SECyTA 2013

XIII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques

BOOK OF ABSTRACTS

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Hotel Beatriz Atlantis & Spa
Puerto de la Cruz, Tenerife
Canary Islands, Spain
October 8-11, 2013

CONTENTS

BIENVENIDA

La celebración por primera vez en Tenerife de la XIII Reunión de la Sociedad Española de Cromatografía y Técnicas Afines (SECyTA) ha sido una apuesta difícil en el periodo de grave crisis económica por la que atraviesa nuestro país, que sin embargo en su momento asumimos con la certeza de que con el esfuerzo y ayuda de todos, investigadores, patrocinadores y la propia Sociedad de Cromatografía, sería posible superar.

El resultado es este libro de resúmenes que ahora tienes en tus manos en el que figuran las contribuciones de investigadores y patrocinadores a SECyTA 2013, y que muestra el éxito de esta apuesta. Estamos seguros que durante estos tres días de reunión podremos disfrutar de los encantos de Tenerife, mientras compartimos resultados y experiencias en las diferentes áreas temáticas que se abordarán durante el congreso (medioambiente, agroalimentación, biotecnología, farmacia, etc.). La organización de este importante evento responde al trabajo conjunto del comité organizador, que en este caso asumimos dos centros de investigación como son la Universidad de La Laguna y el Consejo Superior de Investigaciones Científicas (CSIC), a la labor del comité científico formado por colegas de reconocido prestigio internacional y al trabajo de los miembros de la Junta de la SECyTA, quienes nos han asesorado con sus años de experiencia a la hora de elaborar un programa atractivo para todos los asistentes.

La estructura del Congreso contará con conferencias plenarias presentadas por relevantes investigadores de diferentes ámbitos científicos, comunicaciones orales y sesiones de pósteres. Este año hemos incluido por primera vez sesiones plenarias para jóvenes investigadores que esperamos sean muy atractivas para los futuros investigadores de nuestro país. Queremos resaltar el patrocinio de las casas comerciales que continúan apostando por la química analítica y las técnicas de separación, lo que nos ha permitido llevar a buen puerto este congreso, ofreciendo un buen número de becas para la asistencia de estudiantes, seguir manteniendo los Premios José Antonio García-Domínguez en su novena edición y el de usuarios de la microextracción en fase sólida (SPME), así como ofrecer un estimulante programa social de actividades.

Queremos manifestaros nuestra gratitud por vuestra participación en esta decimotercera reunión y desearos que la estancia en estos días de congreso responda a vuestras expectativas, así como ponernos a vuestra disposición en todo aquello que necesitéis. Esperando que recordéis esta reunión con alegría y satisfacción, os deseamos lo mejor.

Un cordial saludo y ¡bienvenidos a Tenerife!

Chairman
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GENERAL INFORMATION

CONFERENCE VENUE

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38400, Puerto de la Cruz, Tenerife
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Tel: +34 922 37 45 45
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CONFERENCE LANGUAGE

The official languages of the meeting are Spanish and English.

SYMPOSIUM REGISTRATION DESK

The registration desk is located on the 1st floor of the hotel and will be open the following times:

Tuesday October 8, 2013	12:00 pm – 13:00 pm and 14:00 – 19:00 pm
Wednesday October 9, 2013	08:30 am – 13:00 pm and 14:00 – 19:00 pm
Thursday October 10, 2013	09:00 am – 13:00 pm and 14:00 – 19:00 pm
Friday October 11, 2013	09:00 am – 14:00 pm

IDENTIFICATION BADGES

The Organizing Committee requests everybody to wear their identification badges always at the Congress to get admittance to the scientific and social activities.

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DARK PURPLE
Participants
LIGHT PURPLE
Scientific Committee

GREEN
Exhibitors

BOOK OF ABSTRACTS

The book of abstracts is delivered on the USB memory stick upon registration.

EXHIBITION

Exhibits are located on the 1st floor, Beatriz Room. Exhibition hours:

Wednesday October 9, 2013	10:30 am – 18:30 pm
Thursday October 10, 2013	09:00 am – 18:30 pm
Friday October 11, 2013	09:00 am – 13:00 pm

POSTER SESSIONS

Poster sessions and most coffee breaks are located on the 1st floor, Beatriz Room.

Poster boards are labeled with the number corresponding to the abstract number in the Scientific Program.

Presenters must be in attendance at their poster board on the day of their poster session:

Wednesday October 9, 2013	Poster Session 1	Posters PS1-01 to PS1-54
Thursday October 10, 2013	Poster Session 2	Posters PS2-01 to PS2-56

Poster set-up: PS1 posters can be set up on Wednesday morning before 11:00 am. PS2 posters should be set up on Thursday morning before 10:15 am.

Poster tear-down: PS1 posters should be removed on Wednesday evening after 18:30 pm. Any poster remaining after 19:30 pm will be discarded. PS2 posters should be removed on Friday morning before 11:00 am. Any poster remaining after 14:00 pm will be discarded.

SPECIAL ISSUE IN JOURNAL OF CHROMATOGRAPHY A

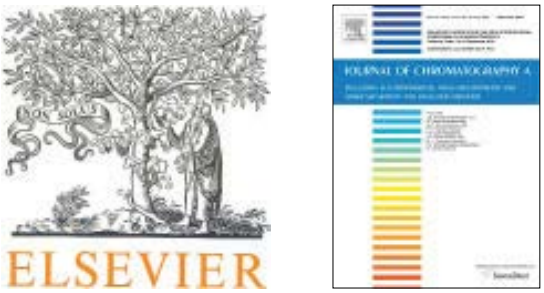
Presenting authors of SECyTA2013 are encouraged to submit a full-length manuscript for potential publication in Journal of Chromatography A. All manuscripts will be subject to the standard, stringent refereeing procedure of the journal. The articles will appear, as soon as possible after acceptance, in the "article-in-press" section in Science Direct.

Individual manuscripts will be published in the first possible regular issue of the journal as soon as possible after acceptance. Each article will have a footnote indicating that is was presented at SECyTA2013, and this footnote will serve as the source for selection and linking to a Virtual Special Issue.

The Virtual Special Issue will be placed on an individual Journal of Chromatography A Special Issue site with links to the papers on Science Direct.

Guide to Authors can be found on: **http://ees.elsevier.com/chroma**

The submission deadline is: November 30, 2013.



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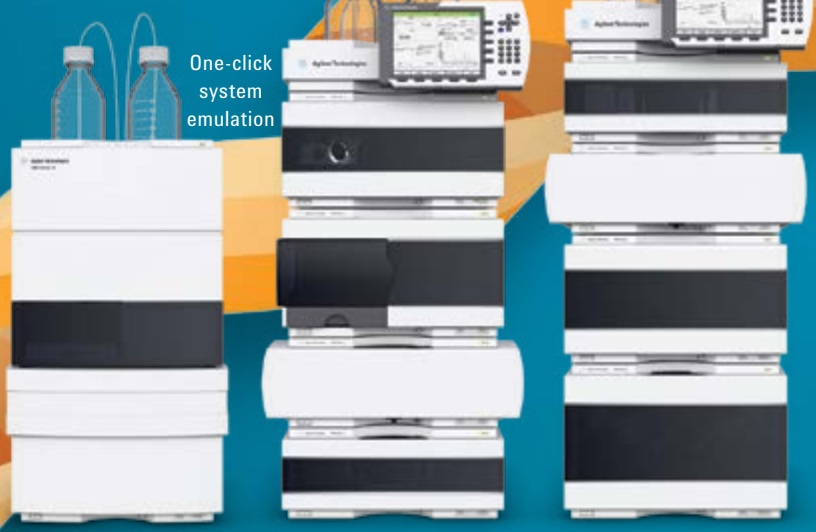

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PLENARY LECTURES

OPL-01

ADVANCED STRATEGIES BY CAPILLARY CHROMATOGRAPHIC AND ELECTROPHORETIC TECHNIQUES COUPLED TO MASS SPECTROMETRY FOR THE CHARACTERIZATION AND QUALITY CONTROL OF FOODS THROUGH THE ANALYSIS OF PROTEINS, PEPTIDES AND AMINO ACIDS

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The current challenges of Analytical Chemistry make necessary to have advanced analytical strategies in which Mass Spectrometry plays a crucial role together with (micro)-separation techniques.

The main goal of this research was to develop advanced analytical methodologies using Mass Spectrometry combined with (micro)-separation techniques in order to obtain the fingerprint or to discover marker molecules enabling the characterization of a system or giving relevant information about it. The developed methodologies were applied in the food analysis field in order to characterize a food and/or to detect marker molecules related with its quality, traceablility or bioactivity.

The separation, identification and quantification of proteins, peptides and amino acids was carried out in different vegetable matrices. In the case of peptides, special attention was paid to the identification of new peptides with biological activity and to the quantification of bioactive peptides in food matrices in order to evaluate their real efficacy/activity. When the compounds investigated were chiral such as in the case of many protein and non protein amino acids, due to the different biological activity that the stereoisomers of chiral compounds can present, analytical methodologies were developed enabling the individual determination of the stereoisomers in order to increase the information obtained from the system and the potential of the developed methods. To achieve these chiral separations, new chiral stationary phases were employed in capillary separative techniques in order to decrease analysis time and cost.

OPL-02

POPULATION-BASED OMICS

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Genetic variants influencing the transcriptome have been studied extensively. However, the impact of the genetic factors on protein expression and the proteome is largely unexplored, partly due to lack of suitable high-throughput quantitative methods. Here we present a unique set of identifications of genetic variants affecting the human plasma proteome achieved by combining label-free high-resolution LC-MS with genome-wide SNP data. We quantified 1,056 tryptic peptides representing 163 proteins in the plasma of 1,060 individuals from two population-based cohorts. The abundance level of one-fifth (19%) of the peptides was found to be heritable, with heritability 0.08-0.43. The levels of 60 peptides from 25 proteins were influenced by cis-acting SNPs. We identified and replicated individual cis-acting SNPs influencing 11 peptides from 5 individual proteins. These SNPs represent both regulatory SNPs and non-synonymous changes defining well-studied disease alleles such as the e4 allele of APOE, which has been shown to increase risk of Alzheimer's disease. In this study, all statistical analyses were performed on the peptide measurements rather than aggregate or derived protein abundances. The peptides directly represent the MS measurements and better capture the protein heterogeneity in the populations. The results show that label-free LC-MS is a viable alternative to SRM, especially for the few hundred most abundant proteins. The composition of the proteome play an important role in the etiology, diagnosis, and treatment of a number of diseases, and a better understanding of the genetic influences on the proteome is important for evaluating potential biomarkers and therapeutic agents for common diseases [1].

[1] Å. Johansson et al., *Proc. Natl. Acad. Sci. U. S. A.* **110** (2013) 4673-4678.

PL-01

DISSECTING AN UNTARGETED METABOLOMIC WORKFLOW

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Metabolomics is a newly emerging field focused on the profiling of small, naturally occurring (endogenous) molecules collectively known as the 'metabolome' [1]. While the genome and proteome represent upstream biochemical events, metabolites are the functional output of cellular reactions and therefore more closely correlate with phenotype. Here an untargeted mass spectrometry-based metabolomics platform will be detailed that provides a basis for optimal study design, sample preparation, chromatographic separation coupled to mass spectrometry [2], and data analysis methods for metabolomics experiments. The application of the technology to uncover novel therapeutic targets and insights into underlying pathobiology will be discussed [3].

[1] G.J. Patti, O. Yanes, G. Siuzdak, *Nat. Rev. Mol. Cell. Biol.* **13**, (2012) 263-269.
[2] O. Yanes, R. Tautenhahn, GJ. Patti, G. Siuzdak, *Anal. Chem.* **83** (2011) 2152-2161.
[3] G.J. Patti, O. Yanes, L.P. Shriver, J.P. Courade, R. Tautenhahn, M. Manchester, G. Siuzdak. *Nat. Chem. Biol.* **8** (2012) 232-234.

CPL-01

GREEN FOODOMICS

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Foodomics, defined for the first time in our research group [1] attempts to provide a global vision on the relationship between food and health through the use of -omics technologies with the mail goal of improving consumer's well-being, health and confidence.

Green Foodomics attempts to contribute to the greenness, sustainability and ecology of Foodomics as a whole.

By definition, Foodomics is a green discipline that tries to provide with new answers to the challenges of our society; aspects related to the sustainability, food quality and safety are basically embedded in the own Fodomics' definition. Other aspects such as those related with the rational design of new foods able to improve human health and to prevent illnesses are basically green by themselves since they will contribute to obtaining safer foods, with lower contamination and chemical risks.

The present talk will present different green alternatives for the production of new functional food ingredients (based on the use of green solvents and the design of integrated processes producing less residues and consuming lower amounts of energy) and how these processes can be also applied to develop greener analytical methodologies to face some aspects related to the food quality, traceability and safety (through the miniaturization of sample preparation techniques, the use of ecological solvents and the development of new separation methods).

Among other examples, some research works developed in our laboratory will be presented dealing with the direct extraction, using SFE (Supercritical Fluid Extraction), of carotenoids (astaxanthin) from *Neochloris oleoabundans* biomass, the isolation, using gas expanded liquids (GXLs), of gamma-linolenic acid from *Spirulina*, the extraction of antioxidants from rosemary using integrated processes of extraction and particle formation (WEPO, Water Extraction and Particle formation On-line) and the use of LCA (Lyfe Cycle Assessment) tool to evaluate the environmental impact of the different extraction and analytical processes.

[1] A. Cifuentes, *J. Chromatogr. A* **43** (2009) 7109-7109.

CPL-02

CHEMOMETRICS AND CHROMATOGRAPHY: FEAR AND WONDER

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Chromatographic techniques often cause a sense of wonder, probably due both to their simple and intuitive fundaments and basic properties, and to their results, which can be highly complex but also useful and easy to understand. However, size and complexity can cause fear instead of wonder, when they are not linked to utility.

A huge amount of data, such as those afforded by chromatographic techniques, apparently difficult to manage, can result in fear which in turn can be transmitted towards Chemometrics. However, the aim of Chemometrics is actually to extract the most useful part of these data, by using mathematical or statistical techniques based on simple and elegant models. Chemometric techniques most frequently applied to chromatographic data are linear and non-linear regression, multiple regression, principal component analysis and discriminant analysis. Their application as a help in solving chromatographic problems is described here, using examples drawn from the analysis of multicomponent samples.

In sample preparation, the different recovery of each component is an important problem when accurate quantitative results are required, while in chromatographic separations overlapping can also cause loss of accuracy, mainly when non-identified components can be present in the sample. Application of different regression modes can improve quantitative estimations.

Precision is also necessary when chromatographic results must be used for characterization purposes. Dispersion of quantitative results can be caused by non-random factors which can be pointed out, and to some extent corrected, from their statistical study.

For classification purposes, discriminant analysis is the technique of choice, but in some cases other techniques can be more suitable or even necessary.

Retention estimation can afford useful qualitative information, or help in the optimization of a chromatographic method: both cases require to apply mathematical approaches.

Chemometrics can help us to enhance the utility of the results of the chromatographic methodology and in this way increase its “sense of wonder”.

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YOUNG SCIENTIST PLENARY SESSION

YS1-01

NATURAL AND SYNTHETIC ESTROGEN ANALYSIS IN MILK, YOGURT AND CHEESE USING HOLLOW-FIBER LIQUID-PHASE MICROEXTRACTION

Bárbara Socas-Rodríguez, María Asensio-Ramos*,
Javier Hernández-Borges, Miguel Ángel Rodríguez-Delgado

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Estrogens are a group of analytes with significance in certain food matrices, particularly in milk and milk derivatives, which are highly consumed. In the case of endoestrogens (estrone, (E_1), estradiol (E_2), estriol (E_3) and their methylated and hydroxylated metabolites) they naturally appear in daily products, while exoestrogens (17α-ethynylestradiol (EE_2), diethylstilbestrol (DES), dienestrol (DS), hexestrol (HEX), etc.) are also introduced in the milk cycle by humans in an attempt to take benefit of their anabolic effects [1].

Current trends in sample preparation are focused on the use of low miniaturized techniques with low solvent consumption. Between them, liquid-phase microextraction (LPME) has become really successful in the last decades, also in the extraction and preconcentration of organic and inorganic compounds from food extracts, despite the complexity of these matrices. Among all LPME techniques, hollow-fiber LPME (HF-LPME) and its variations have been scarcely used in the analysis of estrogens. Despite its inherent advantages like easy handling, high preconcentration and low cost and solvent consumption, the technique has not been previously employed for the extraction and preconcentration of estrogens from milk.

In this work, four natural estrogens (E_1, 17β-E_2, 17α-E_2 and E_3), four synthetic (17α-EE_2, DES, DS and HEX) and a metabolite (2-hydroxyestradiol, 2-OHE_2) have been extracted and preconcentrated from skimmed, semi-skimmed and whole milk, natural and skimmed yogurt as well as fresh cheese. After protein precipitation with acetonitrile containing acetic acid, evaporation and reconstitution in water, HF-LPME using 1-octanol as extraction solvent was applied to further preconcentrate the analytes. Parameters that affect the extraction efficiency (pH of the sample, extraction time, stirring speed, temperature and ionic strength) were investigated. Separation, determination and quantification were achieved by high-performance liquid chromatography coupled to a diode array detector and a fluorescence detector set in series. Calibration, precision and accuracy studies were carried out to validate the methodology for each type of matrix providing LODs in the low µg/L range.

[1] B. Socas-Rodríguez, M. Asensio-Ramos, J. Hernández-Borges, A.V. Herrera-Herrera, M.A. Rodríguez-Delgado, *TrAC-Trends Anal. Chem.* **44** (2013) 58-77.

YS1-02

DIRECTLY SUSPENDED DROPLET MICROEXTRACTION FOLLOWED BY BACK-EXTRACTION FOR DETERMINING HALOACETIC ACIDS IN WATERS INTENDED FOR HUMAN CONSUMPTION

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Haloacetic acids (HAAs) are a family of compounds belonging to water disinfection byproducts (DBPs). Their chemical structures are based on that of acetic acid, in which one or more hydrogen atoms have been substituted by chlorine and/or bromine atoms. They are formed when waters intended for reuse in public supplies are subjected to chlorination under certain conditions. The degree of HAAs formation depend on several characteristics of treated waters, such as organic matter or bromine content, on chlorination process, and on operational factors such as temperature, pH, residual chlorine, and contact time between the disinfectant and the organic matter.

Several epidemiological studies have analyzed the possible association between HAAs exposure and adverse effects on health, mainly those related with reproductive health. Indeed, several HAAs have been classified as possible human carcinogens by the International Agency for Research on Cancer (IARC). Therefore, the monitoring of HAAs in waters destined to human consumption is a priority.

There are several existing methods to determine HAAs in waters. Thus, the 552.2 method of the United States Environmental Protection Agency (US-EPA) utilizes conventional liquid-liquid extraction (LLE) and derivatization followed by gas-chromatography (GC) with electron capture detector (ECD). However, emerging trends in Analytical Chemistry are shifted to the development of extraction methods that eliminate or at least minimize the organic solvent consumption. In this sense, conventional extraction techniques such as LLE are currently not advisable from an environmental point of view.

The present work describes the determination of nine HAAs in waters using the novel microextraction technique directly suspended droplet microextraction, which minimizes the extraction solvent consumption down to microliters and avoids the necessity of a dispersive solvent. The method is then followed by a back-extraction using a low volume of aqueous phase, and further injection in a high-performance liquid chromatograph (HPLC) with diode-array detection (DAD), without the necessity of further clean-up steps.

