metal commonly used in magnetic resonance imaging to improve the sharpness of the images obtained. The specific compounds used are known as gadolinium- based contrast agents (GBCA). After administration, the agents are usually excreted within 24hours through urine eventually reaching the environment via sewage [1]. On the other hand, a prolonged residual gadolinium excretion phase has been described that may increase long-term toxicity [2]. The present work shows the development of a method for the determination of selected GBCA in urine by LC(IC)-ICP-MS.

The separation method was conducted with an anion exchange column ESI CD-Gd-01, using $NH_4NO_3$ as mobile phase. The GBCA's involved in this study were those used in the healthcare system of the Castellón province: Gadoteridol, Gadobutrol and Dotarem. Optimization of the separation conditions was firstly carried out through a broad study of the pH range from 6.5 to 9.5. Afterwards, a more detailed study from 9 to 9.8 was performed as well at different concentrations of ammonium nitrate. The final conditions for the mobile phase were 0.5 mM $NH_4NO_3$ at a pH of 9.2.

The validation was performed at 2 levels of concentration, 5 ng $mL^{-1}$ and 50 ng $mL^{-1}$, in diluted humane urine. Samples were obtained from a healthy volunteer suitably informed. Urine matrix can induce a significant matrix effect on both the ICP-MS instrumental signal and chromatographic retention times. The selected strategy was a 1 to 100 dilution step with deionized water, which provided satisfactory results at the analyzed concentration levels. Recovery values between 80-120% were obtained and RSDs were lower than 20% (in repeatability and reproducibility conditions) at the two concentrations studied.

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## P26

# Characterization of carbohydrates present in microalgae consortia (phytoplankton holobiont) with application in the food and cosmetic industry

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Recently, the study of microalgal consortia, constituted by diverse microalgae or by microalgae and bacteria, presents a great interest due to the role they play in natural ecosystems. In addition to their extraordinary capacity for industrial water purification, these consortia present great potential in other fields, such as cosmetics and nutraceuticals, since they can produce bioactive molecules due to cooperative interactions between the co-cultured microorganisms [1]. Carbohydrates, which represent more than 50% of the total biomass they produce, are one of such bioactives to which a number of properties such as anti-oxidative capacity, anti-aging, etc. have been attributed [2]. Although the structure of carbohydrates produced by various microalgae has been reported, the available data are still scarce [3], mainly for consortia. Therefore, a more exhaustive study of their composition is necessary.

Gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography (LC) with evaporative light scattering detector (ELSD) techniques are very useful for carbohydrate characterization due to their high sensitivity, efficiency and small sample quantities required for analysis. Thus, in this work GC-MS and LC-ELSD methods were developed to evaluate the composition of carbohydrates present in two microalgae consortia: Marine algaemass composed by *Chlorella* sp. and *Tetraselmis* sp.; and Xtreme algaemass, mainly composed by *Dunaliella* sp. GC-MS was used to determine low molecular weight carbohydrates (LMWC) after derivatization to their trimethylsilyl oximes, while monomeric composition of high molecular weight carbohydrates also required a previous acid hydrolysis. Degree of polymerization (DP) of polysaccharides was determined by LC-ELSD using the combination oftwo size exclusion columns. Additional spectrophotometric assays to determine total carbohydrates and content of uronic acids were also carried out. In general, galactosyl-glycerol was the most abundant LMWC detected, while polysaccharides were mainly constituted by glucose units, although fucose, galactose and xylose, among others, were also detected. Regarding DP, polysaccharides up to DP70 were detected.

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## P27

# EVALUATION OF THE MIGRATION OF BPA, ITS SUBSTITUTES AND DIGLYCIDYL ETHER DERIVATIVES IN CANNED BEVERAGES

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Bisphenol A (BPA) is a synthetic compound that has been widely used in the production of plastics, such as polycarbonate and epoxy resins. It is commonly found in items like water bottles, food containers, and the inner coating of metal cans. However, BPA has raised concerns due to its potential to act as an endocrine disruptor compound and, for this reason, its use is regulated [1] and, in some cases, restricted [2] by European Union. Due to these concerns, efforts have been made to find substitutes for BPA in different consumer products. Two of the main common substitutes for BPA are bisphenol S (BPS) and bisphenol F (BPF), but there are many other possible alternatives including bisphenol B (BPB), bisphenol AF (BPAF), fluorene-9-bisphenol (BHPF), bisphenol Z (BPZ), and tetramethyl bisphenol F (TMBPF). Unfortunately, research suggests that all of them may have endocrine-disrupting effects [3] and, consequently, they should be also controlled. The migration of BPA, its substitutes, and diglycidyl ether derivatives of BPA and BPF into canned water and soft drinks is a major issue due to the potential transfer of these compounds from the inner coating of cans to products. Therefore, this work evaluates the migration of BPA, 7 of its substitutes, and 6 diglycidyl ether derivatives in 20 commercial samples of canned beverages purchased in supermarkets in the Community of Madrid.

The migration study was performed using: (i) simulant B (3% acetic acid) for all soft drinks (4 lemons, 2 cokes, 1 bitter, 1 ginger, 1 tonic, 4 teas, and 3 sodas) and (ii) ultra CHROMASOLV™ quality water for UHPLC-MS for canned water (2 still and 2 sparklings) using different contact times: $t_0$ (one hour after filling the containers), 24 h, 4 days, 1 week, 2 weeks, 4 weeks, and 4 months. Aliquots from each time and container were analyzed by ultra-high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UHPLC-QqQ(MS/MS)) using an electrospray (ESI) interface [4].

Regarding the most relevant results, migration for BPA was detected in, at least, one sample at all evaluated contact times, except at 24 h. In the case of TMBPF and BPB, migration began at 4 days, and remained for 2 weeks and 4 months, respectively. For the diglycidyl ether derivatives, the most significant result was the migration observed for $\mathrm{BADGE \cdot 2H_2O}$ at all evaluated times in 35% of the cans analyzed. No significant concentrations were found for BPF, BPS, BPZ, BPAF, BHPF, BADGE·HCl, $\mathrm{BADGE \cdot HCl \cdot H_2O}$, and BFDGE in any of the samples and migration times investigated.

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## P28

# IMPROVED CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY METHOD FOR PROTEIN PROFILING AND AUTHENTICATION OF A1/A2 BOVINE MILK

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Protein profiling of major bovine milk proteins (i.e., whey and casein proteins) is of great interest in food science and technology. Current routine methods for milk protein profiling at the intact level are typically based on capillary electrophoresis with ultraviolet detection (CE-UV), which does not allow confirming unequivocally the identity of the separated proteins [1]. In a previous work we developed, for the first time, a capillary electrophoresis-mass spectrometry (CE-MS) method, which allowed the separation and identification of major bovine milk whey and casein proteins [2]. In addition, high-resolution mass spectrometry demonstrated to be necessary to reliably characterize proteoforms with slight molecular mass differences, including β-casein A1 and A2. These proteoforms are relevant to unequivocally identify milks with specific β-casein compositions (i.e., A1A1, A1A2, and A2A2 milk), which are nowadays of great interest for the dairy industry [2]. However, the main drawback of the previously developed CE-MS methodology is that it needs the use of lab-made hydroxypropyl cellulose (HPC) coated capillaries to prevent protein adsorption on the inner capillary wall. Although being effective in reducing protein adsorption, the coating procedure is complex and time-consuming, and reproducibility between batches tends to be low if done without extreme care. In this study, we developed an alternative and improved CE-MS methodology for bovine milk protein profiling, using deactivated fused silica commercial capillaries. After optimizing several conditions and parameters, the new method showed to be better in terms of repeatability, reproducibility, peak shape, and durability of the capillaries. Under the optimized conditions, the method was applied to the analysis of milk from individual cows (A1A1, A1A2, and A2A2), as well as to study adulterations of A2A2 milk with low levels of A1A1 milk. The developed method showed its potential for protein profiling of bovine milk whey and casein proteins, as well as to be used as a rapid and simple method to screen for possible A1/A2 adulterations.

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## P29

# VOLATILE PROFILING BY SPME GC-MS OF FLAVORED OLIVE OILS PRODUCED BY MALAXATION AND INFUSION

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A traditional practice in the Mediterranean gastronomy is the flavoring of extra virgin olive oil (EVOO) with aromatic plants and spices [1-2]. This procedure has been described to confer EVOO, not only a characteristic aroma and a number of bioactive properties (antioxidant, antimicrobial, etc), but also an improved oxidative stability [3].

In this study, a new methodology by solid-phase microextraction (SPME) followed by GC-MS analysis has been optimized to evaluate for the first time the effect of the processing (infusion *vs* malaxation) on the volatile composition of Calabrian EVOOs (from *Olea europea* Ottobratica olives) flavored with bergamot (*Citrus bergamia*) peel, ginger (*Zingiber officinale*) root, turmeric (*Curcuma longa*) or mace (*Myristica fragrans*) spices.

After selection of the optimal SPME fiber coating (2-cm DVB/Carboxen/PDMS), sample amount (100 µL) and internal standard (tridecane), a 3-level factorial experimental design was used to provide the optimal SPME operating conditions (temperature = 44°C, equilibrium time = 10 min and extraction time = 60 min). This methodology was further applied to the different samples under study with the aim of quantifying a number of volatiles including those previously reported as positively contributing to the aroma of EVOOs (*trans*-2-hexenal, *cis*-3-hexenal, 2-hexen-1-ol, 1-hexanol, etc) or characteristic of the different matrices considered (e.g. *cis*- and *trans*-α-bergamotene and bergamiol for bergamot-flavored EVOO). Compounds associated with undesirable notes (2-heptenal, heptanal, octanal and 2,4-heptadienal) were also quantified.

Irrespective of the flavored EVOO considered, malaxation samples were richer in characteristic volatiles arising from the plant matrix as compared to infusion samples (e.g. 1.16 *vs* 0.80 mg $g^{-1}$ sabinene in mace-flavored EVOO), this effect being particularly evident for sesquiterpenoids (0.23 *vs* 0.018 mg $g^{-1}$ α-curcumene in ginger-flavored EVOO). In conclusion, the SPME GC-MS approach here developed is shown as a green, miniaturized and affordable procedure, of easy implementation both in research and industry laboratories, for monitoring of the volatile composition of flavored olive oils for quality control purposes, among others.

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## P30

# CHARACTERIZATION OF THE VOLATILE PROFILE OF SPENT COFFEE GROUNDS FOR THEIR INDUSTRIAL VALORIZATION

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Spent coffee grounds are the most abundant by-products generated in coffee beverage preparation and instant coffee production. They are generally disposed of in landfills or processed for composting, leading to significant negative effects on the environment. Spent coffee grounds have a high content of medium and high molecular weight bioactive compounds, but there is virtually no information about their volatile fraction [1]. Since they may contain significant amounts of volatile organic metabolites with industrial applications, a more in-depth characterization of their volatile fraction and its possible dependence on the geographical origin and the type of coffee bean processing would be of great interest.

Thus, in this work, an HSSPME GC-MS method was optimized to obtain the volatile fingerprint of spent coffee grounds from Arabica coffee beans (medium roast) from eight countries, namely Brazil, Colombia, Guatemala, Honduras, Ethiopia, Kenya, India, and Indonesia. They were also processed in various ways: natural, semi-washed, washed, and *monsooned*.

The optimal extraction conditions were found to be 1.0 g of sample at 90 °C for 10 min. Under these conditions, 70 compounds were identified and classified according to their chemical family as follows: 16 nitrogen compounds, 15 furans, 11 hydrocarbons, 9 carbonyl compounds, 7 terpenes and terpenoids, 3 sulfur compounds, 2 phenols, and 7 others. The most abundant compounds in all samples were furfural, 5-methylfurfural, 1-furfurylpyrrole, and benzaldehyde, except those from India where 4-ethylguaiacol was found in the highest concentration. Principal component analysis revealed the influence of geographical origin and/or coffee bean processing method on the volatile profile of the spent coffee grounds.

The results obtained suggest the potential application of this by-product in the food and pharmaceutical industries and open new pathways to valorize spent coffee grounds, contributing to the circular economy.