[1] S.D. Richardson, *TrAC-Trends Anal. Chem.* **22** (2003) 666-684.

YS1-03

FORENSIC DISCRIMATION OF PAPERS BY CAPILLARY ELECTROPHORESIS AND MULTIVARIATE ANALYSIS

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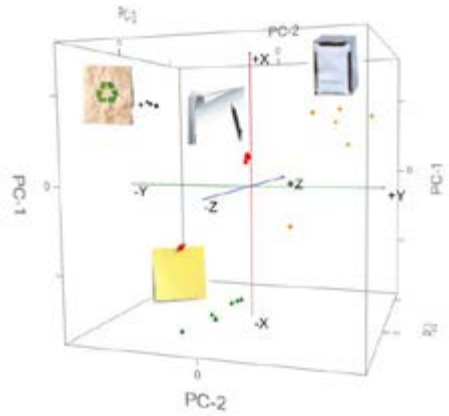
Up to date, most of the documents daily employed around the world are produced in paper. This fact involves these paper documents are part of crime scenes, becoming crucial the deep study of the common paper sources. Forensic studies on documents are focused on searching similarities and differences of color, size, shape, composition, fibres, etc. among papers [1]. However, paper sources are mainly made of cellulose, as consequence, the knowledge of cellulose properties and its analytical determination is necessary for document examination and the generation of high-quality information useful in the forensic field.

Capillary electrophoresis (CE) is a leading analytical technique in the study of cellulose. Cellulose as carbohydrate, the components of cellulose fibres and its degradation products from aging or pulp processes have been massive investigated by CE [2]. Based on this information we proposed the study of cellulose from different commercial paper sources. To the best of our knowledge, this work presents the first approach for the separation and posterior discrimination of different commercial papers through the comparative study of the complete cellulose electropherogram using multivariate analysis (MVA).

First, four different paper sources (white and recycled paper sheets, yellow adhesive notes and restaurant napkins) were pulverized by scratching with a surgical scalpel. Then, 0.30 mg of each powered paper sample were derivatized with 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) and injected in the CE system [3]. The complete electropherograms were normalized, aligned and discriminated by principal components analysis (PCA). Figure shows the 3D PCA plot where the four paper sources were successfully discriminated obtaining four different groups without entanglements.



[1] A. R. W. Jackson, *Forensic Science*, Pearson-Prentice Hall (2007) 230-256.
[2] N. Volpi (Ed.), *Capillary electrophoresis of carbohydrates, Humana Press* (2011).
[3] M. Fernández de la Ossa, M. Torre, C. García-Ruiz, *Anal. Chim. Acta* **745** (2012) 149-155.

YS1-04

FAST SEPARATION OF CAPSAICINOIDS FROM PEPPERS BY REVERSED PHASE ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY

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Capsaicinoids are the pungent compounds responsible for the hot flavour of peppers. Among these compounds, there are two major capsaicinoids, i.e. capsaicin and dihydrocapsaicin, that represent around 90% of total capsaicinoids present in most hot varieties of peppers [1]. Besides these two major capsaicinoids, other minor capsaicinoids have been identified in peppers: nordihydrocapsaicin I and II, homocapsaicin I and II, homodihydrocapsaicin I and II and nonivamide, amongst others, along with more than 20 others in several pepper varieties [2, 3].

Capsaicinoids are widely used in food in most parts of the world due to their pungent properties [4]. Capsaicinoids also have several properties and biological effects regarding human health. Chemopreventive and anticarginogenic properties have been established [5]. Antioxidant properties [6] participation in the regulation of fat metabolism [7] and antiinflamatory properties have also been demonstrated [8]. The huge importance and wide use of these compounds in both food and medicine makes it of interest to develop rapid methods for the determination of these compounds.

A new chromatographic method for the separation of major capsaicinoids has been developed. Nordihydrocapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C) and homodihydrocapsaicin (h-DHC) have been separated by Reversed Phase Ultra Performance Liquid Chromatography. Capsaicinoids were analyzed on a Waters BEH C18 column (50 x 2.1 mm I.D., particle size 1.7 µm). A gradient method has been developed using two solvents: 0.1% acetic acid in water and 0.1% acetic acid in methanol. The developed method allows the full separation of capsaicinoids in less than 3 minutes, with high reproducibility (RSD < 4.3%) and repeatability (RSD < 3.6%). Robustness regarding the total amount of methanol in the sample was determined. Comparison with previous RP-HPLC methods using both monolithic and conventional columns was also studied. Finally, the method was applied in the determination of major capsaicinoids in sixteen hot pepper samples produced in Spain. Microwave-assisted extraction was used to obtain the samples.

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YS1-05

DETERMINATION OF BENZOTRIAZOLES IN WATER SAMPLES BY POLYETHERSULFONE SOLID-PHASE MICROEXTRACTION AND LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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In this work, we investigate the suitability of a commercial available and low cost polyethersufone (PES) sorbent for the microextraction of 1H-benzotriazole (BTri), and four polar derivatives (4 and 5-methyl-1H-benzotriazole, 4-TTri and 5-TTri; 5,6-dimethyl-1H benzotriazole, XTri; and 5-chloro-1H-benzotriazole, 5-ClBTri) from surface and wastewater samples. The performance of liquid chromatography (LC) combined with quadrupole time-of-flight mass spectrometry (QTOF-MS) for the selective determination of target compounds is also discussed.

Parameters affecting the efficiency of the microextraction step, such as sample's pH, ionic strength, stirring speed and extraction lapse of time, and the PES membrane desorption process have been thoroughly investigated.

Analytes were extracted from 15 mL samples, containing a 30% of sodium chloride and adjusted at pH 4.5, using a tubular PES sorbent (5 cm length x 0.7 mm o.d., sorbent volume 42 µL). After methanol desorption and solvent exchange, benzotriazoles were determined by LC-MS, with chromatograms extracted using a mass window of 20 ppm, centered in their $[M+H]^+$ ions. The identity of chromatographic peaks was confirmed with accurate ion product scan (MS/MS) spectra. The method provided limits of quantification (LOQs) between 0.005 and 0.1 ng mL^{-1}, and relative recoveries from 81% to 124% (except for XTri in sewage samples, ca. 60%) with associated standard deviations between 2% and 9%.

The efficiency of the PES sorbent for the extraction of these compounds has been compared with that attained by stir-bar sorptive extraction (SBSE), with polydimethylsiloxane (PDMS) covered stir bars. The PES polymer achieved significant higher responses (5- to 20-fold) for these polar pollutants.

To the best of our knowledge, this research constitutes the first application of both techniques (microextraction using a PES sorbent and LC-QTOF-MS) for benzotriazoles determination in water samples. The method was used to provide data regarding the levels of target compounds in river and urban wastewater samples, including the individual quantification of 4-methyl and 5-methyl-benzotriazole isomers. Obtained results confirmed the ubiquity of benzotriazole, 4-methyl and 5-methyl-benzotriazole in urban wastewater and their incomplete removal at sewage treatment plants.

YS1-06

ADVANCED MONITORING OF PERFLUORINATED COMPOUNDS IN WATER, SEDIMENT AND BIOTA OF THE LLOBREGAT RIVER BASIN (NE SPAIN)

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This study is aimed at the screening of 21 perfluorinated compounds (PFCs) in environmental samples by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), and to identify target compounds at low levels in water, sediments and biota of the Llobregat River in 2010. To extract PFCs from sediment, ultrasonication with acidified acetonitrile followed by an off-line solid-phase extraction (SPE) procedure was used. In the case of river water samples, an SPE with STRATA cartridges was applied. Separation was achieved by means of a conventional analytical column (15.0 cm × 0.21 cm, 3 µm) using (A) water - (B) methanol, both with ammonium formate (10 mM), in a gradient. For fishes PFCs were extracted with an alkaline digestion and clean-up, separation and detection steps were performed using the TurboFlow™ on line technology coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). MS/MS was performed in selected reaction monitoring mode with ESI in negative mode.

The limits of detection (LODs) and limits of quantification (LOQs) of the method were calculated by analysis of spiked river water, sediment, and biota with minimum concentrations of each individual compound at a signal-to-noise ratio of 3 and 10, respectively. The LODs and LOQs of the method in river water ranged between 0.004 and 0.8 ng L^{-1} and between 0.01 and 2 ng L^{-1}, respectively. In sediment LODs were 0.013-2.667 ng g^{-1} dry weight (dw) and LOQs were 0.04-8 ng g^{-1} dw, meanwhile in biota these were 0.006-0.7 pg μL^{-1} and 0.02-2.26 pg μL^{-1}, respectively. Recoveries ranged between 65% and 102% for all target compounds. The method was applied to study the spatial distribution of these compounds in the Llobregat River basin. For this, a total of 40 samples were analysed (14 water, 14 sediments, 12 fishes). Of the 21 target compounds, 13 were identified in water samples (PFBA, PFDA, PFHpA, PFHxA, PFHxDA, PFNA, PFOA, PFPeA, PFTrDA, PFUdA, L-PFBS, L-PFHxS and L-PFOS), and their concentrations ranged between 0. 1 ng L^{-1} (PFNA) and 2709 ng L^{-1} (L-PFOS). Similarly, PFBA, PFDA, PFDoA, PFHpA, PFNA, PFOA, PFPeA, PFTrDA, PFUdA, L-PFBS, L-PFHxS, L-PFOS and PFOSA were identified in sediments samples, with concentrations ranging from 0.147 ng g^{-1} dw (L-PFOS) to 13 ng g^{-1} dw (PFBA). In biota similar PFC were detected, with values between 0.03 and 1738.06 ng g^{-1}.

Acknowledgements
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YS2-01

A 6-HOLE CAPILLARY INSPIRED BY PHOTONIC CRYSTAL FIBERS FOR CAPILLARY ELECTROPHORESIS. FUNDAMENTAL STUDY ON THE HYDRODINAMIC INJECTION

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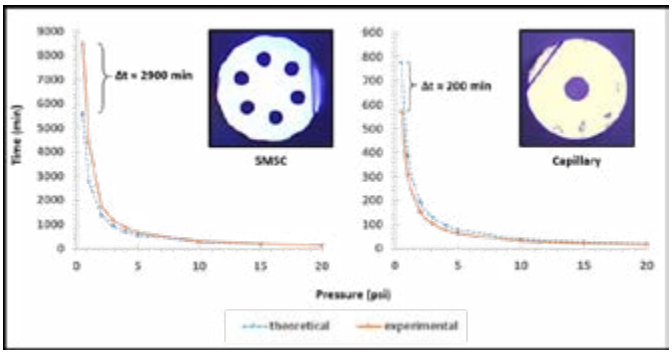
Initially employed in fibre-optic communication, photonic crystal fibres (PCFs) are becoming interesting devices in a wide range of fields [1]. The application of this material in nanoscience, such as components in chemical sensors [2] and microchips [3] could be a representative example of how versatile PCFs may be. Taking advantage of the similarities between PCFs and conventional capillaries for capillary electrophoresis (CE), the study of the performance of these devices could lead to relevant improvements and developments in CE instrumentation. In our previous work, preliminary studies using PCFs and new devices called smart-micro-structured capillaries (SMSCs) were made obtaining promising improvements in some analytical parameters such as peak asymmetry. However, the inherent differences between capillaries and SMSCs promote the need for basic and comparative tests in this cross-disciplinary research.

Firstly, a new SMSC was designed and manufactured using the well-established stack and draw technique. This SMSC had 6 holes of 29.5 µm inner diameter (id) and 290 µm outer diameter, with a transparent coating instead of the conventional polyimide. Then, the SMSC was employed to compare theoretical (from Poiseuille's equation) against experimental times needed for an aqueous solution (V = 400 µL) to flow through the SMSC. A Beckman P/ACE MDQ system as CE equipment was used for this fundamental study on the hydrodynamic injection. Values obtained with the SMSC were also compared to those from a 75 µm id conventional capillary (see figure). Experimental times to flow 400 µL of solution were higher than the theoretical ones when using the SMSC. This leads us to think that a modification of the usual injection equations is needed for a more precise volume injection. Future work with these devices is therefore required.



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YS2-02

SELECTIVE SEPARATION OF BIOACTIVE CARBOHYDRATES USING IONIC LIQUIDS

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Ionic liquids (ILs) are low melting point salts with unique properties such as low vapor pressure and high thermal and chemical stability [1]. These properties make them to be considered a promising recyclable alternative to traditional volatile organic solvents for a high number of applications such as those related to catalysis [1], solvents for extractions of a variety of substances [2], as stationary phases for gas chromatography (GC) [3], etc.

Interest in biological activities of carbohydrates is currently increasing in different research areas such as food, pharmaceuticals, and environmental science. Prebiotic carbohydrates can modulate the activities of the gut microbiota, whereas inositols exhibit different activities mainly connected to insulin-related diseases [4]. Most of these carbohydrates are extracted from natural products, and the presence of other co-extracted sugars which could interfere in their bioactivity makes the fractionation of the extracts mandatory. This is a challenging process considering their similar structures and concentrations. Techniques such as Ion-Exchange Chromatography [5], Activated Charcoal [6] or Pressurized Liquid Extraction [5] have been proposed for the fractionation of carbohydrates, but ILs have not been applied before to this purpose and could represent a good alternative to conventional solvents.

Before evaluating the selective fractionation of carbohydrates in ILs, new solubility data of a wide range of carbohydrates (aldoses, ketoses, inositols and linear polyalcohols) in different ILs ([EMIM][DCA], [EMIM][Ac], [MMIM][Me_2PO_4] and [HMIM][Cl]) at different temperatures was required. Optimization and validation of a derivatization method for their GC analysis was also a prerequisite for the quantitative studies.

Mono- and disaccharides showed higher solubilities values (at 25 ºC) than inositols in [EMIM][DCA], whereas linear polyalcohols were more soluble than the other carbohydrates in [EMIM][Ac] and [MMIM][Me_2PO_4]. The differences in solubilities of aldoses and ketoses in [HMIM][Cl] and [EMIM][DCA] could be also useful for their potential separation. These results indicate the efficiency of ILs in the selective fractionation of bioactive carbohydrates.

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YS2-03

ANALYSIS OF COSMETICS AND PERSONAL CARE PRODUCTS: RECENT DEVELOPMENTS

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To protect consumer health and ensure compliance to existing government regulations, the development of reliable analytical methods for cosmetic analysis is needed. General analytical methods exist, or are to be developed to assess the quality of cosmetics in accordance with the European Union (EU) Cosmetics Products Regulation [1]. In this context, ISO 12787 International Standard [1] defines validation criteria to which analytical results obtained from the analysis of cosmetic products should comply in order to give confidence in performance, reliability and quality of the final result. It proposes an analytical approach for chromatographic analyses in order to obtain an assay result and different validation elements which can be determined for each sample (or sample groups) submitted to the analysis. As cosmetic formulations very often contain complex mixtures of ingredients, chromatographic techniques are the most commonly used for the analysis of such substances. Gas chromatography (GC) and liquid chromatography (LC) coupled to different detectors are the determination techniques most frequently used. In terms of selectivity, mass spectrometry (MS) and tandem mass spectrometry (MS/MS) represent, at present, powerful detection tools for both chromatographic techniques. In order to achieve a good chromatographic performance in cosmetic analysis, sample preparation is a vital factor to be considered to succeed in the development of a new methodology. For most cosmetic samples, it is not possible to simply dilute the sample in an adequate solvent prior analysis, since several sample components would not be solubilized and we would not obtain homogeneous extracts. In addition, the complexity of the obtained solutions would cause chromatographic contamination after few analyses, and coelution of matrix components, making really hard to obtain satisfactory analytical results for the target compounds. To overcome some of these drawbacks, advanced extraction techniques such as supercritical fluid extraction (SFE), solid phase extraction (SPE), and solid phase microextraction (SPME), pressurized liquid extraction (PLE) and matrix solid-phase dispersion (MSPD), have been recently applied for the determination of different additives in cosmetics. These extraction procedures in combination with GC-MS, GC-MS/MS or HPLC-MS/MS have arisen as powerful methods to successfully tackle cosmetic analysis. This presentation gives a quick overview of the most recently developed analytical methods to the determination of the main groups of cosmetic ingredients which have arisen social concern; preservatives, fragrance allergens, synthetic musks, phtalates, and other plasticizers [4-6]. This new chromatographic applications, represent an updated analytical alternative for testing cosmetic products.

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YS2-04

ADVANCES IN COUPLING CSEI-SWEEPING-MEKC WITH DIFFERENT SAMPLE TREATMENTS FOR 5-NITROIMIDAZOLE DETERMINATION IN FOOD, ENVIRONMENTAL AND CLINICAL MATRICES

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Cation-selective exhaustive injection (CSEI)-sweeping is a novel methodology initially developed by Quirino and Terabe [1]. It combines two on-line concentration techniques: field-enhanced sample injection (FESI) with sweeping and it is possible to achieve concentration factors from a thousand- to almost a million-fold in relation to the normal injection in capillary electrophoresis (CE) [1]. It overcomes the lack of sensitivity that has been traditionally associated to CE-UV methods, being a very useful alternative in trace analysis. In spite of this, few methods have been reported using this new sensitive methodology, which involves the need of more contributions to check its advantages and disadvantages.

In this work, we have developed a new method based on CSEI-sweeping for the analysis of 5-nitroimidazoles (5-NDZs) in different matrices. Prior to injection, the capillary must be rinsed with a low conductivity buffer (50 mM phosphate buffer pH 2.5), followed by a plug of a higher conductivity buffer (100 mM phosphate pH 2.5, 50 mbar, ≈ 31.5 % total capillary volume) and a plug of water (50 mbar, 2 s). Analytes, dissolved in a solvent of lower conductivity than that of the separation medium, are electrokinetically injected at 9.8 KV for 632 s in a bare fused-silica capillary (57.2 cm, 50 µm I.D.), and thus the desired FESI effect is obtained. 5 mM phosphoric acid with 5% of methanol was selected as injection solvent to perform FESI. Separation was carried out applying -30 KV at 20°C in 44 mM phosphate buffer pH 2.5, containing 8 % tetrahydrofurane and 123 mM SDS.

Moreover, the proposed method has been applied in different areas as food, environmental and clinical analyses. Besides, different sample pretreatments have been evaluated to be coupled with CSEI-sweeping: solid phase extraction (SPE) has been proposed for egg sample cleaning-up; dispersive liquid-liquid microextraction (DLLME) has been used prior to the analysis of water samples; and untreated human urine and serum samples has been also evaluated. Detection limits at low ng/mL have been obtained for egg and water samples, achieving the recommendations from European Community Reference Laboratories [2]. Detection limits at low µg/mL have been reached for urine and serum samples, which enables the detection of these drugs at their normal levels in biological fluids [3].

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YS2-05

HPLC ANALYSIS AND OCCURRENCE OF FULLERENES IN THE ENVIRONMENT

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Natural sources of nanoparticles (NPs) in the atmosphere include natural event such as volcanic eruptions, forest fires, hydrothermal vent systems and biological processes. However, the natural background of NPs in the atmosphere is low in comparison to those caused by antrhopogenic process like diesel and gasoline fueled vehicles and stationary combustion sources which for many years have contributed to the particulate material in the atmosphere in a wide size range, including NPs. Carbon based nanomaterials (NMs) of different kinds have also been reported to occur in ordinary hydrocarbon flames and the are emitted from common heat sources and the forecast for the next coming years is that emission will increase significantly due to the nanotechnology industry.