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## P31

# ULTRASOUND-ASSISTED EXTRACTION AND PRESSURIZED LIQUID EXTRACTION AS GREEN TECHNOLOGIES TO OBTAIN BIOACTIVE PEPTIDES FROM CITRUS PEELS

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Processing of Citrus fruits, one of the main fruits cultivated worldwide, generates up to a 67 % of waste of the total production [1]. It is well-known that residues from the agri-food industry are rich in bioactive compounds so they can be considered as a source of high added-value compounds. In this context, there is necessary to develop strategies that comply with the principles of green chemistry and allow the obtaining of bioactive compounds that can be used in the pharmaceutical, food or cosmetic industries, among others. In this way, the sustainability of the food chain increases by reducing the negative environmental impact of these citrus fruit residues.

The aim of this work was to develop sustainable strategies enabling the revalorization of citrus peels through the obtaining of protein extracts as a source of bioactive peptides. For that purpose, two different extraction techniques such as ultrasound-assisted extraction and pressurized liquid extraction were employed to obtain protein extracts from the peel of two varieties of lime (*Citrus aurantifolia*). The effect of deep eutectic solvents on the protein extraction yields was also investigated. Subsequently, several enzymes were tested in order to produce small peptides with potential bioactivity and different biological activities were evaluated for the protein hydrolysates using different *in vitro* assays. Finally, the separation and identification of peptides present in the most active hydrolysates were carried out using UHPLC-QTOF-MS.

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# P32

# DEVELOPMENT OF A PAPER-BASED APTASENSOR TO DETERMINE CONCANAVALINE A IN FOOD SAMPLES

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There is a growing interest in the development of highly selective and cost-effective sensors for food control [1]. In this regard, the use of aptasensors (APs) for food safety analysis has seen exponential growth [2]. APs are based on aptamers, which are single-stranded DNA or RNA molecules capable of recognize selectively molecules or compounds. On another hand, Concanavalin A (Con A) is a lectin found in seeds, raw legumes, and tubers, among others, which is responsible for causing typical allergies. In this work, a paper-based aptasensor was developed for Con A determination. For this purpose, an aptamer with a fluorescent tag was applied onto paper, followed by the addition graphene oxide (GO) suspension. The presence of GO leads to a reduction of APs initial fluorescence due to a quenching phenomenon by GO-AP complex formation. Then, Con A addition into the system caused a shift in the equilibrium, resulting in the release of GO and subsequent recovery of fluorescence intensity. Fluorescence measurements were performed onto the paper using a microplate reader in combination with a 3D printed support to ensure a stable position for the paper and reproducible measurements. Fluorescent intensity recovery was proportional to Con A concentration present, achieving a limit of detection of 0.4 mg per gram. Additionally, no interference with other studied lectins was observed, with sample recoveries close to 100%.

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## P33

# ANALYSIS OF PESTICIDES IN CORN PRODUCTS USING COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY: METHOD DEVELOPMENT AND VALIDATION

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The growing popularity of organic food has heightened concerns regarding the use of chemicals and pesticides in agriculture. To address this, scientists have actively developed techniques to monitor pesticide in food. Conventional liquid chromatography sometimes has limitations in providing sufficient resolving power and selectivity when dealing with complex matrices. In this sense, multidimensional and, specially, "comprehensive" two-dimensional liquid chromatography methods offer enhanced resolving power, peak capacity, and reduced matrix effects. In this study, we present for the first time a novel approach using comprehensive two-dimensional liquid chromatography coupled with tandem mass spectrometry for the multiclass analysis of 112 pesticides in corn-based products. A modified "reduced" QuEChERS-based procedure was applied for sample extraction and clean-up. The method was validated assessing linearity, sensitivity, precision, and accuracy, while matrix-matched calibration curves were used. Quantification limits bellow European legislation threshold were achieved; intra-day and inter-day precision were below 12.9% and 15.1%, respectively, and over 70% of the target compounds exhibited recoveries ranging from 70% to 120% with standard deviation values below 20%. Additionally, matrix effect was studied yielding values from 13% to 161%. The proposed method was applied to the analysis of real samples, and three pesticides were detected at trace levels. These findings, expand the horizons for the analysis of complex sample matrices such as corn-derived products [1].

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## P34

# The effect of aging conditions on the changes of the aroma volatile profile and key odourants in garlic by SPME GC-MS and GC-O

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Garlic (*Allium sativum* L.*)* is widely used, not only as a condiment for culinary applications, but also as the basis for garlic supplements due to its wide variety of biological properties. However, the consumption of garlic products is sometimes conditioned by their strong aroma, and its tendency to cause stomach upset in sensitive individuals. To overcome these limitations, raw garlic is subjected to an aging process, usually involving thermal heating (40-90°C) for 1-3 months under controlled humidity conditions (60-80%), resulting on what is known as aged garlic (AG). The organoleptic properties (black-brown color, aroma, flavour, etc) of this AG product are the result of the aging conditions employed [1-2].

In this work, Solid-Phase Microextraction followed by Gas Chromatography-Mass Spectrometry (SPME GC-MS) and by GC-Olfactometry (SPME GC-O) were optimised for the analysis of aroma volatile compounds and key odourants from different AG samples obtained by ABG Cool-Tech™ processing, which employs controlled temperature (<60°C) and humidity conditions (60-90%) for up to 40 days. For comparison purposes, raw garlic and commercial black garlic samples were also analysed. After selection of the optimal SPME operating parameters (sample amount: 0.1 g, equilibrium time: 20 min, extraction temperature and time: 40°C for 20 min) and separations conditions (DB5-Column, injector temperature: 250°C and splitless time: 0.75 min), more than 30 aroma volatile compounds belonging to different chemical classes (sulfides, aldehydes, alcohols, pyrazines, etc) were identified and their concentration was dependent on the processing stage and/or crushing pretreatment considered for aging. Regarding GC-O analysis, key odour active compounds such as diallyl disulfide (sulfur), 2-propen-1-ol (pungent), methyl-pyrazine (green) or furfural (sweet) were identified in the samples under study. Noticeable differences in the odour profile were detected, particularly for samples subjected to crushing during the aging process. In conclusion, the multianalytical approach here considered can be proposed as a green, efficient and reliable approach to select the AG samples with better sensory acceptance by consumer.

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# P35

# Development of a HPLC-DAD-ToF MS methodology for the authentication of Damiana *(Turnera diffussa)* extracts

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Damiana (*Turnera diffussa*) is an endemic shrub from Mexico, that has been historically used in traditional herbal medicine throughout the world, mainly as a sexual stimulant or aphrodisiac, being one of the most used herbs in this type of formulations [1]. Moreover, it has been also described as a traditional remedy against stomachache, lung diseases related to tobacco abuse, bladder and kidney infections, etc. [2] and it is now widely consumed as food supplement (FS). FS for sexual enhancement are some of the most adulterated at present using drugs intended for the treatment of erectile dysfunction [such as phosphodiesterase-5 (PDE-5) inhibitors] [3]. However, the development of analytical methods to detect such adulterations in damiana formulations has hardly been addressed. Therefore, in this work, a new analytical method to detect these potential frauds has been proposed.

Firstly, different solvents (water, methanol and hydroalcoholic mixtures) were evaluated as extractants of damiana leaf compounds, selecting the most appropriate one to allow a comprehensive characterization of these samples. A methodology based on liquid chromatography with diode array detector coupled to time of flight mass spectrometry (HPLC-DAD-ToF MS) using a C18 reverse-phase column under both positive and negative ionization modes was proposed. In addition, PDE-5 inhibitors (sildenafil, tadalafil and analogues) as well as different simulated additions at different concentrations of these drugs to damiana extracts were also analyzed by the developed methodology.

Methanol:water (50:50) extracts of damiana showed the highest number of extracted compounds; the untargeted analysis of these samples allowed the detection of 3000 molecular features in negative ionization mode and 4000 features in positive mode. Phenolic compounds such as flavonoids glycosides and catechins, cyanogenic glycosides such as tetraphyllin B and terpenoids such as tehuetenone A were detected in damiana leaf extracts. HPLC-DAD-ToF MS methodology allowed the analysis of PDE-5 inhibitors and the successful detection of their presence in the intentionally adulterated damiana extracts.

**Acknowledgements**

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### P36

# Development of a QuEChERS-based method coupled to LC-MS/MS for the determination of eight Persistent, Mobile, and Toxic substances in *Cichorium endivia.*

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The reuse of wastewater for irrigation is a potential measure against water scarcity. However, contaminants of emerging concern, especially persistent, mobile, and toxic (PMTs) compounds, may be present in reusable water, due to their inefficient removal by conventional wastewater treatment plants [1]. Consequently, irrigation with contaminated water may lead to an uptake of PMTs by the crops and plants. The possible presence of these compounds in plants requires advanced analytical methodologies capable of determining them.

In this work, mixed-mode liquid chromatography coupled to tandem mass spectrometry (MMLC-MS/MS) has been used to develop a methodology for the determination of eight PMTs in escarole (*Cichorium endivia*). Several experiments were performed to optimize the chromatographic and MS/MS conditions. The selected PMTs (Benzophenone-3, Clarithromycin, Imazalil, Metformin, Sulpiride, Terbutryn, Tiapride and Tramadol) were separated within 8 minutes using mobile phases consisting of water, acetonitrile (ACN) and ammonium acetate (variating between 5 - 20 mM along the gradient). MS/MS, using a triple quadrupole mass analyzer, operated in positive-ion mode. The acquisition of at least two transition per compound allowed accurate quantification and reliable identification of each PMT. To obtain a simple, fast, and cost-effective sample treatment, QuEChERS was employed [2]. Briefly, 10g of escarole was extracted with 10 mL ACN and the citrate kit salts ($MgSO_4$, NaCl, $Na_2HCitrate·1.5H_2O$ and $Na_3Citrate·2H_2O$) followed by a dispersive solid phase extraction (dSPE) clean-up step using $MgSO_4$, PSA and $C_{18}$. Finally, the extract was 2-fold diluted and 5 µL was injected in the MMLC-MS/MS system. Results showed satisfactory overall recoveries (including extraction and matrix effects) in a range of 70-120%.

The methodology was fully validated at the Limit of Quantification (LOQ) and 10 times LOQ level considering the following parameters: linearity, precision, and accuracy. Finally, the methodology was applied to escarole samples irrigated with spiked water and the possible uptake of the PMTs was investigated.

**Acknowledgements**
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## P37

# DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN URINE FROM SPANISH POPULATION, ARE THERE RISKS?

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Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants generated from incomplete combustion of organic matter and in various industrial processes. They can have adverse effects on the immune system, development, and reproduction in humans, and some of them have classified as a human carcinogen or as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC) [1]. Ingestion, due to their production during culinary practises [2], and inhalation of polluted air or cigarette smoke are the predominant sources of PAH exposure. Once PAHs enter the body, they undergo biotransformation and metabolism, and are excreted through urine or faeces, according to their molecular weight [3].

In the present work, 11 urinary PAH metabolites were determined in first-morning urine samples from the participants of the BIOMOVAL project, using liquid-liquid extraction followed by liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). The limit of quantification (LoQ) was between 0.01-0.05 $\mu g \cdot L^{-1}$. Later, PAH exposure was assessed using an internal dose approach, based on the calculation of the ratio (Hazard Quotient, HQ) between the estimated daily intake (EDI), and the reference limit for oral exposure (RfD) for each PAH [4,5]. Finally, the hazard index (HI) was employed to estimate the cumulative risk of exposure to PAH mixtures [6].

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## P38

# Assessment of children's exposure to common and new PFAS through suspect screening by LC-HRMS

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In this study we aim to investigate the potential harmful effects of legacy contaminants as well as contaminants of emerging concern in children. Children belongs to a very susceptible part of the population since the harmful impact that those contaminants could have on their health due to their young age and their chronical exposure may be much more dangerous than in adult population. In this context, we wanted to investigated the exposure of early stage population to commonly known per- and polyflruoroalkyl substances as well as any other new PFAS used as a substitute. For this reason, a non-target methodology was developed for the study of 40 per- and polyfluoroalkyl substances including the legacy PFOA and PFOS, among other 18 commonly known PFASs, and the ones considered as new replacement PFASs such as the short TFA, ADONA, GenX, Capstone A and Capstone B, F53B and PFMBOA, among others, in urine as a non-invasive matrix of 185 donors at age of 8.
The urine samples were collected by each participant and preserved at -60ºC until analysis. Very brief, 100 µl of samples were spiked with a mixture of internal standards and, then, the proteins and part of the salts were precipitated with 100 µl of acetonitrile and shacked for 30 min. Afterwards, the samples were centrifuged and the supernatant collected in a LC-vial for further analysis.
The final extracts were analyzed by means of liquid chromatography coupled to high resolution mass spectrometer Orbitrap by full scan at 70000 of FWHM and data dependent scan of the ions at 15000 FWHM. The separation of common PFASs was achieved using an Hypersil Gold PFP column while the short PFASs were separated in an Atlantis Premier BEH C18 AX column.