Fullerenes have attracted considerable interest in many fields of research and have found numerous applications. Therefore, it is essential to determine the risk that these materials may pose to human health and the environmental and it is of high importance to evaluate their presence in the environment.

In this presentation different analytical approaches based on liquid chromatography coupled to mass spectrometry will be introduced and environmental results ofthe investigation of the occurrence of fullerenes (C_{60}, C_{70}, C_{76}, C_{78} and C_{84} fullerene, C_{60} pyrrolidine tris-acid ethyl ester, [6,6]-Phenyl-C_{61} butyric acid butyl ester and [6,6]-Thienyl C_{61} butyric acid methyl ester) in different environmental matrices (suspended materials of wastewater and river water, air borne particulate, soils and sediments) will be discussed.

The results of these studies will be considered in combination with their toxicological behaviour and recent data about their synergistic effects with other toxic organic contaminants susceptible to be present in the same environmental compartments.

YS2-06

PERFLUOROALKYL SUBSTANCES IN HUMAN DIET AND ACCUMULATION IN DIFFERENT HUMAN MATRICES

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The perfluoroalkyl substances (PFASs) are used in a great variety of consumer and industrial products thanks to their physicochemical characteristics and stability. However, their stability and resistance to degradation makes these compounds persistent in the environment. In addition, PFASs can be bioaccumulated and biomagnified through the food chain. Several studies have report their present in different environmental and humans matrices worldwide [1, 2]. Recent findings of the toxicity of these compounds have been reported [3]. The main aims of this work were to study the occurrence of twenty-one PFAS in human diets from countries representatives of South America, European Mediterranean Countries, Central Europe and Medium East (Brazil, Saudi Arabia, Spain and Serbia), and on the other hand study the distribution patterns of PFASs in different human tissues and fluids including blood, urine, hair, semen, nails and saliva. Therefore, as a previous step, different analytical pretreatments were evaluated for the sample preparation of the different matrices included in this study. The analytical method employed in this work was based on turbulent flow chromatography coupled to liquid chromatography-tandem mass spectrometry (TFC-LC-MS/MS) using a Turbo Ion Spray source operated in the negative mode. The analytical method presented method limits of detection (MLODs) between 0.01-13 µg/L with minimal sample preparation. The method showed high recoveries rates ranging from 30 to 150 % and good reproducibility and repeatability were also shown.

The compounds found in the food samples analyzed in each country were: in Spain PFBA, PFPeA, FDEA; in Serbia PFBA, PFHxA, PFPeA; in Arabia PFOA, PFNA, PFHxA and finally in Brazil PFBA, PFNA and PFOA.

On the other hand, for the majority of the human tissues PFOS and PFOA were the compounds found at higher concentrations. PFHxS and PFOS were the PFAS found at highest concentration in blood. PFBA, PFOS and PFOA were major compounds in hair. PFBA, PFPeA, PFHxA and PFOS were the predominant compounds in semen, whereas PFHxA and PFOS were the more relevant in saliva. The results of this pilot study indicate a potential different tend in the accumulation and distribution of PFAS in human body and highlights the need performing this type of studies including a representative number of donors.

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ENV1-01

EXPERIMENTAL EVALUATION OF VOC REMOVAL EFFICIENCY OF AN ACTIVATED CARBON FILTER FOR THE REDUCTION OF VOC CONCENTRATIONS INDOORS

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Volatile organic compounds (VOC), defined as having a boiling point that ranges from 50ºC to 260ºC, are a highly diverse class of chemical contaminants; and between 50 and 300 compounds may be found in non-industrial indoor environments. VOC can act as irritants to the human organism and have negative health effects, as well as trigger discomfort and odour nuisances. Furthermore, the subgroup of population more sensitive to contaminants (e.g. multiple chemical sensitivity) are at much greater risk of having adverse health effects deriving out of the chronic exposition to low levels of indoor air pollutants. Indoor air purification is one of the main strategies to improve indoor air quality (IAQ).

Activated carbon filters are greatly employed to enhance IAQ. Nonetheless, the reported adsorptive capacities and the removal efficiencies of activated carbon filters are limited to high VOC concentrations and generally focused in few concrete compounds. Additionally, the results published in the literature are often obtained under constant temperature and relative humidity conditions which, in real buildings, may vary significantly between seasons, months and even days. Thus, most filters in the market have not been evaluated in the field, and therefore their real VOC removal efficiencies are unknown.

The present study evaluated the performance of a commercially available activated carbon filter for VOC reduction. The filter was evaluated in a PVC portable inflatable bubble provided with an air purifier system called Airbox Phase II by Zonair3d. The filter was placed in the Airbox Phase II of the bubble, between a pre-filter and a particulate HEPA H14 filter. Simultaneous duplicate samples were taken from the input air duct before the driving system and from the bubble. VOC were dynamically sampled during 2-hour control periods by connecting custom packed glass multi-sorbent cartridge tubes (Carbotrap, Carbopack X and Carboxen 569) to air pump samplers. Additionally, CO_2, CO, temperature and relative humidity were simultaneously and continuously monitored inside the bubble and in the input air duct before the filter. A validated analytical method based on TD-GC/MS was used to quantify a wide variety of VOC families (alkanes, aromatic hydrocarbons, alcohols, ketones, halocarbons, aldehydes, esters, terpenes, ethers, glycols and nitrogenated compounds). No significant differences (*t*-test; $p<0.05$; $n = 275$) were observed between filter efficiencies depending on input air, with average reduction efficiency values of 65 ± 13% and 62 ± 15% for input indoor and outdoor air, respectively. Several aldehydes were desorbed from the filter at low concentrations when outdoor air was used as input air. Ozone removal efficiency was evaluated through a continuous monitoring system, and was 100% in all cases where ozone was present.

ENV1-02

DETERMINATION OF NEUROTOXIC COMPOUNDS IN HUMAN SERUM SAMPLES BY GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY

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Pesticides are widely used in agriculture to prevent or reduce losses by pests and to improve the yields and quality of the products. However, the extensive use of these compounds has been associated to some unwanted effects such as cancer, allergies, neurological or reproductive disorders. Concern on pesticide use and its impact on human health and environmental quality has increased significantly. Measurement of these contaminants and their metabolites in body tissues and fluids may provide useful assessment on the exposure risk of the population.

In the present study a multi-residue method has been developed for the analysis of a wide range of pesticide (pyrethroids, organochlorine compounds, organophosphorous compounds) and polychlorobiphenyls congeners in small-size human samples.

Different cartridges and solvents were tested until optimal extraction of all the analytes was obtained. The analytes were isolated by solid-phase extraction using C18 and silica gel cartridges for clean-up. Methanol, terbutyl methyl ether and water were used for cartridge conditioning and terbutyl methyl ether for the analyte extraction.

The samples were analyzed by GC–MS/MS in electron ionization mode acquiring two MS/MS transitions for each analyte. Chromatographic parameters like injection temperatures, oven temperature program and source MS parameters were optimized to improve selectivity and sensitivity. MS source parameters had significant influence on compound sensitivity and were optimized to increase selectivity without reducing sensitivity. Accuracy and precision were evaluated by using serum samples fortified at two concentration levels with satisfactory results in the majority of cases.

Accordingly, a sensitive and selective analytical method has been developed for the analysis of different groups of neurotoxic compounds in human serum samples.

ENV1-03

NEW AGILENT ONLINE ENRICHMENT SOLUTIONS FOR ENVIRONMENTAL SCREENING ANALYSIS

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Contaminants screening of water samples for environment analysis is becoming more challenging due to increasing regulations. This makes analysts on the need to look for protocols that allows them to reach lowest LOQ. Surface and drinking water had the property of been a clean matrix which allows approach it with an enrichment technique with no clean-up step.

Local regulations and Water EU Framework directive are restrictive enough to be prepared to have the right tools to face these challenges in a routinely base.

Online enrichment techniques are not new and some approaches started to be developed on the 70's. More sensitive technology and new analytical techniques have been changing the way and the frequency this technique is used. Agilent has been using so far a double HPLC pump system to afford this in a comfortable and automated system.

We want to present a new online enrichment solution based on simplicity and flexibility. FlexCube is the core of the system and allows for multiple configurations depending basically on the volume of samples and throughput capacity.

This new systems is an excellent solution to afford real trace analysis on LCMS systems with no need of ultra high-end systems in terms of sensitivity for surface and drinking water.

The flexibility of different valve configuration allows users also to setup a clean-up system affording more complex analysis with derivatization included like Gliphosate/AMPA analysis.

FA1-01

SIMULTANEOUS DETERMINATION OF FREE AND BONDED VOLATILE CARBONYLS IN WINE BY SOLID PHASE MICROEXTRACTION: ASSESSING WINE SHELF LIFE AND AROMA CHANGES IN THE GLASS

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There are increasing evidences [1, 2] indicating that some wine aroma compounds form complexes or adducts with different matrix elements, which can deeply affect to their real distribution to the headspace and hence to the aroma perception. The process can be schematized as:

$$\text{Aroma}_{gas} \leftrightarrow \text{Aroma}_{liquid} \leftrightarrow \text{Aroma}_{complexed}$$

In wine these effects are particularly important for carbonyls which form adducts with SO_2 [3]. In spite that the existence of bisulfite adducts between SO_2 and carbonyls was documented long time ago, and in spite of the fact that some carbonyls, namely methional, phenylacetaldehyde, diacetyl and β-damascenone are amongst the most important aroma compounds of wine (the two first responsible for wine aroma oxidation), there are not reports or tools regarding their measurement of their free and complexed forms. This work presents a new analytical method for the simultaneous analysis of both, free and bonded fractions of the most relevant wine carbonyls.

The method is based on a short and unstirred headspace solid phase microextraction (HS-SPME) with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber, followed by gas chromatography electron impact (EI) mass spectrometry. A SPB-1 sulfur column was used using conditions which make it possible to obtain reliable signals for 12 odor active wine carbonyls. The sample is first spiked with standards and surrogates and incubated in a completely oxygen-free chamber for at least 8 hours. Some of the standards provide constant headspace concentrations not dependent on the wine and are used to estimate actual concentrations of analytes in the headspaces (free fractions). Surrogates behave similarly to groups of analytes and their relative signals to those of the standards are used to estimate the proportion of compound in bonded form. Overall, the method makes it possible to get very good estimates of the free and bonded forms of 12 wine carbonyls. Linearity, reproducibility and accuracy were satisfactory. The method provides first evidence showing that some oxidation related sensory changes are not due to the "de novo" formation of carbonyls, but to their release from their complexes with sulfur dioxide once this is oxidized.

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FA1-02

USING A NOVEL ION SOURCE AND INNOVATIVE ION OPTICS TO IMPROVE SENSITIVITY AND SAMPLE THROUGHPUT IN ANALYSIS OF CONTAMINANTS

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The improvement in quality of life and our expectation to live in a safe and secure environment, consume healthy, safe food and use non-toxic pharmaceutical products is driving the demand for increasing analysis to detect contaminants that are potentially damaging to our health.

The optimization of existing technologies and procedures can improve the efficiency of analysis process, but only a totally new approach adopting emerging technologies can provide a quantum leap with instrumental design capable to reduce sample preparation, increase throughput and provide better detection limits.

In this communication we'll review the results provided by a totally new approach for the analysis of contaminants in different matrixes and what is the impact on laboratory workflow.

Several factors will be considered including the compatibility of spectra with existing data bases, the improved identification of long chain homologues, the possibility to analyze challenging products without derivatization and the analysis of products usually considered not suitable for GCMS.

FA1-03

DEVELOPMENT OF A NEW METHOD BASED ON LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR A MULTI-MYCOTOXIN ANALYSIS IN MAIZE SILAGE SAMPLES

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Mycotoxins are toxic natural secondary metabolites produced by several fungi belonging to the genus *Fusarium, Aspergillus, Penicicillium, Alternaria and Claviceps.* Some of them can infect maize forage and produce mycotoxins during cultivation and ensilage. There is evidence that maize silage is the diet constituent that supplies the bulk of mycotoxins intake in dairy cows and due to their very high chemical and physical resistance, some mycotoxins can be transmitted into animal-derivate products.

The European Union (EU) has adopted rules limiting the maximum levels of mycotoxins in feedingstuffs [1-3] particularly aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, fumonisins and T-2 and HT-2 toxins. EU also suggests increasing the monitoring studies for the presence of these mycotoxins in cereals and products intended for animal feeding. Since these European regulations cover several mycotoxins and the simultaneous occurrence of mycotoxins is commonplace, the European Food Safety Authority (EFSA) recommends developing multi-mycotoxins methods. In this way, liquid chromatography with tandem mass spectrometry (LC-MS/MS) is still one of the most sensitive and robust methods for the analysis of organic contaminants, particularly mycotoxins [4, 5]. The objective of this study was then the development of a new efficient multi-analyte method for the simultaneous determination of mycotoxins in maize silage by LC-MS/MS.

Two different extraction methods were assessed: a very simple and fast pH-buffered sample extraction based on the use of a mixture of acetonitrile/water/acetic acid (QUECHERS) and a conventional extraction method. In an attempt to reach the best compromise for all target mycotoxins, a simple liquid/solid extraction was performed with 1 g of silage and 20 mL of methanol/water (80:20 v/v) and after centrifugation/evaporation the resulting extract was reconstituted in mobile phase. Detection and quantification of mycotoxins were carried out by reverse-phase liquid chromatography coupled to electrospray ionization triple quadrupole mass spectrometry (LC-ESI-MS/MS). A linear gradient of methanol/water containing 0.1% formic acid and 3mM ammonium acetate was used as mobile phase with a Hypersil Gold Aq (100 x 2.1 mm x 3□m) column.

Since maize silage is a complex matrix containing chlorophylls and carotenoids from the leafy parts of the plant, matrix effects are likely to occur into the ion source of the LC-MS/MS system. Calibration curves from spiked extracts were then used to assess the signal suppression/enhancement (SSE) for each mycotoxin. Whereas zearalenone, roquefortine C and mycophenolic acid showed high suppressed signals in presence of matrix, signals of T-2 toxin,

verruculogen, penicillic acid, fumonisin B1 and B2, hydrolysed fumonisin B1 and ochratoxin A were strongly enhanced. The observed SSE emphasized the need to quantitate mycotoxins in maize silage extracts by means of matrix-matched calibration curves. The achieved recoveries from spiked samples ranged from 58 % to 85% and the limits of detection (LOD) ranged from 0.2 (Aflatoxin B2) to 10 μg/kg (roquefortine C). They were much lower than the maxima levels allowed by regulations in feed.

In order to assess the contamination level with mycotoxins in maize silages, the optimized method was applied to 270 samples collected in 30 dairy farms from Galicia. Zeralenone, fumonisin B1, roquefortine C and mycophenolic acid were detected.

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FAC-01

SURROGATION OF PHARMACEUTICAL AND ENVIRONMENTAL PARTITIONING PROCESSES BY CHROMATOGRAPHY AND ELECTROCHROMATOGRAPHY

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The experimental determination of biopartitioning properties of pharmaceutical and environmental interest, such as drug absorption and distribution, bioaccumulation or toxicity, is difficult, expensive and in some instances ethically questionable.

For these reasons, surrogate physicochemical systems capable of estimating biopartitioning properties in a faster, easier and more economic way have become of potential interest. Among them, chromatographic and electrochromatographic systems are very popular because they are cheap, fast and easy to perform. They offer very promising alternatives for the experimental determination of partitioning properties of bioactive compounds because they can be easily obtained from the measured chromatographic retention.

In order to get linear relationships between biological and surrogate chromatographic parameters both measuring systems must have similar partitioning properties. If the two systems are characterized by the same partition model (namely the solvation parameter model [1] in our work), the *d* distance parameter [2] provides a measure of the similarity of the partition processes.

Moreover, the precision achieved for a particular biopartition process surrogated by several possible chromatographic systems can be previously calculated from the precisions of the biological and chromatographic measurements and the similarity (*d*) between the two systems compared [3]. This estimation provides a useful tool to select the best surrogate chromatographic system for any biopartitioning.

In this work, we present the comparison and selection of surrogate chromatographic systems, based in C18 and IAM HPLC columns and MEKC surfactants, for three biopartitioning processes of pharmaceutical and environmental interest, namely fathead toxicity [4], soil-water sorption [5] and skin permeation [6].

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FAC-02

MODELS FOR PREDICTING THE RETENTION AND PEAK SHAPE IN GRADIENT ELUTION CHROMATOGRAPHY

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The optimization of the experimental conditions in gradient elution requires reliable algorithms for the description of the retention and peak profile. As in isocratic elution, the linear relationship between the logarithm of the retention factor and the solvent contents is widely used to predict the retention in gradient elution due to its simplicity and the fact that it is easily integrable. However, this model is only acceptable in relatively small concentration ranges of modifier. For this reason, other models have been proposed, the most familiar being the quadratic logarithmic model, which has the disadvantage that the analytical integration of the general equation for gradient elution is not straightforward. In this work, alternative approaches for modelling the retention in linear gradient elution are discussed. A semi-empirical integration, based on the similarities of the linear and quadratic trends, is proposed. This approach can be also applied to other models, as the polarity model. Another model, which allows an analytical integration, initially proposed for normal liquid chromatography is demonstrated to offer excellent predictions in RPLC gradient elution, with errors similar to the quadratic model (usually below 1–2%).

Based on the half-width changes of chromatographic peaks along one or more gradients, an approach is also reported to predict the peak profile. A unique equation allows the prediction of peak profiles in both isocratic and gradient elution modes with low errors (usually below 2−3%). The same fitted model can be used for a set of compounds exhibiting similar partitioning kinetics in a given column.

The proposed approaches were applied to two sets of probe compounds (diuretics and flavonoids), eluted with acetonitrile-water gradients. The changes in retention and peak shape in isocratic and gradient elution are illustrated through plots of the logarithm of the retention factor versus the concentration of organic modifier at the beginning of the gradient (similar to the familiar plots of the logarithm of the retention factor versus fixed organic modifer concentration in isocratic elution), and plots of the peak half-widths or widths versus the gradient, respectively. The diagrams define triangular regions including all possible values of retention factors or peak half-widths (or widths) inside the selected working ranges.

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FAC-03

INCREASED THROUGHPUT PHARMA R&D AND QA WORKFLOWS WITH MAXIMUM VERSATILITY–HOW GENERIC CAN A UHPLC METHOD FOR POLAR ANALYTES BE?

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Liquid chromatographic (LC) methods for the assay of active pharmaceutical ingredients (API), drug candidates, intermediates, related substances, and impurities are crucial in pharmaceutical development. During early-stage drug discovery, the chemical synthesis route, the impurity profile, and even the formulation of the drug product are not completely established but are rather subject to constant modification. Thus, from pre-clinical to clinical development and, finally, to New Drug Application (NDA) submission, a drug-specific LC method must be modified several times. These samples can contain analytes of interest with a wide polarity range which makes their chromatography challenging. A very promising practice in drug discovery is the use of two separate analyses of the same sample; by reversed-phase (RP) LC on the one hand and hydrophilic interaction LC (HILIC) on the other hand.

In this work, a generic method approach is presented that addresses common changes during the drug development lifecycle and is applicable to each new drug formulation. The analysis combines gradient RPLC in the first stage and, enabled by organic solvent addition to the eluate coming from the RP column, gradient HILIC in the second stage. Coupling HILIC to RPLC has already been introduced [1]. This approach was optimized by combining solvent and flow rate gradients, simple solvent compositions, and UHPLC technology. This allows simplified separation of both hydrophilic and hydrophobic substances within a broad elution window and in a single, short run. Furthermore, Charged Aerosol Detection (CAD) allows near universal detection of non-volatile substances for the generic approach.