The main results showed the presence of common carboxylic acid PFASs such as PFPeA, PFHpA, PFOA, PFNA, PFUdA and the sulfonates PFHxS, PFOS and PFNS. The concentrations were ranging from 4.9 ng/L (PFNS) to 532 ng/L (PFHxS). In addition, some short chain PFASs have been detected in some punctual samples including TFA. The two most frequently detected compounds have been the TFA and GenX, being present in 51% and 50% of samples at quantifiable concentrations, respectively, followed by PFHxS which was present in 34% of the samples.
The values detected in this work were comparable to the ones reported for children but at much lower concentration compared with the ones reported in the literature for adult people [1] and workers from a PFASs manufacture [2]. However, it is important to remark that this study has been focused on 8-years-old children so their exposure to these toxicants has been basically through the diet and, therefore, these concentrations could increase through the years since their exposure could be chronically.

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## P39

# PASSIVE SAMPLING OF ALPINE AIR AND WATER: MULTI-COMPOUND ANALYSIS OF EMERGING AND LEGACY SEMI-VOLATILE ORGANIC POLLUTANTS

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Alpine areas are essential locations for the study of the environmental fate of anthropogenic pollutants. Legacy and emerging chemicals reach them through long-range atmospheric transport and accumulate there due to thermodynamical processes that lead to cold-trapping, which is favoured by the environmental conditions in such regions. In recent years, advancements in the understanding of passive sampling technologies and materials have spurred an increase in their popularity since they help to overcome limitations of traditional sampling strategies in high mountains. Moreover, they have not only been used in remote locations, but also in urban areas and as part of worldwide monitoring networks.

We present a multi-compound study of legacy and emerging organic pollutants in air and water from several high-elevation Pyrenean lakes at the receiving end of atmospheric pollutant transport [1,2,3]. This includes polychlorinated biphenyls (PCBs), organochlorines like hexachlorobenzene, polycyclic aromatic hydrocarbons (PAHs), and the emerging organophosphate esters (OPEs). Polyurethane foam (PUF) and low-density polyethylene (LDPE) passive air and water samplers were deployed over several consecutive periods spanning three years. After a Soxhlet extraction and clean-up (including an HPLC fractionation step), the analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS) or tandem mass spectrometry (GC-MS/MS). Low limits of quantification ($<0.4$ pg/m$^3$ of air, $<5.1$ pg/L of water) allowed the trace-level analysis of pollutant concentrations. The samplers were calibrated using performance reference compounds (PRC), yielding sampling rates with low uncertainties (2–34%). Compared to studies in the same area from over two decades ago, pollutant concentrations in both air and water decreased by different magnitudes depending on their persistence. Gas-phase concentration trends with temperature revealed a prominent influence of secondary volatilization from environmental surfaces (e.g., soils, snow) for OPEs and the less volatile PCBs and PAHs. Yearly average air–water exchange fluxes showed net deposition (PAHs, some PCBs), equilibrium between phases (PCBs, OPEs), and volatilization from water into air (hexachlorobenzene). Alltogether, the general scenario is one of accumulation of organic pollutants with secondary emissions that fluctuate with temperature changes.

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# P40

# WHERE THE RIVER MEETS THE SEA, THE LIE HAS THE TASTE OF TRUTH “SEI DE UM RIO”

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In Portugal, the Tagus estuary (Lisbon city), Sado estuary (Setúbal city), and the Sines harbor, present high anthropogenic activity; making them important sources of direct pollutant emissions into the sea. It is the only coastal area in Portugal that did not reach the “Good Environmental Status” of the EU Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC, 2008) according to the latest environmental report. In this study, **PAHs**, **PCBs**, **DDTs**, **HCHs** and **HCB** were analyzed in sediment cores that were collected in 23 sites (with 9 stratification samples in depth) in this area.

After sampling sediment cores, 5 g of freeze-dried sediment spiked with surrogate standards, ultrasonic extracted in $CH_2Cl_2$/MeOH (2:1 v/v; 3 x 15 min.), hydrolyzed with KOH/MeOH (6%), and purified on 1 g $Al_2O_3$ column. Concentrated extracts were injected into a **GC-MS EI** (Agilent 5975) and compounds were analyzed in SIM mode for the PAHs analysis, while assessment of organochlorines (OCs) was carried out using a **GC-ECD** (Agilent 6890N).

The sites with the highest concentration of all compound groups (max ∑PAHs = 4200 ng/g dw; ∑OCs = 22 ng/g dw) were found in the Tagus estuary, and diluted in sediments at larger distance from the land. Here, terrestrial industry as well as transporting activities are the main emission sources. These sediments show relatively higher presence of organic matter (OM) content and a dominance of fine-fraction profiles, resulting in high adsorption potential for the hydrophobic persistent organic pollutants. The Sines harbor sediment showed higher concentration of light PAHs, that were related to the thermoelectric power plant and oil refinery plant.

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# P41

# DEVELOPMENT OF AN ANALYTICAL METHOD FOR WASTEWATER BASED EPIDEMIOLOGY ON PHARMACEUTICALS

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The monitoring of pharmaceuticals in influent wastewater samples (IWW) is not only valuable from the environmental point of view, but also as a tool to analyze patterns of consumption in the region of the catchment area by the so-called wastewater-based epidemiology (WBE) approach [1,2]. In Spain, some studies [1,3] demonstrated the occurrence of pharmaceuticals in different separate IWW samples. However, there are no studies that monitored different wastewater treatment plants (WWTPs) geographically distributed to obtain a general picture on the pharmaceutical occurrence in this country. Thisstudy developed an analytical method based on solid-phase extraction using Oasis HLBfollowed by liquid chromatography with tandem mass spectrometry to monitor the occurrence of a group of pharmaceuticals including the most representative for therapeutic families in IWW samples. The samples were collected during a week campaign in six different WWTPs located in different cities and towns in Spain.

The developed method provided successful figures of merit with recoveries in IWW ranging from 42% to 139%, and low matrix effect (in general lower than ± 30%), and methodquantification limits (MQL) between 1 ng/L and 24 ng/L for all compounds, except atenolol (58 ng/L). All the studied pharmaceuticals were found in all analyzed samples with concentrations ranging from <MQL to 10,393 ng/L, being the highest concentration found for tramadol. In addition, the population normalized daily load (PNDL) data reveals that the consumption of pharmaceuticals has, in general, similar patterns in all monitored WWTPs. Insummary, the proposed method is a suitable analytical tool for WBE to estimate pharmaceutical consumption.

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# P42

# IMPACT OF SUPER-INTENSIVE OLIVE CULTIVATION AND THE APPLICATION OF ORGANIC AMENDMENTS ON SOIL ORGANIC MATTER COMPOSITION

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Olive cultivation is a crucial driver of the economy in the European Mediterranean basin, particularly in Spain, where it occupies about 2.75 million ha [1]. In recent years, there has been an expansion of super-intensive olive farming, which ensures high productivity but requires irrigation and is expected to increase organic waste generation and water demand. Consequently, it is imperative to adopt more sustainable approaches to manage the agricultural waste generated during olive harvesting and agro-industrial processing. To tackle that challenge, there is a renewed emphasis on utilizing organic amendments derived from agricultural residues. Biochar (B), a highly aromatic carbon obtained through biomass pyrolysis, has the potential to sequester carbon in the soil for extended periods and its unique physical properties increase soil porosity [2]. Nevertheless, B effects on SOM composition and functionality in the medium and long term are largely unknown. Additionally, green compost (Cp) is rich in nutrients and labile organic carbon, making it a valuable soil amendment for improving soil fertility, structure, and moisture retention. The two main goals of this study are: i) To discern the effects of transforming traditional olive orchards into bush-row olive cultivation on soil organic matter (SOM) composition, which plays an essential role in the carbon cycle and on soil quality, and ii) To assess the effects of of B and Cp on SOM composition two years after application. With such purposes, soil samples were taken from super-intensive olive orchards (1650 trees/ha; irrigated 30% water needs) amended with (B), green compost (Cp), a combination of B+Cp, as well as control plots [3]. Soil samples were also collected from traditional olive orchards in the same farm (*La Hampa*, Coria del Río) for comparative purposes. The free lipid fraction and volatile SOM were studied by using GC/MS and analytical pyrolysis (Py-GC/MS) respectively.

Control soils, particularly those from the traditional olive orchards, exhibited higher total and relative abundance of fatty acids (including unsaturated fatty acids) and sterols (including α-Tocopherol). In contrast, the chromatograms of soils amended with B and to a lesser extent with Cp showed higher relative abundance of linear *n*-alkanes. Preliminary analysis of analytical pyrolysis data also indicates a greater soil functionality in the soils from the traditional olive orchards, which also exhibited the greatest total C and N contents.

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## P43

# ASSESSMENT OF THE REMOVAL CAPACITY OF EMERGING ORGANIC POLLUTANTS IN WATER BY BIOCHAR PRODUCED FROM AGRICULTURAL RESIDUES

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Pyrolysis, heating biomass in the absence of oxygen, enables the conversion of biomass residues into biochar [1]. It is an aromatic carbonaceous material with high porosity and adsorption capacity, which can be activated and used in the production of filters that function similarly to commercial activated carbon (AC) filters. This study evaluates the adsorption capacity of biochar produced from rice husk (RH) and almond shell (AS) for the removal of emerging organic contaminants (EOCs) in water. This application could reduce the concentration of EOCs derived from pharmaceuticals and pesticides present in water, avoiding the use of activated carbons (AC) derived from fossil coal or imported from long distances. Additionally, it would reduce the implementation of pyrolysis and activation processes that pose high environmental risks and promote the recycling and valorization of low-value residual biomass. Biocarbons (BCs) were produced from RH and AS at pyrolysis temperature of 750 °C and were activated with steam or KOH (for comparative purposes). The elemental composition, physical properties, and iodine index of the BCs were determined before and after activation. Subsequently, their adsorption capacity of Sulfamethoxazole, Atenolol, Carbamazepine, Diclofenac, Salicylic Acid, and Acetaminophen were measured ($n$=3). These organic compounds represent cationic, anionic, and neutral pharmaceuticals and pesticides, some of which are currently considered as EOCs due to their frequent presence in hospital and wastewater effluents. For this purpose, 100 mg of each BC sample were mixed with 20 mL of a 10 mg $L^{-1}$ solution of each contaminant, shaken for 24 hours and centrifuged. The contaminant concentrations in the supernatant were measured using high-performance liquid chromatography with a diode array detector (HPLC-DAD). The chromatographic column used was a reverse-phase C18 (Novapack, Waters Co.) with specific mobile phases for each contaminant. All the activated BCs were rich in organic carbon (> 75%), and their physical properties were suitable for its use as a filter, although the iodine number was notably lower than that of commercial AC. AS biochars showed slightly lower performance in terms of EOC removal, while the BCs from RH activated with steam exhibited a nearly 100% adsorption capacity for all tested contaminants, with better yields than commercial AC.

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# P44

# INVESTIGATING THE OCCURRENCE OF VERY POLAR AND VERY MOBILE ORGANIC POLLUTANTS IN URBAN WATER SAMPLES

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As the population increases, so does the demand for water, leading to a need for new sources. Groundwater is a viable alternative, but unfortunately it may be contaminated by organic pollutants that come mainly from wastewater and runoff water [1]. Unfortunately, limited studies have been conducted to address these pollutants, particularly very polar and very mobile (vPvM) compounds. These highly polar compounds demonstrate exceptional mobility in water, effectively propagating through the water cycle and resisting technical andbiological barriers. Consequently, they present a high risk of infiltrating the drinking water supply [2]. To bridge this research gap, our study focuses on examining the occurrence of various vPvM pollutants in urban water samples collected from Barcelona. Due to the high polarity of these compounds, their retention in solid-phase extraction (SPE) cartridges is ineffective. Therefore, a preconcentration technique utilizing evaporation was employed to concentrate the samples for analysis. For accurate identification and quantification of these contaminants, High-Resolution Mass Spectrometry (HRMS) was chosen as the most promising instrumentation. Specifically, the Q-Exactive (Thermo Fisher) hybrid quadrupole- Orbitrap system coupled with an Acquity LC (Waters) was utilized. HRMS offers several advantages, including the ability to perform retrospective analysis and detect compounds forwhich standards are currently lacking. By employing this comprehensive approach, our study aims to contribute to the understanding of the presence and potential risks associated with vPvM pollutants in urban water. The results obtained will provide valuable insights for water resource management and the development of appropriate strategies to protect the quality of our water supply.

## Acknowledgements

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## P45

# BIOGENIC ORIGIN OF PRESERVED ORGANIC MOLECULES IN VOLCANIC CAVES BY ULTRA-HIGH RESOLUTION ANALYTICAL PYROLYSIS AND CHEMOMETRICS

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Siliceous speleothems, formed by mineral deposition in lava tubes, are vital for understanding past volcanic eruptions, assessing environmental conditions, and investigating extreme life forms. Molecular characterization of cave samples is also relevant, needing the use of cutting-edge analytical techniques. The analytical pyrolysis (Py) coupled with exact-mass spectrometry (Py-GC/Q-TOF) is a promising one. Analytical Py involves thermal decomposition of organic matter (OM) at high temperatures without oxygen, enabling chromatographic separation and providing valuable information about complex mixtures' composition and molecular structure, including natural and synthetic macromolecules. Advantages of analytical Py include minimal sample preparation, small sample sizes, and detection of various organic materials, even those with low solubility or resistance to degradation. This technique facilitates rapid analysis of scarce or unique organic samples found in extreme environments and Mars analogues. Ultra-high resolution analytical pyrolysis (UH-Py) allows the identification of numerous distinct organic molecules in a sample without pre-treatment. However, due to the large number of chemical structures discovered, investigating each molecule separately is unfeasible. To address this, novel graphical-statistical tools are needed to identify primary chemical and biogenic families in a sample and investigate microorganisms' adaptations to environmental or anthropogenic factors. This communication introduces the integration of UH-Py with multivariate statistical analysis and graphic-statistical tools as an innovative strategy for unsupervised classification and visual analysis of preserved organic matrices in siliceous speleothems from diverse lava tubes. The study focuses on samples from Selvagens Islands, La Palma (Canary Island), Easter Island, and the Galapagos Islands. By combining these analytical techniques, it becomes possible to identify biosignatures, such as microorganisms, and biomarkers indicative of paleoenvironmental and anthropogenic changes, such as wildfires or ecocide. This approach offers a valuable insight into the complex organic compositions within the speleothems and their implications for understanding the history and conditions of volcanic environments.

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## P46

# DIRECT CHARACTERIZATION OF MICROPLASTICS FROM ALENTEJO COAST BY HIGH-RESOLUTION ANALYTICAL PYROLYSIS

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Plastic production has skyrocketed since the 1950s, resulting in over 8.3 billion tons of plastic generated and a projected 1500 million tons by 2050. Plastics have become pervasive but also environmentally impactful, contributing to CO2 emissions and marine pollution. Every year, 4.8 to 12.7 million metric tons of plastic waste enter the ocean, microplastics are small fragmented pieces (< 5 mm) of fragmented plastic debris, microbeads, and fibres, becoming a persistent pollutant in the sea and beaches. They absorb persistent organic pollutants (POPs) and Polybutylene terephthalate (PBTs) and can harm marine life by acting as carriers for pollution. Land-based sources account for 80% of microplastic pollution, while the fishing industry contributes 18%. In this work, we have sampled polluted sand at two depths (0–10 and 10–20 cm) at two sites in Lagoas de Santo André e da Sancha Nature Reserve, Alentejo coast (Portugal): close to the sea (P) and close to the lagoon (L) within a transect of 1.3 km with a separation of 150 m among sites. The main goal of this study was to monitor and identify the type of plastic at the molecular scale to understand its occurrence, composition, and impact to aid in the development of mitigation strategies and a better understanding of their environmental impact. In identifying microplastics techniques such as Fourier-transform infrared spectroscopy, Raman spectroscopy, and Thermogravimetry are commonly used. Another technique widely used in microplastic molecular characterization is nominal mass analytical pyrolysis (Py-GC/MS). Pyrolysis techniques have well-known advantages such as the requirement of small sample size with little or no preparation, being therefore convenient for inexpensive and relatively rapid analyses of scarce or little samples. Here, a novel exact-mass high-resolution analytical pyrolysis (Py-GC/Q-TOF) technique is described and -to the best of our knowledge- for the first time used to identify the constituent monomers and additives in microplastics.

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## P47
# ANALYSIS OF BIOPLASTICS' BIODEGRADATION PRODUCTS BY LC-HRMS

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The global bioplastics market is experiencing consistent growth due to escalating demand for sustainable products and strong policy support for the bioeconomy. Additionally, the bioplastics industry is actively engaged in continuous research and efforts to create innovative materials with enhanced properties and novel functionalities [1]. Polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) have emerged as preferred choices due to their versatility and viable alternatives to conventional plastics. However, it is important to acknowledge that many commercial bioplastics require pre-degradation before complete removal from the environment. Some bioplastics persist as biomicroplastics (bioMPLs), whichcan exhibit similar properties to conventional plastics and decompose more rapidly [2]. These ecological aspects, along with potential toxicity on microorganisms and human health, necessitate further research and investigation in the field. In this outlook, the main objective of this work has been to investigate the biodegradation of two selected bioplastics (PLA and PHB) and two commonly used consumer products made of the above mentioned polymers (PHB - plastic bags and PLA-single use knives). All the materials have been exposed under marine controlled microcosms for two months emulating Mediterranean conditions. Samples were taken weekly, purified and concentrated by solid-phase extraction procedure and, finally, analyzed by means of LC-HRMS equipped with an HESI source working in negative andpositive, separately [4]. All the data was acquired by full scan and data dependent scan, in parallel, at a resolution of 70,000 FWHM. The raw data has been processed by Compound Discoverer 3.1, where more than 15,000 tentative compounds/or interesting masses (m/z) have been postulated. These results have been manually refined reducing the tentative identification to less than 500 compounds. The study showed leaching of bioplastic additives like phthalates and adipates, which decreased over time. PLA monomers increased, and PHB bags degraded faster than PLA knives. This highlights the need to study the effects of biopolymers and new bioplastic consumer items on the environment.

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## P48

# Quantitative method for the analysis of microplastics in natural and drinking water by Py-GC-MS

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Nowadays, the presence of microplastics in aquatic environments raises concerns due to their potential adverse effects on ecosystems and human health. However, standardized methods for the reliable analysis of microplastics are currently lacking, and the concentrations of microplastics in water spanned to 10 orders of magnitude depending on the study [1].

In this study, an analytical method for the analysis of microplastics using pyrolysis gas chromatography mass spectrometry (Py-GC-MS) has been successfully developed. This methodology allows for quantitative (in terms of µg/L), fast (20 minutes per sample) and direct analysis (without the need for sample pretreatment) of the 7 main types of microplastics in water samples. The methodology was validated, and satisfactory quality parameters were achieved for all the compounds. Furthermore, the developed methodology was applied to determine the concentrations and distribution of microplastics in the drinking water supply network of the urban area of Barcelona, that provide drinking water to 3.000.000 inhabitants.

Therefore, the developed methodology provides a valuable tool for monitoring microplastics throughout drinking water supplies, including both drinking water supply networks and aquatic resources as rivers or wells. This methodology anticipates the upcoming European regulations on pollutants in drinking water by proposing a routine-applicable methodology for drinking water quality laboratories.

**Acknowledgements**
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## P49

# LC-HRMS/MS METHODS FOR EFFECT-DIRECTED ANALYSIS OF CECs IN WATERS

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Contaminants of emerging concern (CECs) are chemical substances widely dispersed and ubiquitous in the environment due to their continuous use and low removal during waste treatment. There is no regulation on the emission of CECs in the environment and little is known about their potential adverse effects on organisms exposed.

We focused on the assessment of polar organic CECs in terrestrial waters aiming to identify key drivers of toxicity in complex environmental samples. To address the complexity of mixtures of CECs present in waters, we are developing Effect-Directed Analysis (EDA) monitoring tools. EDA combines adverse biological reaction testing and stepwise fractionation with chemical analysis to prioritize biologically active CECs in waters.

For chemical identification of CECs, we developed Suspect Screening (SS) and Non-Target (NT) analytical methods based on Ultra-high Performance Liquid Chromatography (UPLC) coupled to High-Resolution Mass Spectrometry (HRMS). We used Reversed Phase Liquid Chromatography mode (RPLC), using a C18 chromatographic column, coupled to a HR hybrid mass analyzer (Q-Exactive Orbitrap, Thermo Fisher Scientific) interfaced with an Electrospray Ionization Source (ESI) working in positive and negative ionization modes for broad-range screening of unknown organic polar CECs in water extracts. Data-Independent Acquisition (DIA) and Data-Dependent Acquisition (DDA) modes were evaluated in the Orbitrap analyzer as SS and NT analytical methods, respectively.

The Compound Discoverer 3.1 software (Thermo Fisher Scientific Inc., Waltham, MA) was used to process the complex raw data obtained. Different workflows modifying processing parameters (including RT alignment, mass correction, peak selection, grouping signals (e.g., isotopes and adducts) belonging to the same molecular structure; and cut-off values for signal intensity or signal-to noise ratio (S/N), for RT or m/z) were tested for the optimal identification of bioactive CECs in the SS and NT approaches developed.

EDA using HRMS-based analysis is growing popularity in environmental quality assessment of water bodies as a powerful tool for prioritizing toxic CECs. Though being challenging and time consuming, SS and NT analytical methods are key approaches to disentangle the associated risks of the thousands of known and unknown CECs that are being continuously released to the environment.

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## P50

# OCCURRENCE OF PERSISTENT ORGANIC POLLUTANTS IN BRAZILIAN MANGROVES FROM BRASIL

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Due to their ubiquitous nature, persistent organic pollutants (POPs) have been detected in ecosystems at all latitudes. The presence of POPs in Brazilian mangroves, however, has been scarcely studied despite this unique ecosystem is one of the most widespread in Brazil and provides a wide variety of vital ecosystem services. Therefore, within the framework of a broader research in Brazilian mangroves, here we focused on the assessment of POPs at different depths in sediments from this ecosystem.

In April 2022, five depths were collected of five sediments from the Suricuju and Amazonas rivers and tributaries in the state of Amapá in Brazil. The total of 25 samples were analyzed for PCBs, PBDEs, PCDD/Fs, PeCB, HCB, DDTs, HCHs, and endosulfan. Twenty grams of lyophilized samples were spiked with $^{13}$C-labeled standards and Soxhlet extracted. A first purification of the extracts was based on GPC. Subsequently, two aliquots were generated and underwent further purification by means of the automated system DEXTech+ (LCTech GmbH, Dorfen, Germany) with different sorbent phases. One purified aliquot was used for the analysis of organochlorine pesticides (OCs) and the other one for the rest of target POPs.

PCBs, PBDEs, and PCDD/Fs were quantified by GC-HRMS on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). Positive electron ionization (EI+) was used in selected ion monitoring mode (SIM) mode at 10,000 resolving power. OCs were analyzed with a GC-MS/MS system (Agilent, Palo Alto, CA, USA) composed of a 7010B QqQ spectrometer with an EI high efficiency source, coupled to a 7890B chromatograph. Acquisition was carried out by multiple reaction monitoring (MRM) mode with two MRM transitions for each analyte. Quantification was based on the isotopic dilution technique.

Target pollutants were found with the overall abundance: ΣDDTs > ΣPCBs, PeCB, HCB, ΣHCHs > Σendosulfan > ΣPBDEs > ΣPCDD/Fs. Significant differences across depths were observed for some sediments in their contents of OCs, likely associated with their transport by the Amazon River and atmospheric wet deposition. Conversely, no significant differences were found regarding ΣPCBs, ΣPBDEs, and ΣPCDD/Fs. Furthermore, the current (eco)toxicity risk level was low, although the indices used to assess it do not consider important factors such as the bioavailability of POPs.