The advantages of RP-HILIC-UV-CAD and its potential to become the generic method approach of choice for screening in pharmaceutical discovery and development will be discussed. The performance of the generic method approach will be shown with a model sample and an emtricitabine degradation study.

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NDI1-01

PHLOROTANNINS CHARACTERIZATION FROM *Cystoseira abies-marina* BROWN ALGAE BY COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Algae are now recognized as crucial natural sources able to provide abundant bioactive compounds to the food and pharmaceutical industry. Among them, brown algae are popular for their biological activity mainly related to their high phenolic content.

The phenolic compounds in brown algae are basically phlorotannins. These compounds have been found only in brown algae and they have demonstrated to possess a number of interesting biological functions including antioxidant, antiallergic, antiinflammatory, anticarcinogenic and antidiabetic effects, making these compounds ideal candidates as functional ingredients.

Phlorotannins consist on polymers of phloroglucinol which can be soluble if they are stored in cell organelles or insoluble if they are bonded to the cell wall linked to alginic acid. Depending on the type of linkage between the phloroglucinol units and the number of hydroxyl groups, the phlorotannins can be classified into four subclasses: phlorethols and fuhalols (with ether bond), fucols (with a phenyl bond), fucophlorethols (with both ether and phenyl bonds) and eckols (with dibenzodioxin linkages). Besides, halogenated and sulphated phlorotannins have also been described. The molecular weight of these compounds ranges from 126 (phloroglucinol) to 10^5 Da (polymers).

Considering the complex composition of natural phlorotannins, the use of a single chromatographic method for a complete characterization is not possible; for instance, conventional RP-HPLC methods can only separate the smaller phlorotannins, whereas larger polymers cannot be correctly separated under these conditions. In this sense, comprehensive two-dimensional liquid chromatography (LCxLC) might be a useful tool for the separation of these complex mixtures.

LCxLC is based on the on-line coupling of two different separation modes, improving significantly the separation power with respect to the mono-dimensional technique.

In this work, a LCxLC method coupled to mass spectrometry has been developed for the chemical characterization of phlorotannins from the brown algae *Cystoseira abies-marina*. For this aim, an optimized phlorotannin extraction was carried out, including solvent extraction, an alkaline hydrolysis to obtain both soluble and insoluble phlorotannins fractions, and a solid phase extraction clean-up step prior to LCxLC-MS analysis.

NDI1-03

DISCOVERY OF KEY METABOLIC BIOMARKERS INVOLVED IN ALZHEIMER'S DISEASE PROGRESSION BY UHPLC-MS

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Until date, the mechanisms involved in Alzheimer's disease (AD) are still not clear [1]. The development of new methodologies for an early AD diagnosis is becoming urgent since AD incidence is unceasingly growing. AD is preceded by a mild cognitive impairment (MCI) state followed by dementia [2]. However, up to date, there is no clinical method to determine which MCI cases will progress to AD except for a long clinical follow-up period. Thus, another imperative need arises to identify specifically those MCI patients who will later progress to AD.

In this work, a non-targeted metabolomic approach based on ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) is developed to examine metabolic differences in cerebrospinal fluid (CSF) samples from 75 subjects. Four different cognitive states related to AD progression were considered, namely, healthy controls, MCI subjects who developed AD after a follow-up period of 2 years, MCI subjects whose initial MCI status remained stable during the course of the follow-up, and a group of subjects with AD. After multivariate statistical analysis, accuracy value of 98.7 %, and specificity and sensitivity values above 95% were obtained applying the proposed predictive method. Moreover, some potential metabolite AD biomarkers are revealed in this work.

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ENV2-01

GAS-PARTICLE PARTITIONING OF POPs IN URBAN AIR

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After decades of industrialization, air pollution has become a major environmental issue for both developed and developing countries, because poor air quality has both acute and chronic effects on human health [1]. Some of these adverse effects have been associated to the presence of persistent organic pollutants (POPs) in the air, however, there are several data gaps related to their fractionation in this matrix. The present study was designed to: i) evaluate the presence of some POPs (polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and Dechloranes) in ambient air, and ii) study pollutant air fractionation in the gas and particulate phases.

Urban air was sampled in Madrid (Spain) by using high-volume active samplers. Different particulate matter (PM) fractions were collected using Glass Fiber Filters: TSP, PM10, PM2.5 and PM1. Additionally, polyurethane foam (PUF) was utilised for sampling gas phase.

Prior to the extraction processes, the samples were spiked with ^{13}C labelled recovery standards and then soxhlet extracted for 24 h with toluene. Purification and fractionation stages were performed in an automated Power PrepTM System (FMS, Inc., USA) including multilayer silica, basic alumina, and carbon columns. Instrumental analysis of PCDD/Fs, PCBs and Dechloranes were carried out by HRGC-HRMS, on a Micromass Autospec Ultima NT, operated in electron ionization mode at resolution greater than 10000 (10% valley). PBDEs determination was performed on an Agilent 5973 MSD connected to an Agilent 6890 GC. Identification and quantification were carried out using isotopic dilution for all compounds except for Dec 602, Dec 603, Dec 604, CP and mirex which quantification was performed using $^{13}C_{10}$-*syn* DP as the internal standard.

The major pollutants resulted the NDL-PCB indicators (124- 257 pg/Nm^3), following in decreasing order by DL-PCBs (25 - 63 pg/Nm^3), PBDEs (9 - 33 pg/Nm^3), DP (0.5 – 1.4 pg/Nm^3) and finally by PCDD/Fs (0.2 – 0.4 pg/Nm^3). PCBs were mainly related to gas phase (98 %), while PCDD/Fs (99 %), DP (100 %) and PBDEs (95 %) were associated with particulate matter. Dec 602, Dec 603, Dec 604 and CP were not detected in any of the analyzed samples. This information will be of interest for environmental risk assessments and will result useful for decision-making support systems, at both national and international regulatory scale.

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ENV2-02

OCCURRENCE AND FATE OF EDCS AND RELATED COMPOUNDS IN WASTEWATER TREATMENT USING DUAL COLUMN LIQUID CHROMATOGRAPHY SWITCHING SYSTEM COUPLED TO MASS SPECTROMETRY

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The organic pollutants such as endocrine disruptors (EDCs) are a group of substance that interfere with the endocrine system and disrupt the physiological function of hormones. Some of these contaminants are found in a high variety of products commonly used in the daily life (detergents, in personal care products such as cosmetics, pharmaceuticals and in different industrial formulations).

Wastewater treatment plants (WWTPs) receive these contaminants daily and after different treatments, effluent wastewater discharges returns again into the environment, moreover, sewage sludge are reused in agricultural soil amendment or disposed to the landfill.

For all of these this reason, it is important to evaluate the efficiency of WWTPs. Focusing on EDCs and related compounds, the objective of this work was the determination of these contaminants in sewage sludge samples, influent and effluent wastewater in four representative watersheds of Spain (Llobregat, Ebro, Júcar, and Guadalquivir) during two campaigns in 2010 and 2011. In order to analyze this significant number of samples two different LC-LC-MS/MS techniques were applied.

In the case of sewage sludge a pressurized liquid extraction (PLE) was performed, then an online TurboFlow™ purification technology was applied. For wastewater samples, EQuan™ methodology was used as a preconcentration technique, allowing to inject high volumes of sample.

The results showed widespread occurrence of target compounds, although the level of concentrations of different compounds detected varied considerably depending on the WWTPs, campaign or matrix. Being, for example, in the case of wastewaters samples, alkylphenolic, anticorrosives and orghanophosphorous flame retardants, the 3 chemical groups with higher contribution in terms of concentrations. The natural and synthetic estrogens in free and conjugated forms were found in some points in lower levels in the three types of different samples.

ENV2-03

OPTIMIZING ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS USING NEW SELF-CLEANING ION SOURCE

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Polycyclic aromatic hydrocarbons (PAHs) are included on US Environmental Protection Agency (EPA), US Food and Drug Administration (FDA), and National Oceanic and Atmospheric Administration (NOAA) pollutant lists because of their mutagenic and carcinogenic properties.

Researchers monitoring PAH contamination in environmental samples have faced difficulty in achieving detection limits required by regulatory authorities due to poor peak shape, variation in peak response, and non-linear calibration curves. There is one of the most challenging environmental applications now optimized for the routine laboratory. Labs need to maximize system up-time.

Agilent Technologies, Inc. has introduced a programmable module for in situ source conditioning that addresses these analytical limitations. This presentation will introduce the new module, discuss its operating modes, and review how using a slow bleed of conditioning gas into the MS during analysis helps analysts optimize PAH analysis by single quad GC/MS and triple quad GC/MS/MS systems.

The New Self Cleaning Ion Source provides source cleaning by eliminating MS venting and manual cleaning. Combine this with use of a hydrogen bleed to enhance system performance for PAH analysis to extend time between source cleaning.

FA2-01

RESIDUAL FLAVONOLS IN LORENA (*Vitis labrusca*) SKINS AFTER WINEMAKING PROCESS

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The major part of grape polyphenols comes from the solid grape parts, high proportion of polyphenols still remains in the solid winemaking by-products or grape marc. Grape marc(a mixture of mainly grape skin and seeds) is produced after pressing the previously crushed grapes, in white wine production technology, or after the maceration phase concurrent with fermentation step, in red wine production technology. Polyphenols have been considered as nutraceuticals and a great effort is devoted to find available, natural sources of them [1]. In this work, we have studied the occurring flavonols (a potent grape antioxidant) in a hybrid grape variety developed in Brazil (BRS Lorena, *Malvasia Bianca × Seyval*) for production of white wine. Quantitative extraction of phenolics from separated skins of grape marc (year 2011 and 2012) was achieved using a mixture of methanol-water-formic acid (50:48.5:1.5 v/v/v) (1.0 g of dried skins; 3 x 25 mL; 2 min in ultrasonic bar and centrifugation each batch) and further analyzed by HPLC-DAD-ESIMS/MS [2]. A total of 11 flavonols could be assigned on the basis of, first, their characteristic UVvis spectra and, second, their MS and MS/MS spectra. Was flavonol-3-glycosides were detected: the 3-glucosides of kaempferol (k), quercetin (Q) and isorhamnetin (I); the 3-galactosides and 3-glucuronides of kaempferol and quercetin; the 3-rutinoside of quercetin; and the recently reported 3-rhamnoside of quercetin. And, free aglycons (free kaempferol, quercetin, and isorhamnetin) were also detected, these Quercetin-type flavonols also dominated the flavonol profiles of non-fermented skins (89.44 and 93.44 % in 2011 and 2012). The occurrence of hydrolysis of flavonol-3-glycosides in non-fermented skins after pressing and storage is causes of non-fermented skins showed high proportion of free flavonol aglycons, (55.29 % Molar in 2011 and 34.06 % in 2012: free Q, free K, free I). These results show a content of 297.6 ± 17.7 mg/kg in 2011 and 310.7±22.9 mg/kg in 2012 (as quercetin-3-glucoside equivalents). From the flavonols values determined in the BRS Lorena, concludes winemaking by-products exhibit significant amounts of these bioactive compounds as a potential functional component to be used in industrial applications.

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FA2-02

ANALYSIS OF FAT SOLUBLE VITAMIN CAPSULES USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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The analysis of fat soluble vitamins (FSV) formulations, typically from capsules, can be a challenging task. Current methods employ normal phase and reverse phase liquid chromatography techniques as well as gas chromatography, thin layer chromatography, and colorimetric techniques for these analyses. The use of supercritical fluid chromatography (SFC) in fat-soluble vitamin analysis provides a viable alternative that lowers the use of organic solvents, provides faster analysis times, and maintains chromatographic data quality. SFC is generally considered a cost effective, sustainable and green technology yet widespread adoption of analytical SFC has been hampered by instrumentation which does not perform to the standards established by modern HPLC systems. Using a newly designed analytical supercritical fluid chromatography system, the ACQUITY UPC2 system, a series of FSV formulations were analyzed. The formulations examined contained Vitamin A only, Vitamins A + D, Vitamin E, Vitamin K, Vitamins K + D, and Vitamin D only. Results from these experiments show SFC has the potential to replace many of the separation methods in use today.

FA2-03

IDENTIFICATION AND BIOAVAILABILITY OF ANGIOTENSIN CONVERTING ENZYME INHIBITORY PEPTIDES IN COMMERCIAL SOYBEAN BASED INFANT FORMULAS USING TANDEM MASS SPECTROMETRY

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Hypertension affects about a quarter of world's population and is a major risk factor involved in cardiovascular diseases [1]. Angiotensin I converting enzyme (ACE I) plays an important role in the regulation of blood pressure and its inhibition is at the core of hypertension treatment. Some native peptides in foodstuffs have demonstrated to prevent hypertension and, consequently, there is an increasing interest in this field. Moreover, in contrast to synthetic ACE inhibitors, these peptides have shown to be free from side effects. This work evaluates the presence of antihypertensive peptides in different commercial soybean based infant formulas (SBIF). The characterization of antihypertensive peptides, their identification by tandem mass spectrometry, and study of their bioavailability are also presented.

Extraction of all samples was performed by direct ultrafiltration (UF) using Mwco 10 kDa filters. Five different SBIF were extracted and all presented a high peptide concentration. Antihypertensive activity was measured by the *in vitro* inhibition of the ACE enzyme. The most relevant antihypertensive activity was presented by samples SBIF 4 and 5, with IC_{50} values of 5.88 ± 0.12 µg/mL and 2.45 ± 0.07 µg/mL, respectively. These extracts were further fractionated using selected cut-off filters and fractions from 5-10 kDa, 3-5 kDa, and below 3 kDa were obtained. The highest antihypertensive activity was observed in the peptide fraction from 3 to 5 kDa and below 3 kDa. Peptides in these fractions were identified by HPLC-ESI-Q-ToF and PEAKS software. At least, 36 different peptides for fraction 3-5 kDa and 23 different peptides for fraction below 3 kDa were assigned to the soybean proteome. Moreover, fractions were submitted to a simulated gastrointestinal digestion (GI) to determine peptide bioavailability. In all cases, antihypertensive activity slightly decreased. However, for samples SBIF 4 and 5, antihypertensive activities were still maintained at a high level, showing IC_{50} values of 18.18 ± 0.10 and 4.87 ± 0.13 µg/mL, respectively.

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OAP-01

LINKING ANALYTICAL CHEMISTRY AND ECOLOGY BY CHROMATOGRAPHIC TOOLS

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Analytical chemists can be considered problem-solving researchers. This approach involves constant challenges that only can be addressed with a solid base in extraction and separation techniques. Indeed, chromatographic techniques, in any of their combinations, are extremely useful tools to solve problems in every field. Within this philosophy, our group has established all sorts of collaborations. One of the most exciting and productive lines has been the cooperation with ecologists. For example, analysis of specific organic molecules has represented a key tool for disentangling trophic relationships and community dynamics in ecological studies.

The chemical ecology of living organisms also underlies their effective and successful survival strategies. Thus, the attraction and communication between organisms frequently involves detection of specific semiochemicals (a chemical emitted by an organism that provokes a behavioral or physiological response in another organism of the same or different species [1]).

In this talk we will show some examples of how analytical chemistry helps to shed light into unsolved ecological questions, such as: using GC-MS-FAME profiles for the characterization of microbial communities in solid organic wastes and composts [2]; tracking down microbial communities (structure and function) via fatty acids analysis in soils and solid organic samples [3]; clarifying the top-down effect of macrobiota on soil microbiota [4, 5]. Other study examples involving vertebrates includes the role of nonpigmentary antioxidants on sexual-selected traits [6], the pollution effects on oxidative damage and coloration in wild populations [7], and the effect of stress on phenotypic plasticity during development [8]. These collaborations are still alive and current investigations include the study of lipid metabolism in invertebrates and odor communication in seabirds.

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OAP-02

LA CIENCIA DE LA SENSIBILIDAD SOSTENIBLE: ÚLTIMOS DESARROLLOS TECNOLÓGICOS EN GCMS Y LCMS

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Actualmente, la mayor parte de los Laboratorios de Control de Calidad así como los Centros de Investigación dedicados al análisis de trazas en matrices complejas, se enfrentan a muy diversos retos analíticos. Uno de los más importantes es alcanzar una altísima sensibilidad para cumplir los requerimientos analíticos cada vez más exigentes de las normativas y sistemas de calidad actualmente en vigor en la mayoría de los países.

Existen ya muy diversas normativas analíticas en los campos de alimentación, medioambiente y toxicología, principalmente, que exigen límites de detección (LOD) o de cuantificación (LOQ) a niveles de femtogramos o incluso inferiores. La evolución tecnológica de las últimas décadas en instrumentación analítica y, particularmente, en GCMS y LCMS, ha permitido alcanzar niveles de sensibilidad insospechados hasta hace muy poco tiempo, pero quedaba pendiente la respuesta a una cuestión fundamental: ¿Cómo conseguir que la alta sensibilidad sea constante en el tiempo?

Este es un tema de vital importancia para un análisis cuantitativo riguroso en donde la exactitud, precisión y robustez son absolutamente necesarias en la determinación rutinaria de ultra-trazas en matrices complejas, principalmente en los Laboratorios que analizan una gran cantidad de muestras y requieren unos costes de mantenimiento y operación moderados.

Los recientes e innovadores desarrollos tecnológicos de Bruker en GCMS y LCMS dan una solución eficaz a esta problemática: nuevos diseños de las fuentes de ionización e interfaces que optimizan la formación y transferencia de iones, sistemas de limpieza activa de las fuentes, sistemas de cuadrupolos sin lentes, sintonización simplificada….

Estas recientes innovaciones de Bruker se desarrollarán en este Seminario con ejemplos específicos de aplicaciones y robustez de los análisis.

OAP-03

ANALYSIS OF MOSH AND MOAH IN FOOD AND PACKAGING MATERIAL USING PEGASUS 4D COMPREHENSIVE GC X GC TOF MS

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High values of compounds with alkyl chain lengths between C10 and C56 are often determined in food that is in direct contact to printed paper-based packaging or packaging made of recycling paper. This contamination is caused via migration from mineral oil containing inks. Migration of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) present a health risk and it is one of the food industry's hottest topics when it comes to the contamination of packaged foodstuff.

LECO offers you a complete solution for the analysis of MOSH and MOAH in either packaging material, ink or various food matrices. The solution includes a sample preparation based on the method developed by German Federal Bureau of Risk Assesment (BfR) and GCxGC TOF MS analysis.

This combination represents a fast, robust and sensitive analytical method that allows quantification of target analytes as well as semi quantification using Classifications and associated Scripting.

Comprehensive chromatography offers a new approach to measuring contaminants migration in foodstuff from packaging material. GCxGC is the method of choice for fast, easy and efficient quantification of the MOSH & MOAH fractions.

SP-01

DEVELOPMENT OF DIFFERENT STRONG-CATION EXCHANGE MATERIALS TO SELECTIVELY SOLID-PHASE EXTRACT ILLICIT DRUGS AND PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

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Efficient, selective, clean and cheap sample preparation to achieve low matrix effect and high sensitivity is challenging issue in analytical chemistry when complex samples are analysed. The most commonly used enrichment technique for liquid samples has been the solid-phase extraction (SPE), since their well-known advantages, such as the availability of different sorbents, which might involve capacity (sensitivity) or effectiveness in the removal of interferences from the matrices (selectivity) [1,2]. Moreover, in the last years, the application of mixed-mode polymers as SPE materials has really increased due to their enhancement of selectivity towards the target analytes and the elimination of matrix interferences. To fulfil this mixed-mode demand, different manufacturers as well as some researchers have developed several mixed-mode sorbents and their applications to analyse complex samples, emphasising the clean extract obtained, their great capacity, the effective washing step and thus low matrix effect [2].