## P51

# DETERMINATION OF ORGANOPHOSPHATE FLAME RETARDANTS IN RIVER WATER AND WASTEWATER EFFLUENTS USING SOLID-PHASE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Organophosphate esters (OPEs) are a class of emerging pollutants that are widely used as flame retardants and plasticizers in numerous industrial and household products, such as plastics, polymers, textiles, anti-foaming agents, and electronics. Since the restrictions and phasing out of the uses of polybrominated diphenyl ethers (PBDEs) in the 2000s, OPEs have increasingly been produced around the world to replace PBDEs. Nevertheless, some OPEs and their degradation products have the potential of carcinogenicity (chlorinated OPEs), neurotoxicity, contact irritability, endocrine disruption, and adverse effects on the reproductive system of living organisms. Since these compounds are often added to materials, rather than chemically attached to them, OPEs are easily released into the environment, posing a threat to human health and wildlife. Therefore, OPEs are ubiquitously present in influents and effluents from wastewater treatment plants (WWTPs), surface water, groundwater, and even drinking water, at concentrations ranging from ng/L to low µg/L levels. Therefore, it is necessary to develop sensitive and selective methods to know the risk associated with OPE exposure and to better understand their behaviour and distribution in aquatic environments.

The present work aims to develop a reliable analytical method for the routine determination of organophosphate esters at ng/L levels in river water and influents and effluents of wastewater treatment plants (WWTPs) based on solid-phase extraction combined with gas chromatography-mass spectrometry (GC-MS). For this purpose, an SPE method was developed by loading 500 mL of a water sample into an Oasis HLB cartridge (500 mg, 6 mL) and eluting the target compounds with 20 mL of ethyl acetate. Operational MS parameters were carefully optimized to achieve the highest sensitivity and selectivity on the determination of the target compounds working in selection ion monitoring mode. Quality parameters of the methods provided good linearity, recoveries higher than 90% for all the compounds, and excellent repeatability (RSD% < 14%) and trueness (RE% < 12%). In addition, the SPE GC-MS method provided low limits of quantification (mLOQs) in the range of 0.6-62 pg/L. The methods have been successfully applied to the analysis of river waters and effluents and influents of wastewater treatment plants located within the metropolitan area of Barcelona. The results obtained showed the presence of the target OPEs at concentrations ranging from 2.18 to 133 ng/L in river waters and between 7.20 and 2,687 ng/L in effluents of WWTPs, being the main compounds detected: tris(2-chloroisopropyl) phosphate (TCPP), tris(2-butoxyethyl) phosphate (TBOEP), and 2-ethylhexyl diphenyl phosphate (EHDPP).

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## P52

# IN SILICO PREDICTION AND LC-HRMS ANALYSIS OF BISPHENOL METABOLITES IN URINE SAMPLES

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The presence of bisphenols in the environment has been investigated in several studies over the past decades as they represent a group of endocrine active substances that can negatively affect the endocrine system of organisms. Moreover, the list of bisphenols congeners is progressively growing, as manufacturers are constantly creating new functional analogues with similar properties to the previous ones in order to circumvent the legislative restrictions proposed by different institutions on these forbidden compounds. Nowadays, up to 20 types of bisphenols with different functions, such as anticorrosion agents, raw materials for plastics and polymers, etc., can be counted.[1] Their presence in the environment is ubiquitous, as they can be present in everyday items such as plastics, thermal papers, etc., so, exposure of these pollutants to humans is constant.

In addition to the large number of bisphenols daily used by humans, several other chemical species derived from them are generated when these compounds are metabolized by living organisms. In the case of humans, once in the organism, these compounds can be transformed into metabolites that could be also be harmful, of which there are not many toxicological studies. In order to predict these metabolites, different software have been designed to obtain their chemical structure in silico.

Currently, some targeting studies have been done for known metabolites of the most common bisphenols, such as glucuronide, sulphate or glutathione conjugates.[2] In addition, different LC-HRMS based method for suspect screening for metabolites in urine have been performed.[3]

Under the above theoretical framework, the following research has been carried out with two main objectives. On the one hand, the in-silico prediction of different metabolites of 20 bisphenols present in our everyday life and which could normally be found in the environment has been carried out by different software destined to this function, thus creating a list of supposed compounds coming from bisphenols. On the other hand, a liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) method using a Thermo Fisher Q-Exactive Orbitrap MS system has been developed to quantify 20 bisphenols, but also to confirm the presence of the in-silico predicted metabolites in human urine samples.

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## P53

# Direct injection analysis of haloacetic acids in treated wastewater and drinking water by hydrophilic interaction chromatography coupled to mass spectrometry

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Haloacetic acids (HAA) are a group of disinfection by-products (DBPs) with toxic properties that are formed as a result of water disinfection with chlorine. Due to their carcinogenic and mutagenic effects, the EU Drinking Water Directive [1], the US-EPA [2] and the WHO drinking water quality guidelines [3], regulate/recommend maximum levels for HAAs in drinking water (in treated wastewater, there is not any available legislation). Commo target HAAs are dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), dibromoacetic acid (DBAA), dichloroacetic acid (DCAA), dichlorobromoacetic acid (DCBAA), dibromochloroacetic acid (DBCAA), monochloroacetic acid (MCAA) and monobromoacetic acid (MBAA). The sum of DCAA, TCAA, DBAA, MCAA and MBAA is commonly referred to as $HAA_5$ in legislation. This work presents a rapid and simple method for the determination of 9 HAAs in chlorinated wastewater effluents (WW) and drinking water (DW) using the direct injection (DI) technique, a feasible alternative to traditional and time-consuming solid-phase extraction (SPE). The determination was performed by liquid chromatography coupled to triple quadrupole tandem mass spectrometry (LC-QqQ-MS/MS) using the HILIC mode. This is the first approach analyzing the aforementioned 9 HAAs by DI using HILIC. The proposed method was successfully validated on WW and DW at two concentration levels (10 and 100 µg/L). Recovery was always in the range 84-113 % ($n$=4) with relative standard deviations (RSD) <21 % (intra- and inter-day precision, $n$=3). Limits of quantification (LOQ) ranged from 0.25-25 µg/L. The method was applied to the analysis of real samples including: 4 chlorinated wastewater effluents, 15 drinking water samples and 1 chlorinated groundwater sample. Extremely low concentrations were detected in the drinking water samples (0.4-9.0 µg/L), always below the maximum levels for those regulated HAA. In contrast, wastewater effluent samples showed higher levels (89-2491 µg/L).

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## P54

# ASSESSMENT OF BIOACCUMULATION OF 12 SELECTED TIRE RUBBER ADDITIVES IN EARTHWORMS AND SOIL USING QUANTITATIVE LC-MS/MS AND SCALED-DOWN QUECHERS

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Tire rubber additives (TRAs) are manufactured compounds widely used as antioxidants and antiozonants in the rubber industry. A myriad of dissolved anthropogenic synthetic chemicals, including TRAs, are found in different environmental matrices and recent studies attempt to identify the compounds interaction with matrix associated biota. Recently, 6-PPD-Quinone, a transformation product of a common rubber antiozonant, 6PPD, has been found to be the cause of the acute mortality of coho salmon in Canada. However, no information is currently available on the interaction and toxicity of TRAs in the agro-ecosystem or with macroinvertebrates.

The earthworm *Lumbricus terrestris*, is a common anecic key species living in natural soils used also as bioindicator in soil pollution assessment. They are good indicators of pollution, due to their sensitivity to contaminants. Furthermore, because of their high soil activity, earthworms, which represent 80% of the total soil biota, contribute to the degradation of organic contaminants in soil and plants, but also redistribute them by moving from the deepest areas to the roots. Additionally, earthworms have proven to be a suitable species for measuring soil contamination levels due to their high interaction with soil and their ability to bioaccumulate a variety of anthropogenic organic pollutants. The present work aimed to evaluate the bioaccumulation of 12 different rubber additives in earthworms and soil under controlled conditions. TRAs in earthworm tissues and in soil were extracted by using a modified QuEChERS method whereas the detection was performed using a Waters XEVO TQ-S triple quadrupole mass spectrometer.

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# P55

# MULTI-TARGET ANALYSIS OF POPs IN PASSIVE AIR SAMPLES TO SUPPORT THE GLOBAL MONITORING PLAN UNDER THE STOCKHOLM CONVENTION

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Article 16 of the Stockholm Convention on Persistent Organic Pollutants (POPs) establishes the need to implement a Global Monitoring Plan (GMP) for POPs to evaluate the effectiveness of reduction/elimination measures taken under the Stockholm Convention [1]. To this end, UNEP with financial support from Global Environmental Facility (GEF) has implemented the 2nd phase of the POPs-GMP project under the Convention in 42 countries from Africa, Asia, Pacific and Latin America and Caribbean countries (GRULAC). The project activities included, among others, sampling and data generation of air and water as core matrices for environmental occurrence and transport and human milk or human blood for human exposure [2].

Air samples were sampled throughout two years in ambient air in the 42 participating countries using passive air samplers (PAS) equipped with polyurethane foam (PUF) disks. Each participant country, selected a site, preferentially at a meteorological station, as a monitoring site that met the criteria described in the GMP guideline [3]. PAS samplers were deployed from 2017 to 2018 with four 3-months exposure period. Once the PAS exposure period ends, chemical analysis from 11 countries in Latin American and Caribbean region (GRULAC) were performed in Dioxin Laboratory by GC coupled to high resolution mass spectrometry (GC-HRMS) and using isotopic dissolution method for quantification purposes. The compounds included were PCDD/F, PCB, PBDE, toxaphenes, organochlorinated pesticides, HBCDD, PBB-153 and short chain chlorinated paraffins.

The Dioxin Laboratory of the IDAEA-CSIC actively cooperate with UNEP in the implementation of the GMP project as an expert laboratory in collaboration with MTM (Örebro, Sweden), CVUA (Freiburg, Germany), Vrije Universiteit (Amsterdam, the Netherlands) and RECETOX (Brno, Czech Republic) with the collaboration of UQ Australia only for Pacific Islands, LATU (Uruguay) as executing agency and BRS and WHO (Geneva, Switzerland) as stakeholders.

Results achieved in this project represents a large POPs concentration database, mainly located in rural zones. All data points obtained in this work were used to follow up changes in POPs concentrations regionally with time and to periodically evaluate the effectiveness of measures taken under the Convention.

**Acknowledgements**

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## P56

# INDOOR AIR SAMPLING AND ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS BY GC-MS/MS

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The European project InChildHealth integrates health, environmental, technical and social sciences research to identify determinants for Indoor Air Quality (IAQ) and evaluate their impact in environments occupied by school children in cities from seven countries from Northern, Central and Southern Europe. The project focuses on chemicals, particle concentrations, microorganisms and physical parameters in schools, homes, and sports halls. The IAQ of these environments determines the dose received by the children and may directly influence their health and well-being. An environmental epidemiological study and controlled interventions conducted in schools in three European cities will assess the health effects of multipollutant airborne exposures on respiratory infections, allergies, and neurological and cognitional symptoms.

Here we present the sampling and sample-handling methodology for multi-compound analysis; among them polycyclic aromatic hydrocarbons (PAHs), with the use of SPE cartridges and low-volume pumps. The current presentation focuses on the analysis of PAHs by GC–MS/MS using an Agilent 7000 Series Triple Quad equipped with a HP-5MS 30 m capillary column. The instrument operated under electron impact ionization while multiple reaction monitoring (MRM) mode was used for acquisition. The method was applied for 16 targeted compounds with instrumental limits of detection ranging between 0.05 and 1.5 pg/uL injected. In previous studies, these semi-volatile compounds were linked to basal ganglia and attention-deficit hyperactivity disorder symptoms in primary school children, even at levels well below the legislated annual target levels established in the European Union [1].
For the study, we perform blank, recovery and breakthrough tests for the sampling and analytical methodologies, and collected and analyse indoor samples to determine concentrations of PAHs.