We present different strong-cation exchange (SCX) materials synthesised using different polymerisation strategies, namely precipitation polymerisation, non-aqueous dispersion polymerisation and bulk polymerisation. In addition, the sulfonic moieties were introduced onto the material using different functionlisation approaches. In all the instances, we produce materials that combine enhanced ionic moieties and suitable morphological properties.

All these materials were evaluated as SPE sorbents to extract a group of basic compounds, including illicit drugs and pharmaceuticals, from wastewaters by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The SPE performance was assessed in terms of recoveries and matrix effect; results that were related to the morphology and ion-exchange capacity of each material.

Finally, the developed method with the best SCX material was applied for the determination of these target analytes in different complex environmental samples.

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SP-02

GAS EXPANDED LIQUIDS (GXLs) AS NEW SOLVENTS FOR BIOACTIVES EXTRACTION

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Haematococcus pluvialis is a microalgae known for accumulating the highest levels of a potent natural antioxidant, astaxanthin, that has demonstrated positive health effects. Therefore, numerous studies have focus on the development of novel and efficient extraction techniques that are in agreement with the strong demands in terms of quality of the extracts (purity and antioxidant activity), while complying with the Green Chemistry Principles.

Supercritical CO_2 ($scCO_2$) emerges as an alternative to organic solvents because of its high selectivity and bioactivity-preserving qualities. Nevertheless, astaxanthin is a large molecule with low solubility in $scCO_2$ that usually requires high pressures, long extraction times and a necessary pretreatment (*i.e* grinding) of the microalgae. Ethanol has been used as a green co-solvent for greatly improving astaxanthin yield by avoiding the problems faced by the use of pure $scCO_2$. Nonetheless, it is not clear the relationship that exists between other extraction factors such as pressure and temperature.

In this study we used a Box Behnken experimental design (BBD) that investigated the effects of operating pressure (200-350 bar), temperature (40-70 ºC) and modifier (0-13 %w/w Ethanol) on $scCO_2$ extraction of oleoresin yield, astaxanthin yield and antioxidant activity of the extracts. The experimental design showed that for all response variables the modifier effect was the most significant factor over pressure and temperature. These results lead us to investigate the effects of a further increase in the modifier content, up to the region of gas-expanded liquids (GXL). GXLs have shown to have improved mass transfer through reduced viscosity, increased solute diffusivity and decreased interfacial tension [1].

We studied the effect of temperature (30-60 ºC) and modifier (50-70%) at a fixed pressure (70 bar) on the same response variables. Results showed that increasing temperature generally worsened the recovery of astaxanthin and the antioxidant activity of the extracts. Still, low temperatures and 50% w/w of modifier could achieve more than 90% of recovery of astaxanthin within 2 hours of extraction. Furthermore, in the case of 45ºC and 50% w/w of modifier the recovery of astaxanthin surpassed the recovery achieved by a conventional acetone extraction, hence, validating the use of this new type of green technology for extraction of high valued compounds.

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SP-03

A QuEChERS METHOD TO DETERMINE POLAR EMERGING CONTAMINANTS IN SEWAGE SLUDGE

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Nowadays, different laboratories monitor concentrations of several chemical pollutants in different environmental samples in order to control the pollution. Nevertheless, this work could be tedious depending on the analytical method employed and the matrix analysed. For this reason, several analytical methods are focused on time and cost reduction. One example is QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method, based on the combination of salting-out liquid-liquid extraction and matrix dispersion extraction, initially developed for the analysis of pesticide residues in food matrices and recently applied to different solid matrices.

Sewage sludge is the solid residue obtained in Sewage Treatment Plants and the fate of most organic contaminants discharged from households, industries or hospital wastes, among others. Up to now, the extraction techniques mainly used for the analysis of sludge samples are pressurised liquid extraction, microwave assisted extraction or ultrasound assisted extraction. These techniques provide good extraction efficiencies for several compounds but most of them require a sophisticate equipment or relatively high time and solvent consumption. Moreover, sometimes a solid-phase extraction (SPE) after solvent extraction is required to reduce the matrix interferences and improve limits of detection (LODs). Therefore, QuEChERS based extraction method could improve some of these drawbacks because it only requires a centrifuge and it is a fast method with low solvent consumption. The possibility to perform a dispersive SPE (dSPE) after the extraction with a broad number of sorbents is an easy option to clean-up the sample.

QuEChERS method has been recently applied to determine a broad number of pharmaceuticals in sewage sludge with very promising results [1]. Nonetheless, this method has not been yet applied for other families such as benzotriazole, benzothiazole and benzenesufonamide derivates. These compounds are well-known aquatic contaminants released from several industrial and domestic uses but the information about their occurrence in sewage sludge is limited to few papers [2]. The aim of this study is to develop an efficient QuEChERS extraction method (including the study of different dSPE sorbents to reduce the matrix effect) followed by UHPLC-(Orbitrap)HRMS to determine five benzotriazole, four benzothiazole and five benzenesulfonamide derivates in sewage sludge samples.

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ENV3-01

FLAME RETARDANTS IN AIR FROM RURAL AND URBAN AREAS IN SPAIN

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Polybrominated diphenyl ethers (PBDEs) are additive flame retardants used in a wide array of consumer products. Technical penta- and octa-BDE mixtures were banned in Europe and the US in 2004 [1]. Furthermore, the Stockholm Convention (SC) listed them as Persistent Organic Pollutants (POPs) in 2009 due to their adverse effects on humans and ecosystems health [2]. Conversely, the deca-BDE was partially restricted in 2008 for electronic and electrical uses in Europe. It will be voluntarily phased out by the end of 2013 in North America. Yet, today it is mostly unregulated and in use worldwide [1].

In 2008, Spain started a POPs monitoring programme in air based on the use of PUF (polyurethane foam) disks following the guidelines of the Global Monitoring Plan for the implementation of the SC. PUF disks as passive air samplers allow for semi-quantitative evaluations of atmospheric POP levels. They are commonly used in air monitoring studies due to the advantages they present such as low cost and easy manipulation [3]. In this study, PBDE concentrations measured in air from urban and rural locations during the period 2008-2012 throughout the Spanish territory are presented.

PUF disks were placed in stainless steel domed chambers at six rural/remote and four urban sites chosen as sampling points. PUFs were deployed and collected every 3 months around each season's change. Samples were Soxhlet extracted during 24 h with petroleum ether. A subsequent clean-up step was performed on glass open tubular columns with multilayer acidic silica gel. Quantification of PBDEs was achieved by GC-qMS operating in the electron capture negative ionization mode (ECNI). Sixteen PBDE congeners were investigated: BDE-17, -28, -47, -66, -100, -99, -85, -154, -153, -184, -183, -191, -183, -197, -196, -209.

PBDE median values (and ranges) of 2.56 (0.01 – 464) pg/m^3 and 7.98 (0.07 – 557) pg/m^3 were measured in the air of remote and urban areas, respectively. Our results suggest that PBDEs in Spanish air are usually higher in urban compared to rural areas. High concentrations variability among sampling localities was observed. Total PBDE concentrations did not show significant temporal or seasonal trends. In contrast, BDE-209, which was found as the most abundant congener in all locations, increased significantly from 2008 to 2012 in both remote and urban areas. This is likely explained by the increasing use of the deca-BDE formulation as a result of the European ban on the penta- and octa-PBDE commercial mixtures. These results agree with the increase in PBDE concentrations, mostly accounted by BDE-209, detected also in other biomonitoring studies conducted in Spain [4].

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ENV3-02

DETERMINATION OF METHYL SILOXANES IN SOILS AND BIOTA SAMPLES FROM THE ANTARCTIC REGION

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Low molecular weight cyclic (cVMS) and linear (lVMS) volatile methyl siloxanes are a class of synthetic compounds, which belong the class of silicones. These compounds are very stable and are used in a plethora of applications. cVMS are used as carriers/thinners for the more viscous siloxanes that are meant to remain on treated surfaces. In industry applications, are used as lubricants, slips, hydraulic, transmission fluids, moisturizers, anti-foaming agents and plasticizer in silicone joint sealants in the construction sector. In consumer products, are used on textiles and in polishes for its water repellent property and as an antifoaming agent in washing powder. Also in food, dimethyl siloxanes are used as antifoaming agents in the production of beer, jam, juices, deep-frying fats and oils and as anti-clotting agents in powdered food. Many of these compounds are also used in personal care products including cosmetics, deodorants and soaps among others [1-5]. These compounds are also present in the industrial releases associated with the manufacture of high molecular weight silicon polymers. As a result of their wide use, siloxanes are presumably spread into the environment (~10×106 kg year-1 in the US) according to Dow Corning estimation [6], by both via point sources and via diffuse sources and may be found everywhere in the environment. Recent studies have suggested that siloxanes may have direct or indirect toxic effects on various biological processes. Consequently, a number of cVMS are currently under review for priority pollutant classification in North America and Europe. Therefore, the occurrence, fate and behaviour of linear and cyclic methyl siloxanes should be assessed and characterized in the environment.

During the recent years some works have studied the presence of methyl siloxanes as environmental contaminants. Due to their high volatility, cVMS have been identified in both outdoor and indoor atmosphere [7, 8]. Dimethylsiloxanes have been detected in sediments and biota [9, 10, 11] and freshwater [12, 13] and due to their volatility and stability they can also be transported to remote areas, as the Arctic region [11] by long range environmental transport (LRET). However, till now no work have informed about their occurrence in the Antarctic environment. In this work we have investigated the presence of eight compounds (hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), Decamethylcyclopentasiloxane (D5), dodecamethylcyclohexasiloxane (D6), octamethyltrisiloxane (MDM), decamethyltetrasiloxane (MD2M), dodecamethylpentasiloxane (MD3M), and tetradecamethylhexasiloxane (MD4M)) in soils, phytoplankton and krill from the Antarctic region.

cVMS were detected in almost all the soils samples analyzed but at very low concentrations ranging from the limit of detection and 50 ng/g. D5 and D6 were the compounds found to be at higher concentrations, as can be explained because are less volatile compounds whereas

D3 and D4 are less retained in soils. On the other hand, lVMS were detected in all the samples in concentrations ranging between the limit of detection and 0.5 ng/g.

In Phytoplankton cVMS were detected in all the samples but in general trends at lower concentrations ranging between the limit of detection and 10.0 ng/g. On the contrary as in soils, D3 was the compound at higher concentrations with a mean concentration of 4.3 ng/g. In addition, in this case lVMS were also detected in all the samples at pg/g concentration levels (between the limit of detection and 120 pg/g). As observed in the cVMS results, the most volatile lVMS was the one found at highest concentration, although MD4M was also found at exceptionally high concentrations in some samples, even surpassing the hundreds of pg/g.

For Krill samples cVMS were detected in all the samples in concentrations in the range of ng/g (between 4.02 and 31.0 ng/g). D4 was the compound found at highest concentrations, with a mean value of 16.1 ng/g while D6 was detected at a medium concentration of only 11.1 ng/g. The results show a good correlation (R^2>0.7) between D5 and D6. MDM was the only lVMS to be detected: Three samples showed quantifiable concentrations of MDM in the pg/g order.

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ENV3-03

NUEVAS PERSPECTIVAS EN EL DESARROLLO DE MÉTODOS MULTI-RESIDUOS: SIMPLIFICACIÓN ANALÍTICA EN EL DESARROLLO DE MÉTODOS Y REVISIÓN DE RESULTADOS MEDIANTE GCMS/LCMS TRIPLE CUADRUPOLO

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La tecnología Triple Cuadrupolo (TQ), tanto en GCMS como en LCMS, operando en modo MRM, es normalmente la elegida para una precisa cuantificación en el análisis de trazas de multi-residuos en matrices complejas. Esto es debido a su alta especificidad y capacidad para realizar, simultáneamente, cientos de transiciones MRM para un gran número de compuestos.

Sin embargo, el desarrollo de métodos MRM para multi-residuos suele ser complejo, necesitando la optimización de múltiples parámetros y la edición de métodos de adquisición y procesamiento con un enorme número de compuestos que requiere una gran atención y dedicación por parte de los analistas.

Por otra parte, la tecnología actual permite análisis muy rápidos lo que implica la necesidad de una revisión exhaustiva de un gran número de análisis con una gran cantidad de compuestos cada uno, diariamente. Los analistas se enfrentan a una difícil tarea para evitar el error humano, además de consumir un tiempo precioso para otras actividades más productivas en el Laboratorio.

Para ayudar a resolver esta problemática, Bruker ha desarrollado una serie de tecnologías basadas en los últimos avances de software en tratamiento de datos y revisión automática de resultados masivos.

La tecnología CBS™ ("Compound Based Scanning") permite la edición automática de métodos MRM a partir de base de datos suministradas por Bruker, sin necesidad de optimizar ningún parámetro operacional. CBS optimiza automáticamente también el tiempo de "scan" para cada transición programada en un método con objeto de obtener el máximo número de datos para cada pico cromatográfico y máxima sensibilidad.

El nuevo software PACER™ presenta un algoritmo matemático único, basado en la excepción, para la integración y revisión automática de resultados de una forma rápida y fiable evitando el error humano durante el análisis de un gran número de muestras.

En definitiva, con estos nuevos desarrollos, cualquier cromatografista puede utilizar la tecnología Triple Cuadrupolo MRM como cualquier otra técnica de rutina en su trabajo diario en el Laboratorio.

NDI2-01

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Daniel López Rodríguez

Micron Analítica, Madrid, Spain

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NDI2-02

STRUCTURAL IMPLICATIONS OF THE EFFECT OF PYROLYSIS TEMPERATURE ON THE CHROMATOGRAPHIC PATTERNS OF THERMAL DEGRADATION PRODUCTS FROM HUMIC ACIDS: CONSIDERATIONS ON THEIR ORGANIZATIONAL LEVELS

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Analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py–GC/MS) is a proficient technique in the structural research on soil organic matter because its outstanding potential for the breakdown of C=O or C–C bonds [1]. Pyrolysis releases a large variety of products with wide range of polarity from soil macromolecules. The potential of analytical pyrolysis for unbiased structural analysis is the subject of controversy. Rigorous authors tend to consider that pyrolysis is only valid for fingerprinting purposes in samples analyzed in the same conditions. Due to the above limitations, the structural information inferred from Py–GC/MS analysis of heterogeneous macromolecular material, such as soil humic substances, requires comparisons between mild and drastic degradation methods. Pyrolysis offers the possibility of modifying the intensity of the final degradation by controlling temperature and time of the combustion [2]. In this study Py–GC/MS at 500 ºC and 700 ºC was carried out in a Pyrojector (SGE instruments) connected to a Finnigan Trace GC Ultra with a Trace DSQ mass spectrometer, using a 30-m HP-1 column. Oven temperature was 50 ºC for 1 min, then increased up to 100 ºC at 32 ºC min^{-1}, from 100 to 320 ºC at 10 ºC min^{-1} and isothermal at 320 ºC. The most significant differences between pyrolytic patterns in terms of temperature were the practical lack of methoxyphenols and the release of a conspicuous alkyl series in samples at 700 ºC. The latter series consisted of fatty acids (mainly C12 to C18 with strong even-to-odd C-number preference) and hydrocarbons (alkanes and alkenes in the C7–C21 range and no C-number preference). The differences are interpreted as: a) most of the information concerning lignins and other methoxyphenol-releasing biomacromolecules is lost after Py at 700 ºC, b) the temperature-dependent aliphatic enhancement observed suggest that most of the alkyl products (C7–C17) are not thermoevaporation products but pyrolytic fragments from a condensed humic organizational level. From the above results, Py at 500 ºC was found appropriate to analyze comparatively young and structurally labile humic acids with lignin-like structures, whereas Py optimized at higher temperature (700 ºC) provides more reliable data on the condensed alkyl domain in humic substances.

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NDI2-03

EVALUATION OF THE IONIZATION BEHAVIOUR OF BROMINATED FLAME RETARDANTS UNDER GC-APCI-MS (QqQ and QTOF)

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Halogenated flame retardants [e.g. polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBP-A)] are widespread in the environment due to their use in a variety of electronics, clothes and furniture for enhancing fire safety. Due to their persistence, bioaccumulative and toxic properties, authorities have restricted the use of PBDEs. The European Union (EU) banned Penta-BDE and Octa-BDE mixtures in 2004 as well as Deca-BDE in electric and electronic products in 2009. Maximum concentration limits of 0.1 % by mass have also been set for Penta-BDE and Octa-BDE in products placed on the market [1,2]. As a result of these regulations, the use of alternative flame retardants is increasing, known as Novel Brominated Flame Retardants (NBFR). Decabromodiphenyl ethane (DBDPE) is used as a substituent of Deca-BDE [3], while BTBPE has been announced as a potential replacement for technical Octa-BDE [4] and the use of phosphate flame retardants is also increasing. Due to the lack of data on the identity and levels NBFRs currently in use, the development of proper, broad screening techniques for these compounds and possible unknowns is highly desirable.

Owing to its sensitivity in full-scan acquisition mode and high mass accuracy, HR full spectrum acquisition techniques, such as HR-TOFMS, have been increasingly used in the last decade in environmental sciences for both targeted and untargeted analysis. The combination of a soft ionization technique capable of generating molecular ion dominated spectra with HRMS is especially suitable for compound identification.

In this way, the new soft ionization APCI source (commercial name APGC) has already been satisfactorily applied in our research group to GC-amenable compounds such as pesticides [5-7] and very recently to dioxins and dioxin-like PCBs.

In this study, APCI is assessed as ionization source for the analysis of brominated flame retardants using HR-(Q)TOF as well as QqQ mass analyzers in combination with HRGC separations.

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PS1-01

DETERMINATION OF ENDOCRINE DISRUPTING CHEMICALS IN HUMAN BREAST MILK SAMPLES BY STIR-BAR SORPTIVE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The group of compounds commonly called endocrine disrupting chemicals (EDCs) covers a wide range of synthetic and natural substances, able to alter the normal hormone function of wildlife and humans, consequently causing adverse health effects. Bisphenol A (BPA) and its chlorinated derivatives, benzophenones (BPs) and parabens (PBs), belong to this group of compounds [1, 2].

The aim of this work is to develop and validate an accurate, sensitive, simple and cost-effective method for the identification and quantification of EDCs in human breast milk samples. Bisphenol A and its main chlorinated derivatives, six benzophenones and four parabens, were selected as target analytes.

The introduction of a stir-bar sorptive extraction (SBSE) procedure, and subsequent solvent desorption with an organic solvent prior to GC-MS analysis is proposed. The use of tandem MS allows the unequivocal identification and quantification of these compounds. The main parameters affecting the SBSE (ionic strength, stirring speed and extraction time), the nature of the organic solvent employed for the solvent desorption procedure; the main parameters affecting the derivatization step (temperature, percentage of derivatization agent and time) and the optimal instrumental conditions for CG-MS/MS analysis were evaluated and optimized.

Quality parameters such as linearity, accuracy in terms of trueness and precision, sensitivity and selectivity were examined with good results. Very low limits of detection and quantification were obtained with a good precision and linearity. Mean recoveries obtained in the trueness study, were very close to 100% in all cases.

In this work SBSE demonstrates to be an effective technique for the extraction of ECDs in breast milk samples, in a simpler and less labor intensive than traditional methods.

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PS1-02

STIR-MEMBRANE SOLID-LIQUID-LIQUID MICROEXTRACTION FOR THE DETERMINATION OF PARABENS IN LYOPHILIZED HUMAN BREAST MILK SAMPLES

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Parabens belong to the group of endocrine disrupting substances which are able to alter the normal hormone function and, consequently, causing adverse health effects. Since breast milk is the main route of exposure of environmental chemical for breastfed infants, its analysis is of special scientific interest. The isolation of the target analytes from this complex biological sample is a critical aspect even when a chromatography technique is employed for sample analysis due to selectivity and sensitivity issues.

In the present communication, a new extraction procedure evolved from stir membrane extraction technique is proposed for the isolation and preconcentration of four parabens from lyophilized human breast milk before their final chromatographic analysis. The extraction procedure allows the simultaneous solid-liquid extraction of the parabens from the lyophilized samples and the subsequent liquid-liquid extraction of the organic extract in order to improve the selectivity and sensitivity of the determination. The main variables affecting the extraction procedure has been evaluated and optimized.

The integration of both extractions allows not only the improvement of basic analytical properties but also the simplification and miniaturization of the sample treatment. In addition, the sample throughput is enhanced since several samples can be extracted at the same time. The new proposal, which potential has been probed, is highly versatile and it can be easily adapted to face up different analytical problems.

PS1-03

MONITORING OF ROUTINE CONTROL ANALYSIS OF ALBUMIN SOLUTIONS

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The major protein of plasma or serum is albumin. Albumin maintains the colloidal osmotic pressure of human serum and is a transport protein for many hormones, ions, vitamins and other factors.

As part of the regulation of biological medicinal products, Article 114 of Directive 2001/83/EC relating to medicinal products for human use, as amended by Directive 2004/27/EC of the European Parliament and the Council provides that a Member State laboratory may, but is not required to, test a batch of a medicinal product derived from human blood or plasma before it is placed on the market. Thus, an Official Medicines Control Laboratory (OMCL) is required for the authorization and approval.

The analysis of this medicine is performed by size-exclusion chromatography, a chromatographic technique which separates molecules in solution according to their size. Its specifications are described in European Pharmacopoeia (Eur. Ph.); the columns used contain silica-based, with 5µm particles and 250Å pores which permits the protein and large peptide separations in the range 10,000 to 500,000.

In this study we summarize the routine analysis for albumin in our blood products laboratory. We have studied more than 1,200 samples of albumin, analyzed between 2008 and 2012 and more than 180 results for an internal control (IC) used in this chromatographic assay.

Although Eur. Ph. doesn't define any suitability test for this assay, we have several in-house requirements for an internal control, performed before analysing the samples: The retention time of peak due to polymers and aggregates (PA) is less than 14 minutes; the relative area of this peak (%PA-IC) meets the acceptance interval according to validation data (2.44 to 3.57 at the 95% confidence level) and its relative standard deviation is not more than 8.0%.

Other statistical study has been performed with the results of %PA-IC, considering the different chromatographic columns used in each assay and the possible variation over the time. We have also checked the variation for the IC results with ANOVA and we have found that the p-value for the factor "columns" was lower than to 0.05. Therefore, there were statistically significant differences among the columns used at the 95% confidence level. No significant effect is shown during its lifetime.

PS1-04

A CAPILLARY ELECTROPHORESIS METHODOLOGY FOR THE CHIRAL DETERMINATION OF DULOXETINE IN PHARMACEUTICAL FORMULATIONS

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Duloxetine is a balanced and potent dual reuptake inhibitor of serotonin and norepinephrine (SNRI) effective in the treatment of major depressive disorder (MDD). MDD affects 121 million people across the world and, according to the World Health Organization, it is the fourth leading cause of disability worldwide. Duloxetine is a chiral drug with an asymmetric carbon being both enantiomers potent norepinephrine and serotonin reuptake inhibitors, although the S-enantiomer was found to be slightly more potent [1]. Since this drug is commercialized as a pure enantiomer, chiral methodologies are needed to ensure the quality control of its optical purity.

In this work, the separation of enantiomers and enantiomer migration order of duloxetine were investigated in CE using various cyclodextrins (CDs) as chiral selectors. The best results were obtained for 2-hydroxylpropyl-β-CD and methyl-γ-CD. The different enantiomer migration order obtained for duloxetine with these two CDs was investigated.

A chiral method enabling the separation of duloxetine enantiomers was developed and applied to the quantitation of duloxetine and its R impurity in different pharmaceutical formulations. In order to develop the chiral method, a phosphate buffer containing 2-hydroxylpropyl-β-CD was employed. The influence of the concentration and pH of phosphate buffer, the concentration of cyclodextrin, the voltage and the temperature on the enantiomeric separation was studied. Under the optimized conditions the obtained resolution was above 4 in less than 20 min.

The analytical characteristics of the developed method were evaluated in terms of linearity, selectivity, accuracy, precision and sensitivity, with a LOD of 0.4 mg L^{-1} for each enantiomer, which was enough to detect enantiomeric impurities up to 0.4 % of R-duloxetine with respect to the major enantiomer (S-duloxetine).

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PS1-05

TOXICITY ASSAY IN INTACT HT-29 HUMAN COLORECTAL CANCER CELLS BY CAPILLARY ZONE ELECTROPHORESIS WITH DIODE ARRAY DETECTION

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Determination of the cytotoxic effect of any potential bioactive food ingredient or drug is essential to characterize its biological activity. Cytotoxicity assays are also widely used in safety testing of potentially harmful substances found in the environment or food chain. For screening purposes, in vitro cytotoxicity methods are preferred over in vivo methods with animals since the latter apart of bringing about ethical considerations, are slow, costly and labor intensive. At present, the most commonly used in vitro cytotoxicity testing methods are based on flow cytometry, microplate-based spectrophotometry, and microscopy. Recently, capillary electrophoresis (CE) with diode array detection (DAD) has also demonstrated to be an excellent alternative for the cytotoxic analysis of intact HeLa cells [1,2]. In the present work, the CE-DAD procedure based on trypan blue exclusion, developed by Ren et al. [1] has been adapted to the analysis of intact HT-29 human colon cancer cells with the aim of testing the cytotoxic effect of different chemicals and food ingredients. To achieve this, a thorough study of the parameters and steps (staining time, washing routine, centrifuge force, time of fixation with paraformaldehyde, and filtering) affecting the preparation of HT-29 cell samples prior CE-DAD analysis was carried out. Also, the CE-DAD separation and detection parameters (capillary inner diameter, wavelength, acquisition frequency, etc.) were optimized in order to enhance the detection sensitivity and improve the feasibility of the method. The optimized procedure was tested on samples prepared with different % of non-viable cells. A correlation coefficient (R^2) of 0.9931 was obtained when theoretical % non-viable cells values were compared with those obtained from the CE-DAD analyses in the range of 0-100% non-viable cells. The method demonstrated acceptable intra-day and inter-day precision for the determination of % non-viable cells in culture samples, providing values around 8 %RSD (n=3) and 9 %RSD (n=6), respectively. Also, the % deviation values from the theoretical ones ranged between 1 and 9 %. The good possibilities of the proposed method in terms of quantitative determination of the cytotoxic effect of bioactive compounds in HT-29 cells was confirmed by testing 5-fluorouracil, carnosic acid and polyphenol-enriched extracts from rosemary leaves.

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PS1-06

IN VIVO HUMAN METABOLISM STUDIES ON MEPHEDRONE

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The stimulant designer drug mephedrone is a derivative of cathinone - a monoamine alkaloid found in khat - and its effect resembles that of 3,4-Methylenedioxy methamphetamine (MDMA). Abuse of mephedrone has been documented since 2007; it was originally a 'legal high' drug, but it has now been banned in most Western countries.

The metabolism of mephedrone has been studied mainly in rats [1] or in vitro using human liver microsomes [2]. Some of the metabolites found in these studies have also been found in human urine samples submitted for drug testing, but no dedicated study with human volunteers has been performed for studying the *in vivo* metabolism of mephedrone in humans.

Here we present the preliminary results obtained for human metabolism observed in six healthy volunteers who received different doses of mephedrone ranging from 50 to 200 mg. Urine was taking before administration and along the following 24 hours based on the fast metabolism expected for this type of drugs.

In this study, the discovering and identification of the Phase I and Phase II metabolites of mephedrone was based on ultra performance liquid chromatography coupled to hybrid quadrupole-time of flight mass spectrometry, operating in the so-called MS^E mode. In this acquisition mode, two accurate-mass full spectra are acquired sequentially. The first one, without applying collision energy and therefore obtaining information about the intact molecules present in the urine samples. The second, applying a collision energy ramp, promoting fragmentation and obtaining accurate-mass fragment ions useful for metabolite identification. When necessary, product ion spectra were also acquired in MS/MS mode.

The aim of the study was double. On the one hand, to establish which is the real human metabolism of mephedrone compared to other models (rats, microsomes,…). On the other hand, to define which is the best biomarker of mephedrone consumption to be used in the new field of sewage epidemiology [3], where drug consumption by a population is indirectly measured through the levels of their metabolites in influent wastewater.

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PS1-07

DEVELOPMENT OF AN ANALYTICAL METHOD FOR SIMULTANEOUS DETERMINATION OF MYCOTOXINS IN HUMAN PLASMA BY LC-MS/MS

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Mycotoxins are toxic compounds produced by fungi that contaminate food and raw materials which can reach man or animal and affect their health. In order to evaluate exposure and risk assessment for human health, it is necessary to develop an analytical method that can measure the bioavailability of these mycotoxins for humans.

The occurrence of mycotoxins in food is continuously being studied; there are many analytical methods for the quantification of mycotoxins [1] in different matrices and quite a few studies regarding the prevalence of different mycotoxins in human food. However, an analytical method for the analysis of mycotoxins in human plasma is needed for a proper evaluation of exposure and risk assessment.

The presence of different families of mycotoxins has been studied in different human biological fluids (plasma, serum, urine, etc.) [2 ,3] and in animal fluids also [4, 5]. Due to the variety of human consumption, the main source of mycotoxin exposure, and the variety of mycotoxins present in different foods, there is a need to develop an analytical methodology for simultaneously evaluating the levels of different mycotoxin families in a single sample.

The aim of this study was to develop an analytical method using LC-MS/MS (QqQ), to be applied when studying the simultaneous presence of T-2, HT-2, deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol, zearalenone, aflatoxins (B1, B2, G1, G2, M1), fumonisins (B1, B2 y B3), OTA, ochratoxin B, patulin, citrinin and cyclopiazoic acid in human plasma.

Mycotoxin extraction procedure was examined in order to combine the best recovery for mycotoxins and the least matrix effect in the detector. Procedures such as protein precipitation, liquid-liquid extraction using different solvent combinations, SPE, QuEChERS and OSTRO were evaluated.

Chromatographic separation was carried out in reversed-phase. After ESI ionization, MS (triple quadrupole) detection was used in dynamic MRM, using at least tree transitions (Q, q, q) for each mycotoxin.

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PS1-08

DETERMINATION OF PHTHALATE METABOLITES IN HUMAN URINE SAMPLES BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Phthalate acid esters (PAEs) are well known chemical compounds widely used in today's society as plasticizers in a wide range of consumer products including food packaging and food containers, toys, personal care products, cosmetics, adhesives, paints, detergents, building products (flooring, sheeting, and films), lubricating oils, carriers in pesticide formulations, PCB substitutes, solvents, some medical devices, medical equipment, etc. [1]. During the last years, their activity as endocrine disruptors, their impact on the normal development of living organisms, as well as their teratogenic activity in both humans and animals, has made them to be considered as new emerging contaminants.For the general population, the oral route of exposure has been considered the major route, including inhalation of air (indoors and outdoors), ingestion of food, incidental ingestion of soil, and ingestion of dust (indoors), as well as direct contact with products that contain phthalates. Once ingested, phthalate diesters are metabolized, initially hydrolyzed in the intestine to their corresponding monoester (primary metabolites), which are then absorbed [2], and could be further oxidized in the body (secondary metabolites), and excreted quickly via urine [3].

We present here the results of the determination of 9 PAE metabolites (MPAEs) in urine samples of 21 volunteers (12 female and 9 male) from the Community of Madrid: 6 phthalate monoesters (monomethyl phthalate [MMP], monoethyl phthalate [MEP], mono-iso-buthyl phthalate [MiBP], mono-*n*-butyl phthalate [MBP], monobenzyl phthalate [MBzP] and monoethylhexyl phthalate [MEHP]) and 3 secondary metabolites of DEHP (5-OH-mono(2-ethylhexyl) phthalate [5-OH-MEHP], 5-oxo-mono(2-ethylhexyl) phthalate [5oxo-MEHP] and 5-carboxy-mono(2-ethylpentyl) phthalate [5cx-MEPP]). Briefly, the sample preparation procedure consisted of an enzymatic deconjugation of the glucuronide phthalates, followed by a purification step with an OASIS® HLB SPE cartridge (Waters, MA, USA). LC chromatographic separation was achieved using an Acquity UPLC BEH-phenyl column (50 mm x 2.1 mm, 1.7 µm, Waters). A precolumn was used, before the injection valve, to eliminate interferences from the system and the mobile phase. For the determination, a triple quadrupole Xevo-TQS (Waters) working in MRM mode was used. Total concentrations, calculated as the sum of the 9 MPAEs determined, ranged between 65 and 906 µg/L. Among the metabolites, the highest concentrations (median (range)) were found for MEP 69 µg/L (25 – 605 µg/L) followed by MiBP 23 µg/L (4 – 106 µg/L) and 5-cx-MEPP 22 µg/L (6 – 195 µg/L). The results found in the different urine samples investigated were similar to other published studies from Germany, US, Taiwan and China [4].

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PS1-09

DETERMINATION OF PESTICIDES AND METABOLITES IN CEREAL-BASED BABY FOODS AND WHEAT FLOUR BY MEANS OF HOLLOW-FIBER LIQUID-PHASE MICROEXTRACTION

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Cereal derivatives such as processed infant cereals and flours are an important energy source for the nutrition of infants and young children. However, the high consumption of cereal-based baby foods may be a significant source of daily exposure to pesticides. Therefore, and because of the high preconcentration factors required to achieve the very strict maximum residue limits, the determination of pesticides in infant foods is often complicated. Moreover, the presence of fats often requires an additional clean-up step to obtain appropriate extracts. In this sense, the miniaturized format of liquid-liquid extraction called liquid-phase microextraction (LPME) appears as an alternative to traditional sample preparation protocols. Among them, hollow fiber (HF)-LPME was introduced by Pedersen-Bjergaard and Rasmussen in 1999 [1] as a promising method owing to its low cost, great reduction in the volumetric ratio of the acceptor and the sample phases, high enrichment factors, excellent sample cleanup ability, total elimination of sample carryover and reproducibility. In this approach, analytes are extracted from the aqueous sample onto a HF, generally made of polypropylene, impregnated with a water immiscible solvent.

In this work, a new HF-LPME procedure has been proposed for the determination of 13 pesticides (some of them European Union (EU) priority pesticides in baby foods, i.e., terbufos, disulfoton, fensulfothion, cadusafos and ethoprofos), and some of their metabolites in two commercial cereal-based baby foods and one wheat flour previous ultrasound-assisted extraction (UAE) by gas chromatography with nitrogen phosphorus detection (GC-NPD). The main parameters affecting the extraction efficiency were investigated such as sample pH, ionic strength, stirring rate, extraction temperature and time as well as the desorption procedure. The optimized method was validated in terms of calibration, precision and accuracy. Limits of detection (LODs) achieved were between 0.29 and 3.20 μg/kg. The extraction of Milli-Q water, as an example of the applicability of the procedure to aqueous samples, allowed achieving LODs in the range 0.01-0.04 μg/L. All these values allow the determination of these pesticides at the levels required by the EU legislation. The proposed UAE-HF-LPME-GC-NPD method provides desirable features such as low-cost, speed, elimination of carry over effects, small volume of organic solvent and high analyte preconcentration.

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PS1-10

DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES IN PROCESSED CEREAL SAMPLES (GOFIO) DURING STORAGE

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Organophosphorus pesticides (OPPs) are widely used as preharvest insecticides, but they can also be applied directly to the commodity during storage for protecting from pests because of their relatively low rates of degradation which, on the contrary, may be hazardous to human health. This issue is particularly important for cereals since they are the traditionally most consumed foods at the basis of all regional diets in the world. They are also used for food products such as processed infant cereals and flours. The degradation of most pesticides in cereals occurs through oxidative mechanisms and their persistence can be enhanced by natural antioxidants like phenolic acids. Gofio, a milled toasted cereal with an appearance similar to flours widely produced in Canary Islands, is known to possess a high nutritional content. However, up to now, no studies have been carried out regarding the degradation of pesticides in this matrix.

In this work, the effect of storage on breakdown of a group of 11 OPPs (ethoprophos, cadusaphos, dimethoate, terbufos, disulfoton, chlorpyrifos-methyl, fenitrothion, pirimiphos-methyl, malathion, chlorpyrifos and fensulfothion) was evaluated during three months of storage in maize and wheat gofio. Their decomposition in four possible transformation products (disulfoton sulfoxide, malaoxon, terbufos sulfone and disulfoton sulfone) was also examined. For this purpose, pesticide-free maize and wheat gofio samples were treated with the pesticides and they were stored in the darkness at ambient temperature in a closed container. A multiresidue analysis based on a QuEChERS method previously developed [1] and subsequent gas chromatography equipped with nitrogen-phosphorus detection was performed for the simultaneous determination of these pesticides and their metabolites over time. Results demonstrated that degradation was slower in maize gofio than in wheat gofio and no metabolites were detected in any of the samples. After three months of storage, the reduction of residues ranged between 32% (cadusaphos) and 86% (disulfoton) for maize gofio and 40% (cadusaphos) 92% (disulfoton) for wheat gofio.

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PS1-11

OPTIMIZED EXTRACTION AND QUANTIFICATION OF SULFORAPHANE IN BROCCOLI

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The potentially protective role of cruciferous vegetables, including broccoli, and active components present in these vegetables, such as glucosinolates and their break-down compounds (isothiocyanates), has been extensively studied. A great deal of research on functional foods like anticarcinogens has focused on broccoli and on a single bioactive component within broccoli, namely, sulforaphane (SF), which it was found to be the most potent naturally occurring inducer of the detoxification of potential carcinogenic substances [1].

In order to obtain the highest amount of SF as possible from several broccoli parts, we have decided to check the influence of several parameters that could affect to the formation of SF as the hydrolysis temperature, pH, volume and time. Moreover, several solvents were tested to perform the extraction of SF from broccoli with the aim of achieving the best efficiency and environmental friendly extraction as possible. Finally, it was required the use of a solid phase extraction procedure with silica cartridges. Moreover, a new, economic and fast liquid chromatography coupled to a diode array detector (LC-DAD) method was developed to determine SF in broccoli. It has been used a reverse phase (RP) analytical column, Synergi Hydro-RP, and isocratic elution mode of the mobile phase, which was composed by ammonium formate in water and acetonitrile, in order to decrease as much as possible the chromatographic run and obtain the best resolution between the analyte and some matrix peaks. The entire method was validated following different International guidelines determining the selectivity, limits of quantification (LOQ) and detection (LOD), as well as the linearity, precision and recovery. Finally, the proposed method has been applied to the evaluation of SF in several broccoli parts samples from six different cultivars (Ramoso calabrese, Parthenon, Marathon, Nubia, Naxos and Viola).