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# P57

# Relevance of reductive photo- and bio-transformation processes in intermittent Mediterranean river

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This work aims at investigating specific attenuation pathways of pharmaceuticals and pesticides in Mediterranean intermittent rivers by combining lab- and field-scale studies. These environmental settings, which are rich in organic matter due to the lack of dilution of WWTP effluent discharges and rich in Cu due to repeated spraying of Bordeaux mixture, have been poorly investigated for pharmaceuticals attenuation so far.

Poorly photodegradable and biodegradable pharmaceuticals such as fluconazole, oxazepam and venlafaxine or pesticides such as fluopyram and trifloxystrobin were found to attenuate in two river stretches with short hydraulic residence times (< 3 h). Lab-scale photodegradation experiments under simulated solar irradiation and biodegradation using column experiments helped for a better understanding of the mechanisms of degradation. This was made possible thanks to the implementation of non-target screening workflows employing liquid chromatography high-resolution mass spectrometry (LC-HRMS) for the tentative identification of suspected or unknown transformation products without the use of reference standards.

Elucidation of transformation pathways of selected pharmaceuticals and pesticides revealed reductive transformation routes including eter cleavage, reductive dehalogenation and deamination. Photoreductive transformation routes were ascribed to the formation of a Cu/Fe composite material under solar irradiation with photocatalytic properties. The role of Cu was to trap the electron in the conduction band of the iron-based photocatalyst, which promoted separation efficiency of electron-hole pairs as well as enhanced photoreduction processes at the expense of oxidation ones [1].

Reductive biotransformation routes were related to redox zonation in simulated hyporheic zones. Interestingly, several compounds including venlafaxine and fluopyram showed increased biodegradation rates after the desiccation period. Faster dissipation could indicate that bacteria capable of degrading these compounds were ubiquitously present and intermittent flow could stimulate short term biodegradation of pharmaceuticals.

**Acknowledgements**
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# P58

# Characterization of pesticide contamination and environmental impact in Doñana and Tablas de Daimiel National Parks

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National Parks are protected areas which are nowadays threaten by the agricultural activities carried out in the surrounding areas. These activities involve the application of pesticides which represent a huge contribution to the overall contamination of National Parks, especially organochlorine pesticides or pyrethroids insecticides, as well as other pesticides currently prohibited but still present in the environment and possibly, used illegally. In view of this situation, we developed a methodology to determine the presence and fate of nonpolar pesticides in water and sediments from the Doñana and Tablas de Daimiel National Parks in Spain, and we evaluated the risk that their presence may pose to non-target organisms in the aquatic environment which is constantly exposed to these pollutants.

Twenty sampling points were chosen in both areas, including control areas, greenhouses, vineyards, olive groves, rice fields, and other crops in the period between April and July 2021. The methodology was developed for the analysis of 33 pesticides in water and 27 pesticides in sediments [1] by means of gas chromatography coupled to tandem mass spectrometry (GC-MS/MS), obtaining high sensitivity and selectivity for each compound.

In the Doñana National Park, the results showed the presence of 10 pesticides in water samples and 18 pesticides in sediment samples, with levels ranging between 8 and 601 ng/L and 7.5 to 326 ng/g, respectively, while in the Tablas de Daimiel National Park, pesticide levels reached lower concentrations (up to 276 ng/L in water and 102 ng/g in sediments). The pesticide profile is similar in the two Parks, with the predominance of pyrethroid contamination, in particular cyhalothrin, fenvalerate and permethrin in water samples and organochlorine pesticides in sediment samples. Some detected pesticides are not allowed for their use, suggesting possible illegal practices. The impact assessment revealed a moderate/high risk for individual pyrethroids and a general high risk in almost all the samples due to the contribution of each compound to the overall contamination. These results highlight the need of studying possible synergistic effects of pollutants that can negatively affect non-target organisms, and focus on the possible impact on the reproductive capacity of the organisms living in the National Parks to preserve their conservation.

**Acknowledgements**

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## P59

# OPTIMIZATION OF A SOLID PHASE EXTRACTION PROCEDURE FOR THE SIMULATENOUS ANALYSIS OF PRIORITARY AND EMERGING CONTAMINANTS IN CATALAN SEAWATER

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Priority Substances (PSs) and Contaminants of Emerging Concern (CECs) have been a focus ofstudy during the last decades due to their presence and the damaging effects they have on the aquatic ecosystems and human health [1]. These compounds are used in a broad range of fields, from agriculture to medicine, thus they are easily dispersed in seawater.

In Catalan waters, the RD 817/2016 and UE 2020/1161 regulate the presence of PSs and CECs, respectively. In order to quantify the concentration of different pesticides, drugs, plasticizers,biocides, and surfactants in seawater from the Littoral Coast and Ebro Delta bays, a method based on Solid Phase Extraction (SPE) has been optimized. The main goal is to extract 47 different compounds with one single procedure.

To do so, four different trials have been done, basing the initial procedure on the one described by Fauvelle and Castro-Jiménez [2]. In all cases, HLB cartridges were conditioned with 2 cycles of 3 mL UPLC MeOH and 2 cycles of 3 mL UPLC Water. In the first trial, seawater samples were passed directly through the cartridges and, after extraction, the cartridges were rinsed once with 3 mL 0.01 N HCl and eluted with 2 x 3 mL MeOH:EtOAc 50:50 5% $NH_3$, 2 x 3 mL MeOH:EtOAc 50:50 2% F.A and 3 mL MeOH:EtOAc 50:50. In the second test, seawater was passed through the cartridge diluted 1:1 with MQ Water, rinsed twice with 3 mL 0.01 N HCl, and eluted analogously. In the third trial, the SPE was done following the second procedure, but the elution was changed for 2 x 3 mL MeOH. Finally, in the last test, the seawater samples were diluted with MQ Water, rinsed once with 3 mL 0.01 N HCl and eluted with 2 x 3 mL MeOH. All the eluates were evaporated to dryness, reconstituted with $H_2O$:MeOH 90:10 and analysed by LC-MS/MS.

The results show that the highest recoveries are obtained when the cartridges are rinsed once with 0.01 N HCl after passing all the seawater. Moreover, when the elution is done with MeOH, more compounds are extracted. It is also important to highlight that diluting the sample before the SPE helps diminish the amount of salt retained in the cartridge and, therefore, the amount of salt present in the analysis vial.

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## P60

# FINGERPRINTING VOLATILE ORGANIC CARBON SOURCES AND assessment of THEir role IN THE FORMATION OF OZONE AND SECONDARY ORGANIC AEROSOL IN RURAL, SUB-URBAN AND INDUSTRIAL AREAS

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Volatile organic compounds (VOCs) can be emitted from both anthropogenic and biogenic sources. These compounds play an important role in atmospheric chemistry by reacting with $OH^-$ and $NO_3^-$ radicals in the presence of sunlight and by their oxidation producing intermediates that can lead to the formation of secondary pollutants such as $O_3$ and secondary organic aerosol (SOA). Some of these compounds have toxic and carcinogenic effects involving a risk for the ecosystems and humans.

The present study is aimed to determine the main VOC sources in three different model locations of rural (Mollerussa), sub-urban (Vic) and industrial (Santa Perpetua de Mogoda) areas, and to assess their role in the formation of $O_3$ and SOA in different seasons.

Sampling was performed by air pumping through two types of stainless-steel sorbent cartridges. Thermal Desorption Gas Chromatography-Mass Spectrometry (TD-GC-MS) in full scan mode allowed the identification of 42 compounds by retention time and mass spectrum interpretation. Multivariate Curve Resolution - Alternatives Least Squares analysis of the obtained database was used for source apportionment in these sites. Using this approach, seven sources were identified, explaining 86% of the total variance: C1 volatile gasoline compounds from traffic emissions, C2 industrial effluents, C3 monoterpenes accumulated under low mixing layer height, C4 biogenic compounds in conditions of high mixing layer height, C5 agricultural fuel compounds, C6 low vapour pressure compounds and C7 diesel oil emissions. The highest diversity of sources was identified in the rural site. Ozone formation was dominated by anthropogenic VOCs in winter and by 1-pentene and isoprene in summer. SOA was mainly related to the occurrence of aromatic VOCs at all investigated sites and seasons.

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# P61

# STUDY OF CATALYTIC OZONATION AS AN ADVANCED TREATMENT FOR REMOVAL OF PHARMACEUTICALS IN WASTEWATER

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The presence of pharmaceuticals in the aqueous environment is a largely studied issue, due to the usual occurrence of these compounds in environmental waters and the risk associated. The main source of pharmaceutical contamination are wastewater treatment plants (WWTPs) discharges since the conventional treatments applied by WWTPS are not designed to entirely degrade these compounds [1]. Hence, it is necessary to implement advanced treatments to enhance the elimination of these contaminants. Catalytic ozonation treatments have demonstrated to be highly effective [2]. In this work, an LC-MS/MS multiresidue method developed for pharmaceuticals was applied to treated effluent wastewater (EWW) samples to study the effects that catalytic ozonation produces in real situations. Special attention was paid to the widely used analgesic ibuprofen. For this compound, an additional study was made to identify possible TPs formed during the above-mentioned treatment making use of LC-HRMS.

Both, distilled water and EWW samples were spiked with ibuprofen and subjected to different catalytic ozonation processes. Treated samples were collected at different time intervals throughout the process. These samples were analyzed firstly for ibuprofen and later for around 40 pharmaceuticals using direct injection followed by LC-MS/MS [3]. The results demonstrate that catalytic ozonation is an appropriate treatment to be implemented in WWTPs to improve the removal efficiency of these organic pollutants.

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## P62

# Occurrence and spatial distribution of pharmaceuticals in Mediterranean intermittent river basins

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Most river basins are subjected to several anthropogenic inputs, including wastewater treatment plant (WWTPs) discharges and urban and storm runoff waters, affecting water quality. Even though WWTPs are used to manage and treat wastewater, the WWTP effluent may still contain several wastewater borne pollutants including several contaminants of emerging concern (CECs), as conventional WWTP do not fully elimate these. In these cases, pharmaceuticals and personal care products (PPCPs) are often prevailing, as they are continuously introduced in surface water and hence are seen as pseudo-persistant contaminants in the aquatic environment where their contamination profiles are often quite constant in concentration [1]. In this study the presence of several CECs was analysed in five countries; Spain, France and Italy, located in Southern Europe and Algeria and Tunisia, located in Northern Africa. In Tunisia and Algeria, WWTPs are sometimes over-exploited and many industries directly release their wastewater to the river basins. In addition to this, the lack of regulations regarding CECs concentration in surface water and the limited monitoring makes it interesting to investigate their presence and impact. Hence, different intermittent rivers from each site were sampled and possible differences between Southern Europe and Northern Africa were investigated. Samples were extracted by means of a solid phase extraction procedure using a homemade multilayer mixed-bed cartridge containing a mixture of four different sorbents with different selectivity to cover a wide range of polarities. A total of 81 target CECs, selected based on their occurrence and ubiquity in the aquatic environment were screened and quantified using high-resolution mass spectrometry Q-Exactive Orbitrap. For the separation of the analytes, liquid chromatography was performed using Acquity UPLC HSS T3 column [2]. The presence and potential differences in contamination levels across the five countries was investigated. The studied river basins from France and Algeria reported lowest concentrations and statistical analysis were performed to study the potential differences per CECs class between countries, and the most remarkable class were the industrial compounds where significant differences between Tunisia and the other four countries were observed. Regarding specific contaminants, caffeine concentrations were outstanding in Tunisia compared to the rest of countries, and Italy presented remarkable concentrations of antihypertensives, with ibersartan and valsartan acid concentrations being statistically different from the rest of the studied countries.

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# P63

# Identification of volatile organic compounds responsible for odours in wastewater treatment plants by HS-SPME with GC-(Q)Orbitrap

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The population living near WWTPs can be greatly affected by air pollution from gaseous streams, especially when it manifests itself in the form of odours. Strategies used to treat water in WWTPs generate solid and gaseous waste that pollute the air due to the presence of many volatile rganic compounds (VOCs), which are the main causes of odour nuisance [1]. Although some of them could be found at low concentrations, odour thresholds, i.e. concentrations at which an average test person can no longer detect the odour, are in some cases several orders of magnitude lower. Consequently, it is essential to develop analytical methodology for the accurate determination, to identify them in order to contribute to design strategies for preventing their presence and spread in populated areas [2].