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PS1-12

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS AND FATTY ACID RATIOS OF ARCHAEOLOGICAL POTTERY FROM THE VACCEAN NECROPOLIS OF "LAS RUEDAS" IN PINTIA

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Archaeological residue studies have generally targeted the recovery of lipids, since they are stable and do not degrade as quickly as other compounds. In general, lipid profiles are distinct between different plant and animal species. However, two processes act to obscure these differences in archaeological pottery. First, cooking exposes lipids to heat, which causes degradation. Secondly, although lipids are stable relative to other organic compounds such as DNA and proteins, oxidation and hydrolysis do contribute to their decomposition. Thus, when using ratios of lipids to identify foods, a goal should be to examine the ratios of compounds that oxidize at similar rates, as fatty acids. For this reason, it has been postulated that the use of ratios of fatty acids that degrade at roughly the same rate can be useful to identify very general categories of foods.

The purpose of this work has been to develop a gas-chromatography-mass spectrometry method (GC-MS) that allows the best and fast separation and determination of 39 fatty acids. It should be remarked that the fatty acids from archaeological pottery samples were analyzed by derivatization to their methyl esters (FAME) using KOH and BF_3, and afterwards, they were isolated by means of a solid-liquid extraction with hexane. The GC-MS analyses were carried out using a gas chromatograph equipped with an automatic injector and a single quadrupole mass spectrometer. The column used for the experiments was a BPX70 (60m, 0.25mm, 0.25µm), and the carrier gas was helium at a flow rate of 1.1 mL/min. The proposed method was validated in terms of selectivity, limits of quantification (LOQ) and detection (LOD), linearity, precision and recovery. Finally, the developed method has been applied to analyze fatty acid residues in archaeological samples (pottery) obtained in the Vaccean necropolis of "Las Ruedas" in Pintia (Valladolid, Spain) in order to identify the content of those ancient pots.

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PS1-13

QUANTIFICATION OF ESSENTIAL OILS IN ANIMAL FEED MEAL BY SOLVENT-EXTRACTION WITH GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AND QUALITATIVE ANALYSIS BY SOLID PHASE MICROEXTRACTION

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Essential oils (EO) are aromatic compounds extracted from plant material usually by steam distillation. They have oily appearance and are non-soluble in water, soluble in organic solvents as methanol or hexane and volatile liquids [1]. In recent years, EO have been explored as an alternative to antibiotics to manipulate rumen fermentation mainly to improve nutrient utilization and performance by ruminants [2]. EO can be either added directly to the ruminant's diet or included in inorganic matrices such as diatomaceous earth. However, these compounds have low stability, being necessary to quantify their persistency in the diet over time. The aim of this work was to develop an easy method to quantify the concentration of essential oils in ruminant's diet. In addition, a qualitative analysis by SPME (solid phase micro-extraction) to determine the presence of EO in the diet is suggested.

Samples of a conventional diet in which four different essential oils: diallyl disulfide, thymol, carvacrol and eugenol were added at 50 ppm, were used for optimization. The solvent-extraction was performed by orbital agitation. Three different solvents (methanol, hexane and ethyl ether) and three agitation times (5, 15 and 30 minutes) were used, comparing the recovery rates using C16:0 methyl ester as internal standard. For quantification BHT was used as internal standard, added at the beginning of the extraction. An additional step of concentration by vacuum evaporation of the solvent for 10 minutes was performed. Hexane was chosen as solvent for the extraction procedure, with 5 minutes of agitation. The extraction and concentration steps were also carried out for calibration standards (fortified samples from 10 to 100 ppm).

For the MS-MS analysis, using an ion trap, the main mass of each compound was selected and several collision energies were tested, looking for the ones which give a spectrum with around 10% of the precursor ion. Quantification was done with the total area of the resulting fragments. Identification was done based on the retention times and on the comparison of the MS-MS spectra with that of known standard. Limit of detection, limit of quantification, linear range, precision and accuracy were calculated for each compound. This quantitative method was performed in samples used in ruminants´ diet which contained EO.

In order to check whether a sample contains EO, a qualitative analysis by SPME was carried out as follows: 30 min headspace extraction of 1 gram of sample at 40ºC with a fiber of Carboxen/PDMS, and MS detection in Full Scan mode. Identification was done based on comparison of the MS spectra with that of known standard and a reference library.

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PS1-14

ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (UHPLC-MS/MS) ANALYSIS OF BENZOPHENONES IN PACKAGED FOOD

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The presence of contaminants in food is a problem which has become relevant in the last years due to its high social impact. Plastic packages are capable of retarding and preventing detrimental changes in the packed food because they act as barriers against oxygen, light, microorganism, etc. Benzophenones (BPs) prevent scents and colors damaging of food products. So they are frequently added to plastic, paper and board as UV blockers. However, there is an increasing concern on the migration of chemicals from packaging on to the foods they protect. Most of the chemicals are not considered to be dangerous at the trace amounts found, but consumer concern and general fears about daily exposure to a cocktail of compounds have led to the European Union to establish regulations for controlling food contact materials and to minimize migration levels.

In this work a fast ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method is proposed for the analysis of seventeen BPs in packaged food. The chromatographic separation was achieved using a C_{18} Thermo Accucore (150 x 2.1 mm, 2.6 µ) column at 45 ^{o}C and MeOH:ACN:formic acid-ammonium formate (25mM, pH 3.75) as mobile phase (400 µL min^{-1}). This chromatographic system was coupled to an ESI source in working in both positive and negative mode. Multi-stage mass spectrometry (MS^n) in an ion trap (IT) was used to study the fragmentation of this family of compounds and the most characteristic product ions were correctly assigned combining the structural information obtained in an ion trap and triple quadrupole. For quantitation SRM in the triple quadrupole is proposed and the method was applied to both aqueous and fatty samples. A QuEChERS-like approach was applied for aqueous matrices, while for fatty samples a liquid-liquid extraction procedure was used.

Finally, a quadrupole-Orbitrap (Q-Exactive) working in different scanning modes (full scan, all ion fragmentation, target-MS^2, etc.) was used for confirmation preventing false positives and to identify possible untargeted plastic additives.

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PS1-15

POLYPHENOLIC CONTENT AND IN VITRO ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM WHITE GRAPES MARC

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Industry byproducts of plant origin represent an abundant source of bioactive compounds. In this contribution, solid wastes from the white wine industry were subjected to evaluation as potential sources of antioxidant phytochemicals on the basis of their content in phenolics and in vitro antioxidant activity.

Monovarietal grape bagasse of twelve varieties of *Vitis vinifera* L. from Galicia (NW Spain) have been analyzed in order to get the total polyphenolic content and the concentration of the major polyphenols present in each bagasse type. Phenolics have been extracted by means of an optimized Pressurized Solvent Extraction methodology [1] and further analyzed by HPLC-DAD.

The studied grape marc samples (all of them from the 2012 vintage) were divided into two groups representing, respectively, byproducts from native varieties obtained in wineries during the normal process of commercial white winemaking: Albariño, Treixadura, Godello, Loureiro and Caíño Blanco; and experimental bagasses, obtained for research purposes from the *Ribadumia Enological Station* (Pontevedra): Torrontés, Blanca Lexítima, Gewurztraminer, Chardonnay, Riesling, Sauvignon Blanc and Pinot Blanc. The phenolic characterization of the first group has been well established due to the number of representative samples. The second group has been screened in order to get a global idea about the polyphenolic profile of the corresponding grape marcs. In addition, in vitro antioxidant capacity has been determined for all samples by the antiradical activity measure procedure based on DPPH. The correlation between the phenolic content (total and individual key polyphenols) and the antioxidant activity is deeply investigated and discussed because it is not always as direct as it can be supposed.

The results showed that extracts from white grape marcs, whatever the native variety, contain large amounts of polyphenols (average 41 mg GAE/g dw), in spite of slight differences in the individual polyphenolic profile. The average antiox activity is about 3 mM Trolox/g dw, higher than numbers reported for white grape marc from other varieties, and even, red grape pomace and other agri-food solid wastes tested [2].

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PS1-16

GRAPE PHENOLICS DISTRIBUTION DURING WHITE WINEMAKING

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White wines are exclusively produced by the fermentation of grape juice, without the presence of the solid parts of the berry (skins and seeds). It is the absence of skin contact in the alcoholic phase, and not the color of the grape, that distinguishes white from red winemaking. This fact does not mean that white winemaking does not include any maceration (solubilization of solid components in juice). A certain degree of maceration occurs in the absence of alcohol during the pre-fermentation phase, at the time of juice extraction and clarification. Thus, prefermentation treatment conditions control the passage of compounds responsible for the qualities and flaws of grapes into must. [1]. Once grapes are harvested, phenolic composition in the wine becomes dependent on processing in the winery. Depending on the grape variety and the must manipulation during production, the final wine will have its characteristic polyphenolic profile.

The importance of phenolic compounds in red wines is so great that the phenolic composition of white wines is often overlooked. The critical phenolic components for white wines are not as well understood, the sensory effects of these compounds have not been studied in the same depth, and the effects of viticultural and winery processes on white phenolics are not as widely known as for red grapes and wines.

In this context, the phenolic composition of different white grape varieties and the characteristic polyphenols of their corresponding monovarietal white wines (2010 & 2011 vintages) have been determined by means of HPLC-DAD analysis. Five autochthonous grape varieties from Galicia (NW Spain) have been considerd, namely Albariño, Godello, Treixadura, Caíño Blanco and Loureiro. Pressurized Solvent Extraction has been applied to grapes [2], whereas wines have been directly injected. The obtained data have been analyzed in order to know how the grape phenolics are distributed between the wine and the grape marc for each particular variety.

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PS1-17

COMPARATIVE PERFORMANCE OF GAS CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADUPOLE-MASS SPECTROMETRY FOR THE DETERMINATION OF FUNGICIDES COMMONLY USED IN VITICULTURE

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Fungicides are widely used pesticides commonly employed in viticulture to avoid fungi infection of *Vitis* plants. Their use is specially frequent in rainy regions with high humidity levels such as Galicia (Northwestern Spain). To a greater o lesser extent, these chemicals can be transferred from grapes to wine during the winemaking process, depending on the type and concentration of fungicides applied to grapes and other operational and climatological conditions. European Union (EU) has regulated the Maximum Residue Limits (MRLs) for fungicides in grapes [1]. Although pesticide MRLs have been suggested for wine in order to guarantee as much as possible the safety of the beverage, the presence of fungicides in wine is not still regulated, which is a matter of concern for both consumers and producers. Considering that the contents in wines are significantly lower than in grapes, sensitive and selective analytical methods are required to detect pesticide residues in wine. The complexity of the grapes and wine matrices poses a high challenge in fungicides analysis. Gas chromatography (GC) and liquid chromatography (LC) coupled to different detectors are frequently used as determination techniques. The need for higher selectivity and sensitivity, as well as the necessity for confirmation, have been successfully achieved by coupling both chromatographic techniques with mass spectrometry (MS) and tandem mass spectrometry (MS/MS). Triple quadrupole (TQ) mass analyzers are the most employed instruments, since sensitive detection of the target analytes can be achieved working in the selected (or multiple) reaction monitoring (SRM/MRM) mode. In this work, GC- and LC- ESI-tandem MS were compared for the quantitative analysis of several fungicides commonly used in viticulture. The performance of both techniques was evaluated in terms of detection limits (LODs), precision and linear working range. For most fungicides, higher sensitivity was obtained using LC-TQ MS, with LODs at the low pg mL^{-1}. In general, LC-tandem MS has been proven to provide better performance than GC-tandem MS for the analysis of the target fungicides. Some compounds such as procymidone and iprodione could not be adequately determined using LC-ESI-TQ MS, whereas other compounds like fenhexamide underwent a significant improvement when using LC- TQ MS. Iprodione showed a good response by using LC-APCI-TQ-MS. Both LC and GC coupled to MS detectors have demonstrated to be adequate for the analysis of the target fungicides.

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PS1-18

CLASSIFICATION OF OLIVE LEAVES AND FRUITS ACCORDING TO THEIR GENETIC ORIGIN USING CAPILLARY GEL ELECTROPHORESIS PROTEIN PROFILES

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Proteins are compounds of high informative value and could constitute a helpful tool to differentiate genetic varieties. However, the isolation and characterization of protein fraction derived from olive leaves and fruits remain a difficult task due to the low crude protein content present and the high levels of interfering components [1]. Enzyme-assisted protein extraction can be an alternative method because its mild extraction conditions and low environment impact [2, 3]. In this work, the method based on the use of an enzyme in assisting the extraction of proteins from olive leaf and fruit has been developed. The type of enzyme employed (cellulase, lipase and phospholipase) and different extraction conditions (solvent composition, enzyme content, pH and temperature) were compared and its influence on the recovery protein was examined. Under optimum conditions, proteins present in leaves and fruits belong to different genetic origin were analyzed by capillary gel electrophoresis (CGE). Several differences between protein profiles were evidenced. To reduce the variability associated with the total amount of proteins recovered from the samples and to minimize other sources of variance also affecting the sum of the areas of all peaks, normalized rather than absolute peak areas were used. To normalize the variables, the area of each peak was divided by each of the areas of the other peaks. With the CGE data, a linear discriminant analysis model capable of classifying the samples according to their genetic origin was constructed. Using a 95% probability, all the objects were correctly assigned classified ($\lambda_w < 0.3$) with an excellent resolution among all of the categories.

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PS1-19

CHROMATOGRAPHIC ANALYSIS OF OLIGOSACCHARIDES DERIVED FROM MALTULOSE OBTAINED BY DEXTRANSUCRASE B 512 F

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The development of strategies for the synthesis of new oligosaccharides with functional properties is currently in great demand in the food, pharmaceutical, feed, and cosmetic industries. Among the different methods of oligosaccharide production, the enzymatic synthesis is perhaps one of the most interesting. Different approaches have been developed by using several donors and acceptors and enzymes from microbial origin, being transfer reactions one of the main sources of new prebiotic oligosaccharides. Dextransucrase (EC.2.4.1.5.) is a glycosyl transferase that catalyzes effective synthesis of various oligosaccharides of different structural nature by using sucrose as the glycosyl or fructosyl moiety donor and carbohydrates with low molecular weights acting as acceptors. Therefore, the objective of this work was to synthesize and characterize the oligosaccharides derived from maltulose (MUS) using Dextransucrase B 512 F from *Leuconostoc mesenteroides.*

Oligosaccharide synthesis in the presence of sucrose (donor) and maltulose (acceptor) was carried out by incubating 300 g/L of carbohydrates (1:1) with 0.8 U/mL of enzyme at 30 ºC and pH 5.2 during 48 h. Formation of oligosaccharides was investigated by taking aliquots from the reaction mixture at suitable time intervals up to 48 h. Analyses of MUS in reaction mixtures were performed by HPAEC-PAD, GC-FID and MALDI-ToF MS. Analysis of oligosaccharides by HPAEC-PAD was carried out using a CarboPac PA-1 column (250 mm × 4 mm) connected to a CarboPac PA-1 (50 mm × 4 mm) guard column. The elution, at a flow rate of 1 mL/min, was in gradient. Oligosaccharides from reaction mixtures in form of trimethylsilyl oximes derivatives were analyzed by GC-FID using a ZB-5HT Inferno™ fused silica capillary column (15 m long x 0.25 mm i.d. x 0.10 µm film thickness). The initial oven temperature was 150 ºC and raised to 250 ºC at a heating rate of 10 ºC/min, raised again to 380 ºC at 3 ºC/min, remaining at this temperature for 7 min.

Chromatographic analyses showed the presence of a considerable number of oligosaccharides which could be assigned as tri-, tetra-, penta- and hexasaccharides. One of trisaccharides was identified as α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Fru (panulose) by comparison with the corresponding standard. MALDI-TOF-MS analysis of reaction mixtures revealed the presence of oligosaccharides with a degree of polymerization up to 12. Maltulose and sucrose decreased up to a 30 and 11%, respectively. The content of glucose, fructose, and all quantified oligosaccharides increased during all incubation assays. During reaction, the level of total oligosaccharides increased, reaching yields of 35%. To our knowledge, this is the first study about MUS formation using dextransucrases.

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PS1-20

CHARACTERIZATION OF CHITOOLIGOSACCHARIDES OBTAINED BY ENZYMATIC HYDROLYSIS OF CHITOSAN USING BRANCHZYME®

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The remarkable biological properties described for chitooligosaccharides (COS) make them very attractive for their application in different fields such as medicine, biotechnology and food industry [1]. The extent of acetylation and molecular weight has been described as the most important factors influencing COS properties, thus a characterization of their structures is required for obtaining controlled products [1]. COS can be produced from chitosan by enzymatic processes, however, the expensive cost of chitosanases limits its wide application on industrial scale. The use of cheaper non-specific enzymes is nowadays gaining more importance. Branchzyme® is a relatively inexpensive commercial preparation containing a branching glycosyl-transferase from *Rhodothermus obamensis.* To the best of our knowledge, it has not been used to produce COS, therefore, the aim of this work was to obtain COS using the enzymatic commercial preparation Branchzyme® and to characterize them.

Aliquots of chitosan (2% in 0.1 M acetic acid), pH 5.3, were hydrolyzed with 40 µL/mL of Branchzyme® at 50°C for 24h. Two fractions of COS (low and high molecular weight oligomers, L-COS and H-COS) were obtained by precipitating with ethanol aqueous solutions (up to 90%) and characterized by size exclusion chromatography (SEC-HPLC); matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS) and gas chromatography (GC-FID). The degree of deacetylation (DD) was determined by Fourier transform infrared spectroscopy (FTIR).

As expected, DD values obtained for native chitosan and COS were very similar (73% and 72%, respectively), since DD is not altered during enzymatic hydrolysis and isolation of chitosan [2]. COS obtained by the depolymerization reaction of chitosan with Branchzyme® were composed by complex mixture of chitooligomers with degree of polymerization (DP) between 2 to 20, without free glucosamine (GlcN) or N-acethyl-glucosamine (GlcNAc), and with a major peak between 0.65-0.90 kDa. Among them, L-COS ranged from DP2 to DP5, DP3 being found in the highest amounts. Meanwhile, H-COS with DP from 5 to 20 presented the highest concentration of DP 7 and 8. The occurrence of oligomers of GlcN and GlcNAc showed a ratio 3:1.

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PS1-21

COMPARISON OF THE VOLATILE COMPOSITION OF AROMATIC VARIETIES OF PISCOS FROM THE MAIN PISCO-PRODUCING REGIONS IN PERÚ BY CHEMICAL ANALYSIS AND GAS CHROMATOGRAPHY-OLFACTOMETRY

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Forty five samples of commercial pisco made with Albilla, Muscat and Torontel grapes in different areas of Perú have been evaluated by gas chromatography-olfactometry (GC-O) and chemical quantitative analysis. The GC-O study has revealed that the Peruvian pisco made of these varieties of grapes has an aroma profile formed by nearly 30 odorants. The odorant 2,3-pentanodione was detected only in the Albilla sample, producing significant differences. The chemical analysis of the volatiles of these samples has shown volatile profiles quite typical of a wine distillate. The Torontel samples (considered the most aromatic variety) were marked by high levels of terpenes and β-damascenone, the Albilla samples showed the lowest values of terpenes, which may explain their low scores obtained during the sensory analysis, while the Muscat samples showed intermediate levels of this family of compounds and were characterized by high concentrations of benzyl alcohol and β-phenylethanol. Furthermore, an evaluation of the sensory contribution of terpenes in these piscos was carried out, demonstrating that linalool is the most odor-active terpene, exceeding its odor thresholds in 34 out of 45 piscos analyzed. However, the other terpenes should also be considered as important contributors to the aroma of piscos because a certain degree of cooperation between the components of this family has been demonstrated. The quantitative analysis data allowed us to distinguish pisco samples according to their variety and could explain some sensory differences observed depending on the nature of the grapes.