In first place, HS-SPME method in combination with GC-(Q)Orbitrap has been developed for the determination of VOCs in samples related with WWTP (air and water itself). A multivariate approach was used for the optimization of the following parameters: fiber type, sample volume, salt addition, extraction temperature and extraction time. The responses of a total of 65 VOCs present in a WW sample were used for setting the final optimized parameters: DVB/CAR/PDMS fiber using 3 mL of water sample with the addition of 0.6 g of NaCl at 45ºC for 60 min. The fiber was finally desorbed in the split/splitless injector of the GC-(Q)Orbitrap instrument at 280ºC for 5 min. Accurate mass full spectrum acquisition data was acquired, with the electron ionization source. Once the HS-SPME-GC-(Q)Orbitrap strategy was optimized, this was applied to a set of WW samples and the results obtained allowed to increase the list of compounds until a total of 138 tentative identified VOCs following a non-target processing workflow that includes the annotation in NIST library.

In second place, the investigation of a characteristic fingerprint from a WWTP located on the Mediterranean coast was faced. For this purpose, the air from 3 different points from the WWTP (primary decanter, biological reactor and air 100 m from the WWTP) were sampled using a static assembly that contains the SPME fiber and allows its contact with the flowing air during 1h. Results obtained showed the detection of around 80-120 VOCs in the different samples. Among them, only 5 were common in the three sampling points, and 26 VOCs were found to coincide in the two tanks. In this way, it is feasible to conduct an analysis to ascertain the compound that bears the most substantial influence in the perception of discomfort arising from odour, and in addition to establishing a distinct chemical signature for each WWTP.

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# P64

# FEASIBILITY OF DEEP EUTECTIC SOLVENTS FOR FAT REMOVAL IN THE ANALYSIS OF POLYCHLORINATED BIPHENYLS IN BIOTIC SAMPLES

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Polychlorinated biphenyl (PCBs) (POPs) are well-known persistent organic pollutants regulated by the Stockholm Convention because of their toxicity, persistence in the environment, capability for bio-accumulation and bio-magnification through food webs, and capacity for long-range transportation [1]. Conventional sample preparation methods in use for the determination of PCBs in fat-containing matrices, such as biotic tissues and fatty foodstuffs, are laborious multistep procedures that typically start with an exhaustive extraction of the analytes from the matrix to ensure proper detectability at the low levels currently detected in the environment. Due to their lipophilic nature, PCBs are usually co-extracted with relatively large amounts of lipids that should subsequently be removed before final instrumental determination by gas chromatography coupled to mass spectrometry (GC-MS) [2].

The present study evaluates the feasibility of different deep eutectic solvents (DESs) based on choline chloride (ChCl) for fat removal during the analysis of PCBs in biotic samples. A fortified pork meat sample containing 18% fat (fresh weight, fw) was used for method development. Once optimized, the proposed miniaturized methodology involved only 0.150 g of the freeze-dried sample and allowed complete fat elimination by treatment with 2.5 g of $[ChCl]:[Ox \cdot 2H_2O]$ 1:1 molar ratio at 60 °C followed by analyte back extraction with 4 mL of *n*-hexane. Satisfactory recoveries (above 87%) were obtained for the investigated priority PCBs, with a repeatability better than 5% and an intermediate precision lower than 14% [4]. The practicality of the new method for the intended determination was demonstrated by satisfactory comparison of the results obtained when analyzing the target compounds in naturally contaminated foodstuffs with different fat contents with those obtained applying a more conventional reference procedure [3].

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## P65

# OLEATES OF BOTANICAL CROPS AS INGREDIENTS IN ECO-COSMETICS

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Plants are a source of botanical ingredients with application in cosmetic formulations based on their traditional use. In recent years, growing scientific evidence confirms the cosmetic properties of various extracts and ingredients obtained from plants [1]. The market demands for products that include these phytochemicals-based formulations are increasing. In addition, their use involves the revaluation of co-products and by-products generated in the commercial exploitation of various plants in the agriculture, food and forestry sectors, and searches for complementary ways to use previously cultivated plants as spices or infusions, or even other wild ones. This is an innovative concept that gives extra value and facilitates the approach to a circular economy.

This study covered the following typical Galician organically cultivated botanicals: *grelo* flowers (*Brassica rapa var. Rapa*), yarrow petals (*Achillea millefolium*), laurel leaves (*Laurus nobilis*), *xesta* pudia flowers (*Genista florida*) and filipendula flowers (*Filipendula vulgaris*). Extracts from these plants contain valuable bioactive compounds such as terpenoids. This work has focused on the analysis of volatile compounds from oleates obtained at company level. To extract these substances, we propose a simple and rapid procedure based on solid-phase microextraction (SPME), in which different experimental conditions were tested, followed by subsequent desorption into a gas chromatograph coupled to mass spectrometer (GC-MS). The aim was to determine the aromatic profile of these samples.

In addition, the most remarkable properties of the phytochemicals present in the botanicals under study were investigated as valuable and multifunctional ingredients for cosmetic use [2], to design new original and ecological cosmetic preparations that respond to the current demands of society. Finally, it was checked if any of the identified fragrance compounds were included in the list of 82 substances declared as contact allergens by the SCCS [3].

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### P66

# Development of on-line aptamer affinity solid-phase extraction direct mass spectrometry for the rapid analysis of α-synuclein

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Simplifying the analytical procedure and enhancing detection sensitivity is a continuing demand in analytical chemistry, especially when analyzing peptides and proteins in minute amounts. Capillary electrophoresis-mass spectrometry (CE-MS) is a powerful microseparation technique for the analysis of polar and ionizable biomolecules. CE provides many advantages such as high separation efficiency and resolving power, while electrospray ionization-mass spectrometry (ESI-MS) allows detailed characterization, sensitive detection, and accurate quantification [1]. However, with the aim of simplifying the instrumental set-ups and speeding up the analyses, different authors have proposed to disregard the chromatographic or electrophoretic separation, to fully trust in the capabilities of direct MS detection [2].

Direct MS analysis of complex samples, such as biological fluids, typically results in poor outcomes due to matrix effect. Therefore, an appropriate sample preparation step is still necessary for sample purification [2]. In this study, we present on-line aptamer affinity solid-phase extraction direct mass spectrometry (AA-SPE-MS) for the rapid purification, preconcentration, and characterization of α-synuclein (α-syn) [2], a protein biomarker with various post-translational modifications related to Parkinson's disease. Valve-free AA-SPE-MS is easily implemented using the typical SPE microcartridges and instrumental set-up necessary for on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry (AA-SPE-CE-MS) [1]. The essential requirement is substituting the application of the separation voltage by a pressure of 100 mbar for mobilization of the eluted protein through the capillary towards the mass spectrometer.

The AA-SPE-MS method is optimized and validated, and its performance is compared with direct MS, CE-MS, and AA-SPE-CE-MS to disclose the advantages and disadvantages of AA-SPE-MS for the rapid and sensitive targeted analysis of protein biomarkers in biological fluids.

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## P67

# A Direct Comparison of Applying Helium & Hydrogen Carrier Gases with HS-SPME-GC-TOF-MS Analysis of Aroma Active Compounds in Whisky

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The use of solid-phase microextraction (SPME) hyphenated to gas chromatography and mass spectrometry (GC-MS), for the analysis of aroma active volatiles is widely accepted and applied as an effective technique to provide insights during characterisation, quality screening and process development of food and beverage raw materials and products.

Additionally, SPME is a relatively green analytical approach due to considerable reduction in solvent volumes, the amounts of sample and extraction time required.

With growing emphasis on green analytical approaches, increased analysis throughputs and reducing costs, there is significant interest in using hydrogen carrier gas instead of helium for GC-MS workflows. Helium costs have been increasing year on year in the last decade, whereas hydrogen can be safely supplied on-demand, via generators and allows superior gas chromatographic performance, enabling increased analysis speeds whilst maintaining or improving separation efficiency.

However, unlike helium, hydrogen is a reactive gas and the possibility exists for the formation of artifacts within the analytical system. Previously, a comparison of helium and hydrogen carrier gas, being used in conjunction with various SPME fibres, reported hydrogenation of unsaturated species to varying extents, when using hydrogen, depending on the fibre phase type and desorption conditions used. [1]

In this study we analysed the same whisky sample using helium and hydrogen, together with a range of SPME fibre phase chemistries and desorption conditions. A number of aroma-active compounds with varying degrees of unsaturation were chosen to investigate the occurrence and extent of hydrogenation between conditions. Additionally, the relative detection intensities and analysis precision of these compounds as well as other important aroma compounds were compared.

Here we report the degree of hydrogenation observed when using hydrogen and mixed-bed SPME fibre phases (i.e., CAR/DVB/PDMS), compared with 100% PDMS phases. Differences in extraction selectivity for some key aroma active compounds when using different SPME phases are also presented.

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## P68

# 3D-PRINTED BIOMIMETIC BLADE STIRRER FOR IN-VITRO ESTIMATION OF BIOAVAILABILITY DATA OF PLASTIC-BORNE EMERGING CONTAMINANTS

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3D printing technologies have opened new avenues in distinct sub-disciplines of analytical chemistry including sample preparation. In fact, 3D printed scaffolds are amenable to chemical derivatization/ modification to construct functional platforms that are fit for purpose.

In this contribution, versatile biomimetic platforms are fabricated by covalent attachment through a series of consecutive organic reactions of biomolecules, including phospholipids (phosphatidylcholine), membrane proteins (alfa-glycoprotein) and plasma proteins (human serum albumin) to 3D printed blades that are readily integrated into stirring devices. The idea behind is to mimic the chemical composition of cell membranes of the human small intestine and blood vessels for the in-vitro elucidation of molecular interactions with plasticizers and plastic additives, such as bisphenol A, diethyl phthalate, acetyl tributyl citrate or N-Butylbenzenesulfonamide from which estimation of human bioavailability data of accidentally ingested contaminants is aimed at. The extractability of the distinct biochemically modified blades (adsorbed concentrations will be calculated by HPLC/GC) will be introduced as a key parameter in multiple linear regression algorithms to which other molecular descriptors of the target compounds are to be accounted for. In-vitro bioavailability estimates will be correlated with in-vivo literature data to assess the goodnessof fit of the proposed models.

The 3D printed prototype features high versatility for accommodating a variable number of blades (up to four in the present design) with a tailorable blade geometry and surface area to vary the amount of immobilized biomolecules on the basis of the role of the distinct cell components on compound bioavailability.

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# P69

# IDENTIFICATION AND QUANTIFICATION OF MULTI-ANTIGENIC PEPTIDES ENCAPSULATED IN PHOSPHATIDILSERINE-LIPOSOMES FOR AUTOIMMUNE DISEASE TREATMENT

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In recent years, the prevalence of autoimmune diseases (AIDs) has increased, affectingindividuals of all ages [1]. Currently, available treatments are palliative and based on immunosuppressive strategies. In this regard, a novel nanotechnology platform involving phosphatidylserine-liposomes (PS-liposomes) is being developed by Ahead Therapeutics to achieve peripheric immunotolerance towards the specific encapsulated antigens. This is plausible using a physiological mechanism of tolerance generation by biomimicking apoptotic cells with PS-liposomes [2]. This strategy has demonstrated efficacy in different autoimmune diseases. In the case of Multiple sclerosis (MS) and Type 1 Diabetes (T1D), the feasibility of multi-antigenic peptides encapsulation in PS-liposomes is being studied to further increase the treatment's efficiency. For this purpose, different autoantigens previously related with MS and T1D diseases in the literature [3,4] were encapsulated in PS-liposomes. Among the critical quality attributes in liposome formulations, determining the encapsulation efficiency of each antigen is crucial as it can be closely related to the efficiency of the treatment.

In this study, a methodology capable of identifying and quantifying in a single run the different autoantigenic peptides within PS-liposomes was developed for each pathology by reversed-phase high-performance-liquid-chromatography with ultraviolet detection (RP- HPLC-UV). Due to the high similarities in the physicochemical properties of the different peptides, achieving good separation was challenging. Several HPLC parameters were evaluated, such as the stationary phase, column temperature, mobile phase composition, gradient conditions, and detection wavelength. Moreover, different methods to extract and quantify free and total antigens within PS-liposomes were tested to determine encapsulation efficiency. Finally, the optimized HPLC-UV method was transferred to reversed-phase high- performance liquid chromatography coupled to mass spectrometry (RP-HPLC-MS) to identify antigen's related substances and degradation products.

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## P70

# Natural deep eutectic solvents for the green analysis of biological matrices: Determination of testosterone and progesterone in saliva

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In bioanalysis, the search for fast, affordable, and non-invasive sample preparation procedures has become on the major priorities for clinical laboratories. In addition, to the extent possible, these procedures should be green and sustainable to reduce the environmental impact of the analytical process.

In this communication, a new method for the determination of testosterone and progesterone in human saliva is presented. The method is based on dispersive liquid-liquid microextraction (DLLME) as preconcentration approach, using a natural deep eutectic solvent (NADES) as extraction phase, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as measurement technique.

First, the molar ratio of the components of the NADES (*i.e.*, menthol and hexanoic acid) was studied to select the best composition for the extraction of the target analytes. Then, a multivariate optimization process was performed to evaluate the optimum conditions for the DLLME step, studying the optimum agitation method and evaluating the need for a disperser solvent, besides unifactorial studies of the agitation time and the ionic strength. With the optimum parameters, the method was validated obtaining good analytical features, such as low limits of detection (5-8 ng $L^{-1}$), enrichment factors of 9.9 and 13 for testosterone and progesterone, respectively, good precision with relative standard deviation below 15%, and relative recoveries between 93-108%. Finally, the method was successfully applied to saliva samples from different volunteers.

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# P71

# Development and comparison of SPE and DLLME methods for the determination of benzodiazepines and opioids in urine samples

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In recent years, the determination of illicit drugs has gained increasing attention for the scientific community since the consumption of drugs is nowadays considered as one of the main public problems, being the opioids and the benzodiazepines some of the most consumed drugs worldwide [1,2].
As a consequence of their consumption, these compounds can reach different parts of the human body, and in last year's methods focused to the analysis of these substances mainly based on chromatographic techniques in matrices as urine have been developed [3,4]. However, due to the complexity of this matrix and the low levels of concentration at which these drugs are usually present (in the ng/L or µg/L range), different sample pre-treatment strategies have been used [5]. The sample pre-treatment step has a relevant importance on the greenest of the analytical methods, for this reason on recent years, some pre-treatment techniques based on green analytical chemistry principles have been developed [6].
In this study, two different methods based on different pre-treatment strategies have been developed and compared for the determination of a group of benzodiazepines and opioids in urine samples. A method based on solid phase extraction (SPE) was developed using ExtraBond SCX as sorbent and 7 ml of 5% of $NH_4OH$ in methanol as elution solvent, achieving recoveries in the range between 9 and 107%. Another method, based on dispersive liquid-liquid microextraction (DLLME) was also developed, using 200 µL of chloroform and 500 µL of ethyl acetate as extractant and dispersant solvents respectively, provide recoveries between 14 and 86%. The second strategy can be considered greener according to the principles of the green analytical chemistry, since it requires low volumes of solvents, the extraction time is shorter, and the energy consumption is lower in comparison with the first method developed based on SPE.
The methods were validated on urine samples and then applied to 12 urine specimens from women who were starting a detoxification program, confirming the suitability of the developed methods in toxicological and forensic analysis. The analyzed urine samples showed that polyconsumption is a general trend for the women who initiated this detoxification program and among the different drugs the most usually detected were from different types, being the most habitual methadone, tramadol, morphine or diazepam.

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# P72

# HOLM OAK ACORN BIOACTIVE EXTRACTS AS ANTIFUNGAL THERAPIES IN FOREST PATHOLOGY

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Holm oaks (Quercus ilex) are infected by a fungus, Phytophthora cinnamomi, which causes the “holm oak drought” leading to massive death of absorbing roots. Fungal invasion of plants is usually accompanied by corresponding protective reactions resulting in the activation of phyto-defensive systems, such as the synthesis of polyphenols. These compounds are considered as phytoarsenals due to their recognized bioactivities[1]. The main objective encompasses the development of natural extracts with fungicidal, antimicrobial and antioxidant properties from acorns to treat plant infections, increasing their productivity.

Bioactive extracts will be obtained using simple and sustainable extraction methodologies such as MSPD, Matrix Solid-Phase Dispersion, with minimal solvent consumption, prioritizing GRAS, Generally Recognized Safe solvents. This technology generates minimal amounts of waste while allowing optimal and scalable extractions at industrial level. The feasibility of MSPD for quantitative and selective extraction of components from various matrices has been demonstrated through a series of application studies in a wide range of fields[2]. The qualitative and quantitative characterization of the bioactive compounds in these extracts will be performed using both liquid and gas chromatography, coupled to different mass spectrometry detectors including low and high resolution mass instruments, that allow to identify not only compounds of interest (target), but also to perform untargeted approaches identifying possible compounds not previously detected in the extracts with interesting properties.

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## P73

# ADVANCED EXTRACTION TECHNIQUES IN COMBINATION WITH HYDROPHOBIC NATURAL DEEP EUTECTIC SOLVENTS FOR THE IMPROVED EXTRACTION OF BIRCH (Betula sp.) BARK BIOACTIVES

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Nowadays, the recovery of high-value bioactives has emerged as a profitable and sustainable approach for revalorization of agri-food and forestry byproducts. Birch (*Betula* sp.) bark is discarded in large quantities by the wood industry and is a rich source of lupane-type triterpenoids with different biological and pharmacological activities [1]. Although solid-liquid extraction (SLE) has conventionally been applied for their recovery, advanced extraction techniques such as microwave-assisted extraction (MAE) or ultrasound-assisted extraction (UAE) have scarcely been explored for their faster and more efficient extraction [2,3]. Moreover, the potential advantages in terms of greenness provided by the combined use of these techniques with hydrophobic natural deep eutectic solvents (h-NADES) have not previously been evaluated.

The aim of this study was the optimization of MAE and UAE methods, using thymol:1-octanol (4:1 molar ratio) as solvent, for the simultaneous extraction of betulin (Bet) and betulinic acid (BAc). First, for a fixed sample: solvent ratio (1 g : 10 mL), SLE, UAE and MAE under identical operating conditions (60°C for 17.5 min) were compared, with UAE and MAE providing a better extraction performance over SLE (Bet: 24 - 47%; BAc: 22 – 24% respectively). A 3-level factorial experimental design was further used for the selection of the optimal MAE and UAE experimental conditions (62°C for 21 min and 61°C for 24 min, respectively), with MAE outperforming UAE by 51% for Bet and by 52% for BAc. Under these conditions, the extraction efficiency with the number of MAE cycles (1-3) was also evaluated. As 86% Bet and 85% BAc were recovered after the first extraction, cycle 1 was selected as a tradeoff to provide enough bioactive recovery and high throughput. Application of this optimized MAE method to six commercial birch bark samples evidenced the variability in the content of these bioactives, ranging 12-33 mg $g^{-1}$ for Bet and 0.85-2 mg $g^{-1}$ for BAc. Finally, AGREEprep software was used to evaluate the sustainability of the MAE method here developed. It can be concluded that MAE, by using h-NADES as solvent, is a promising approach for the sustainable and efficient extraction of birch bark bioactive triterpenoids within a biorefinery frame.

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## P74

# DISPERSIVE LIQUID-LIQUID MICROEXTRACTION WITH DEEP EUTECTIC SOLVENTS FOR THE DETERMINATION OF EMERGING CONTAMINANTS

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Emergent contaminants or contaminants of emerging concern (CEC) can be defined as naturally occurring, manufactured or synthetic chemicals or materials that have now been discovered or are suspected of being present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of living beings [1]. These compounds end up in waters and due to their low concentrations, preconcentration and separation are required before analysis.

In this study, we develop a dispersive liquid-liquid microextraction (DLLME) methodology using deep eutectic solvents (DES) as an extracting agent for the analysis of flumequine, naproxen, β-estradiol, 17-ethinylestradiol, triclosan and mefenamic acid. The separation and quantification of the compounds is performed with HPLC- DAD-FLD.

A deep eutectic solvent is a liquid formed from the interaction of a hydrogen bond donor and a hydrogen bond acceptor whose melting point is considerably lower than that of its individual components. Some of the main properties of DES are their thermal stability, low volatility, and modifiable polarity. Here, mixtures of thymol/lidocaine or menthol/lidocaine are tested for the DLLME of the target compounds using DES:acetonitrile mixtures.

The enrichment factor of the extraction method is optimized by design of experiments. Five variables (the time of agitation, time of centrifugation, ratio DES:acetonitrile, sample volume, and extractant volume) are considered initially. Only the two last ones are found to significantly affect the extraction.

In the validation step of the HPLC method, good linearity is found, with limits of quantification between 0.89 $mg\cdot L^{-1}$ – 2.98 $mg\cdot L^{-1}$ depending on the analyte. Intra-day precision (relative standard deviation) below 20% is obtained, with standard deviation for the retention time under 0.2 min.

The DES-DLLME method developed is applied to water samples taken from the Mapocho River (Santiago de Chile). After verifying the absence of the target compounds in the water samples, the enrichment factor of each analyte is calculated and the matrix effect is discussed.

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# P75

# EVALUATION OF MATRIX SOLID PHASE DISPERSION EXTRACTION FOR THE DETERMINATION OF PLASTIC ADDITIVES IN GILTHEAD SEABREAM

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Microplastics (MPs) and nanoplastics (NPs) are widely spread in the environment generating significant concern due to their potential impact on environmental health. In general, it can be said that the MPs/NPs are already part of the food chain by means of mollusks, arthropods, mammals, birds, amphibians, reptiles, fish, etc. These can ingest plastic fragments and/or entangled in them and drown, also can be injured or block the digestive organs reducing ingestion because of a false feeling of fullness, affected in its energy and nutrition, low growth rate, blocked enzyme production, decreased fecundity and on some occasions resulting in the death [1]. Moreover, once plastic particles are inside the animal, they can release their chemical additives, used to improve some properties of plastics and to eliminate or mitigate others that are undesirable [2] (Coleman, 2017; Murphy, 2003) because these are not chemically bound to the plastic matrix [3].

This paper highlights the development and validation of a straightforward solid-liquid extraction clean-up procedure in combination with a matrix solid-phase dispersion method using high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) detection enabling facile, precise, and reliable identification and quantitation of a total of six bisphenols and phthalates in gilthead seabreams. Under the optimized condition, the developed method showed good linearity (R2>0.993) for all target compounds. Recoveries obtained were between 70-92%. Relative standard deviations (RSDs) for reproducibility (intra-day) and repeatability (inter-day) were less than 9% and 10%, respectively. The validated analytical method was successfully used for the determination of mentioned bisphenols and phthalates in gilthead seabream samples, and could be used for routine analysis.

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# DEVELOPMENT OF ANALYTICAL METHOD FOR THE EXTRACTION AND DETECTION OF NONYLPHENOLS IN SEAFOOD BY HPLC-FL

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The number of emerging pollutants present in terrestrial and marine ecosystems has increased significantly in recent years. Some of the emerging contaminants include plastic additives such as ethoxylated nonylphenols and their derivatives. These products belonging to the family of alkylphenols and are used to manufacture detergents, dispersants, emulsifiers or moisturizers, in both industrial and domestic, plasticizers and some of their derivatives as ultraviolet light stabilizers in plastics. Approximately 500,000 tons of these compounds are produced in the world and 60% is discharged into the aquatic environment. Nonylphenols and their derivatives are some of the most outstanding environmental pollutants. Therefore, their toxicity on ecosystems, aquatic organisms and on food is of concern [1].

In this work, a method for the separation and quantification by HPLC-FL of seven nonylphenols has been developed: Nonylphenol (NP), Nonylphenol 40-Tergitol type 40 (NP40), Nonylphenol 9-Tergitol type 9 (NP9), Nonylphenol monoethoxylated (NPEO1) , Nonylphenol diethoxylate (NPEO2), 4-Nonylphenol (NPEO4) and 4-n-Nonylphenol monoethoxylate (4NP). Analytical separation of these seven nonylphenols has been achieved for the first time during this study, and there is no previous bibliographic record. This method has been applied to real liquid and solid samples, such as liquid from vacuum packed mussels and the liquid obtained during steam cooking of the mussels. Also, raw mussel samples have been analysed using the matrix solid phase dispersion technique.

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