PS1-22

DETERMINATION OF FREE VOLATILE SULFUR COMPOUNDS (VSCs) IN WINE BY DIRECT HEADSPACE ANALYSIS AND STUDY OF THE INTERACTIONS BETWEEN MERCAPTANS AND DIFFERENT WINE COMPONENTS

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Volatile Sulfur Compounds (VSCs): MeSH (methanethiol), EtSH (ethanethiol), DMS (dimethylsulfide), DES (diethylsulfide), DMDS (dimethyldisulfide) and DEDS (diethyldisulfide) together with hydrogen sulfide (H_2S) show similar sensorial characteristics described as rotten eggs and cabbage [1]. These compounds usually appear during bottle aging giving rise to problems commonly defined as “reduction”. The origin of this problem is so far not known. In this work we investigate the existence of complexed non-volatile forms of these compounds which may be ultimately responsible for their release upon the reductive conditions usually found in a wine bottle.

The method consists on the direct injection of the wine headspace into a Gas chromatograph (GC) equipped with a pFPD detector (pulsed Flame Photometric Detector). Separation is carried out with a DBWax-etr column. Samples are previously spiked with internal standards belonging to different chemical classes and left to equilibrate in an absolutely oxygen free atmosphere (O_2 levels <10 ppm) and the signals are compared to those obtained in the analysis of a standard synthetic wine sample. The different operating parameters have been studied and optimized. The final method offers a reasonable sensitivity, precision (DSR 5%) and accuracy and good detection limits (below 1ppb).

The method has been applied to the study of the interactions that VSCs exert towards different compounds in the matrix. In some wines, mercaptans are so strongly complexed that they cannot be detected in the headspace, even after the addition of up to 50 ppb. In clear contrast, in some wines the headspace signals are absolutely equivalent to those measured in synthetic media, suggesting that there is no interaction. Thioethers do not seem to form complexes. Our studies show that Cu^{2+} and to a lower extent Fe^{2+} and even lower Zn^{2+}, are responsible for the complexes, while Ni^{2+}, Mn^{2+} or Fe^{3+} cannot complex mercaptans in wine conditions.

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PS1-23

DEVELOPMENT OF ANALYTICAL METHODOLOGIES BY UV-VIS ABSORPTION SPECTROPHOTOMETRY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETECTION OF ADULTERATIONS IN SAFFRON

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Saffron is the most expensive spice in the world and highly valued both in cookery and in the food industry due to its coloring properties, alluring aroma and pleasant bitter taste. It is also highly appreciated for its biological and medicinal properties. Consequently, saffron has undergone a wide range of adulterations and authentication is a permanent challenge. The authenticity of saffron is an important matter in consumer protection, quality assurance, active properties and economic impact.

Adulterations are done in order to increase saffron weight with foreign matters, or to enhance its color with natural or synthetic colorants. Since the 90's, additions of water-soluble food dyes and oil-soluble azo dyes in saffron have been occurred. More recently, a new adulteration by gardenia additions may have reached the European market.

In this study, samples of authentic and suspicious saffron were analyzed, in styles and ground, and from different geographical origin. Two methods for the detection of adulterations were developed using different extraction media, ethanol or aqueous buffer at pH 2.5 or pH 9.0. The first is a simple spectrophotometric method based on the comparison of the second derivative of the absorption spectrum, which allows identifying adulterations using different absorption maxima. The second is a high-performance liquid chromatographic method using UV detection at different wavelengths. Different chromatographic columns (C18, HILIC and Ciano) were tested to find the best separation conditions. The chromatograms obtained with the C18 column using an aqueous buffer at pH 9.0 as extracting medium showed the highest number of significant differences between authentic and adulterated saffron, being the chromatographic profile of the different authentic samples very similar.

PS1-24

A DERIVATIZATION METHOD FOR GAS CHROMATOGRAPHIC ANALYSIS OF *Rhodiola rosea* EXTRACTS AND THEIR CHARACTERIZATION BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Rhodiola rosea is a plant belonging to the Crassulaceae family, widely used as popular medicine around the world. A number of metabolites like phenyl propanoids, phenylethanol derivatives, flavonoids, monoterpenes, triterpenes and phenolic acids are found in good yields in *Rhodiola* roots. Several activities such as antioxidant, antiaging, immunostimulant, antidepressant, radioprotective or anticarcinogenic, among others, have been attributed to this plant and many of these functions have been adscribed to phenolic compounds such as salidroside, rhodiolosides, p-tyrosol, and glycosides like rosavin or rosarin [1]. Analysis of these bioactives has been generally achieved by HPLC and CE methods [2-3]. However, taking into account the complexity of bioactive substances in *Rhodiola rosea*, GC could be an appropriate technique considering its high resolution and detection sensitivity. Moreover, the use of comprehensive two-dimensional gas chromatography (GCxGC) usually results in a significant increase of peak capacity and in an improvement in sensitivity, and to the best of our knowledge it has not been applied before to the analysis of *Rhodiola* extracts. For both techniques a previous derivatization step of *Rhodiola* compounds is mandatory and it should be carefully controlled to avoid degradation of glycosides. Therefore, in this work we have optimized a derivatization procedure based on the formation of trimethylsilyl (TMS) oximes of *Rhodiola* root extracts for their quantitative GC-FID analysis. Evaluation of the use of GCxGC-TOF MS for their comprehensive characterization has been also carried out. Formation of TMS-oximes is usually done following a two-step derivatization process based on the use of hydroxylamine chloride in pyridine at 75 ºC and hexamethyldisilazane and trifluoroacetic acid at 45 ºC [4]. However, degradation of phenolic glycosides (rosavin, rosarin and rosin) was detected under these conditions, probably due to the acidic media. Among the different reagents assayed, trimethylsilylimidazole with trimethylchlorosilane showed the best results, being this combination chosen for further quantitative analysis by GC-FID. A qualitative study of derivatized *Rhodiola* extracts was carried out by GCxGC-ToF MS. Promising results were achieved by this technique which provided better resolution than one-dimensional GC and allowed the possibility of characterizing well-resolved unknown compounds from their mass spectral data. Further work could be done to improve the chromatographic resolution using different column combinations.

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PS1-25

HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY FOR THE ANALYSIS OF IMINOSUGARS AND OTHER CO-EXTRACTED CARBOHYDRATES FROM NATURAL SOURCES

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Iminosugars are sugar-mimic polyhydroxyalkaloids found in plants (*Moraceae*, *Hyacinthaceae*, etc) and microorganisms (*Streptomyces, Bacillus*, etc), potentially useful as drugs for treatment of diabetes, viral infections, etc. due to its glycosidase inhibitory capability. As iminosugars are polar compounds, hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC-MS) might be the analytical technique of choice for their analysis [1]. However, separation of these bioactives from other co-extracted carbohydrates, whose content in natural extracts should be controlled for the therapeutic use of iminosugars, is not generally achieved [2]. Moreover, most of these works are focused on the analysis of a few of these bioactives, mainly deoxynojirimycin and fagomine, and studies regarding complex mixtures are scarce [3]. Recently [4], we have evaluated the use of different HILIC stationary phases to solve these problems; ethylene bridge hybrid (BEH) with trifunctionally-bounded amide phase showed the best results.

In this work different conditions (binary acetonitrile/water gradients, flows and chemical modifiers) have been assayed to optimize a method for the separation of iminosugars and other co-extracted carbohydrates using the BEH column. Optimal conditions were selected on the basis of the best peak symmetry and better resolution. The method has been applied to the analysis of *Hyacinthus* sp., *Morus* sp. and *Aglaonema* sp. extracts.

The best separation between iminosugars and other carbohydrates was achieved using 0.1% acetic acid; however, non-gaussian peaks were obtained for monosaccharides. Using 0.1% ammonium hydroxide as modifier, a higher efficiency was achieved, although lower resolution between iminosugars and other interfering carbohydrates was obtained. Regarding the use of different concentrations of ammonium acetate (2, 5 and 10 mM) in the aqueous phase, the best results (sensitivity, good peak shape and resolution values) were obtained for 5 mM. When this concentration of ammonium acetate was used in both mobile phases, similar peak symmetries with lower retention times were obtained. The HILIC method thus optimized proved to be useful for the intended chromatographic analysis of the target bioactive iminosugars present in the natural sources under study.

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PS1-26

CAROTENOID CONCENTRATIONS OF ANDEAN LANDRACE POTATOES AS AFFECTED BY COOKING

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Besides the well-known role of β-carotene as a provitamin A carotenoid and of lutein and zeaxanthin in the prevention of cataract and age related macular degeneration, there are some studies that claim that the antioxidant activities of carotenoids are a key factor in reducing the incidence of many diseases including cardiovascular diseases and cancer. The concentrations of total and individual carotenoids were determined by spectrophotometry and HPLC, in a group of Andean landrace potato accessions with diverse intensities of yellow flesh colors for evaluating the effects of boiling on the carotenoid concentrations of native Andean potato accessions with different intensities of yellow flesh color. Based on previous research [1], which indicated that carotenoid profiles and concentrations in potato are correlated with the intensity of yellow flesh color, light yellow, intermediate yellow and deep yellow fleshed accessions were selected to determine the effect of boiling on total and individual carotenoid concentrations.

Total carotenoid concentration of raw deep yellow fleshed accessions was higher than that of intermediate yellow fleshed accessions, and this higher than that of light yellow fleshed accessions The peaks of violaxanthin and antheraxanthin, that were prominent in the accessions with light yellow and intermediate yellow fleshed tubers, almost disappeared in chromatograms of the same potatoes when they were analyzed after boiling. Zeaxanthin an antheraxanthin were the predominant carotenoids in the deep yellow fleshed varieties used in this study. The peak of antheraxanthin was also reduced after boiling. Changes in the carotenoid concentrations due to boiling varied significantly among accessions. Boiling significantly reduced the violaxanthin and antheraxanthin concentration of all the accessions. However, the lutein and zeaxanthin concentrations of boiled tubers were not affected or were higher than the concentrations in raw tubers. An intermediate yellow fleshed accession showed the highest lutein concentration (above 200 µg / 100g fresh weight) and the deep yellow fleshed accession showed the highest concentration of zeaxanthin (above 1000 µg / 100 g, fresh weight) in raw and boiled tubers. Boiled potatoes of deep yellow fleshed varieties are a significant source of zeaxanthin (above 500 µg per 100 g fresh weight). Therefore, yellow fleshed potato could be recommended as a source of lutein and zeaxanthin, although additional research on bioavailability of potato carotenoids is required to have a more useful and complete information.

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PS1-27

PHENOLIC COMPOUND CONTENTS AND ANTIOXIDANT ACTIVITY OF PURPLE FLESHED POTATOES AS AFFECTED BY BOILING

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Total phenolic (TP), phenolic acid (PA) and total anthocyanin (TA) concentrations, and in consequence the antioxidant activity (AA) determined in food crops, are affected by different factors, including variety and the production environment. Furthermore, the methods of analysis used can influence the final determined amount of antioxidants and thus on the measured AA. Parameters having a great impact on the final determined amount of antioxidants notably include the extraction solvent composition and the extraction time.

The effect of boiling on the concentrations of TP, TA and PA as well as on the AA was determined by spectrophotometry and HPLC in a group of native Andean purple fleshed potato accessions. The methods of extraction for analysis of each parameter were previously assayed and optimized for raw and cooked freeze dried potato samples.

The concentration of methanol strongly influences the extraction of TP and antioxidants in raw and cooked potato samples. 80% methanol has the highest yield for extracting TP and antioxidants to be evaluated for AA of raw samples while 60% methanol gives the highest yield for cooked samples. Five minutes of sonication was enough for efficient extraction of the phenolic compounds in all the potato samples studied.

Chlorogenic acid (CA) was the predominant PA in raw and boiled potato samples of the four purple fleshed accessions, caffeic acid was also present in all the raw samples but drastically decrease in all the cooked samples. The ANOVA for TP, TA and CA concentrations indicated significant differences for the interaction between type of sample (raw or cooked) and the accession. For the four purple accessions, the concentration of TP and the AA determined in boiled tubers was higher than in raw tubers. However, with exception of the deep purple fleshed accession (Guincho), the TA and CA concentrations determined in raw and boiled tubers were not significantly different.

Boiled purple fleshed potatoes are a good source of TA and shows high AA. Since potato is an important food staple and the number one vegetable crop in the world the contribution of potato to the intake of the health-promoting phenolics (anthocyanins and chlorogenic acid) can be significant. Research regarding the bioavailability of the different anthocyanins in potatoes as well as of other food is required to assess their availability and roles in the diet.

PS1-28

USE OF AN IONIC LIQUID AS MICELLAR MEDIUM IN THE ANALYSIS OF CARBAMATES BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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A new micellar electrokinetic chromatography (MEKC), employing an ionic liquid (IL) as running aqueous buffer has been proposed for the analysis of eleven carbamate (CRBs) pesticides in juice samples. These pesticides, introduced in the early 1950s, are widely used as insecticides or fungicides. They are neutral compounds in a wide range of pH, making them a good model to evaluate the potential of ILs as micellar media in MEKC.

ILs have many applications derived from their properties, such as high ionic conductivity, low volatility and vapor pressure, non-flammability, high thermal stability, wide temperature range as a liquid phase, good solvation power for many organic and inorganic materials and a wide range of electrochemical stabilities [1]. Up to now the use of ILs in the field of capillary electrophoresis has been focused on their used as modifiers of the electroosmotic flow (EOF) [1, 2]. In this work, the applicability of 1-dodecyl-3-methylimidazolim tetrafluoroborate as pseudostationary phase in MEKC with UV-detection for the analysis of CRBs has been evaluated. Separations were performed in an extended light path fused-silica capillary (64.5 cm × 50 µm I.D., 56 cm effective length); the separation buffer consisted of 100 mM $NaHCO_3$/NaOH and 20 mM IL (pH 9.0); the temperature of the capillary was kept constant at 25 ºC and a voltage of -22 kV was applied (negative mode); the detection of the analytes was performed at a wavelength of 200 nm. Samples were introduced by hydrodynamic injection (50 mbar, 7.5 s). At optimum conditions the analysis time was less than 12 minutes.

In addition, a dispersive liquid-liquid microextraction (DLLME) procedure has been proposed for the extraction of these CRBS from juice samples. Variables affecting DLLME efficiency were optimized, selecting: 5 mL of sample solution, 850 µL of chloroform as extraction solvent, 1500 µL of methanol as disperser solvent. The sedimented phase was evaporated to dryness using a stream of N_2 and reconstituted with 250 µL of the running buffer. The solution was filtered and injected into the electrophoretic system. Under these conditions, a very clean extract was obtained. Following this treatment, sample throughput was approximately 10 samples per hour, obtaining a preconcentration factor of 20.

Matrix-matched calibration curves were established using tomato juice as representative matrix (from 25 to 250 µg L^{-1}) and detection limits ranging from 0.5 to 5.0 µg L^{-1} were obtained. The method is being applied to other kind of juices.

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PS1-29

PHENOLIC PROFILE OF PRESSURIZED LIQUID EXTRACTS FROM DIFFERENT READY-TO-EAT *BABY-LEAF* VEGETABLES

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Baby-leaf vegetables are a ready-to-eat product that offers the consumer a nutrient rich fresh product. Being mostly used as salad vegetables, they are associated with a healthy and convenient image that attracts consumers [1]. Their richness in antioxidant compounds are one of their nutritional advantages, being their phenolic content frequently related to their antioxidant capacity. Baby-leaves products are prepared of young leaves, harvested at very early stage of maturation and in an active metabolic stage. Phenolic compounds are a large group of phytochemicals, ubiquitous in plants, produced as secondary metabolites, via the shikimic acid pathway. They have, at least, one aromatic ring with one or more hydroxyl groups attached, being commonly found conjugated to sugars and organic acids which have been recognizable by their antioxidant properties [2].

Pressurized liquid extraction (PLE) is a technique that combines elevated temperature and pressure with liquid solvents to increase the extraction efficiency of bioactive compounds from a solid sample. It uses less organic solvents and requires shorter periods of extraction time, when compared to conventional extraction methods. This technique has already been applied with high efficiency to the extraction of phenolic compounds from solid vegetable samples.

In this work, a PLE method was optimized to extract the phenolic compounds of several freeze dried samples of baby leaf vegetables (green and red lettuce, spearmint, pea shoots watercress and wild rocket). Two solvent mixtures (methanol/water and ethanol/water) with different proportions (50%, 70% and 90%) of organic solvent, and also different extractions times (5, 10, 15, 20 min) were used to determine the best conditions to extract phenolic compounds. All PLE extractions were made at a fixed temperature (70ºC) and pressure (1500 psi). The best conditions to achieve a higher quantity of phenolics were selected based on their spectrophotometric quantification by the Folin-Ciocalteau method. To study the phenolic profile each diluted extract was injected in an HPLC-DAD system, being the phenolics identified based on the comparison with commercial standards retention times and their UV-Vis spectra. Moreover, additional information was obtained by analyzing the extracted samples by HPLC-MS/MS. The use of a 70% methanol solution combined with two extraction cycles of 10 min were the best conditions selected for the extraction of phenolic compounds. Each sample showed a particular phenolic profile, according to their specie.

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PS1-30

COMPARATIVE STUDY OF THE EXTRACTION OF PHENOLIC COMPOUNDS FROM *Sargassum muticum* USING ALKALINE OR ENZYMATIC HYDROLYSIS AS PRE-TREATMENT FOLLOWED BY PRESSURIZED LIQUID EXTRACTION

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The brown seaweed *Sargassum muticum* has been recognized as a potential source of phenolic compounds, mainly phlorotannins, which had shown high antioxidant potential *in vitro*. Several researches have suggested that phlorotannins are likely to be integral structural components of brown-algal cell walls. Possible linkages between the cell wall (alginic acid) and phlorotannins through ester bonds and hemiacetal bonds, both of which are covalent bonds, would mean that strong extraction conditions are required for their isolation.

The present study compared the extraction yields of phenolic compounds from *Sargassum muticum* as well as their antioxidant activity using alkaline and enzyme-assisted hydrolysis followed by a pressurized liquid extraction (PLE). Alkaline and enzymatic pre-treatment was employed in order to degrade cell wall polysaccharides to oligosaccharides prior to the extraction of phenolic compounds from the residual biomass. Alkaline degradation was performed using NaOH 1.0 M at 2.5 h and enzyme assisted extraction was completed using Alcalase (protease) and Viscozyme (carbohydrase) at their optimum hydrolysis conditions at 2 and 4 h. The residual biomass was separated from hydrolyzed polysaccharides and submitted to extraction process by pressurized liquid extraction. Results in terms of amount of total phenols and antioxidant activity were also compared to those attainable without previous hydrolysis.

The obtained results showed that the extraction yield of phenolic compounds using viscozyme or alcalase were similar, independently of the treatment duration. On the other hand, antioxidant activity was higher using alcalase than viscozyme. In the residual biomass, the phenolic compounds extracted were higher when the pre-treatment was done with Viscozyme (11.464 mg GAE/ g algae) than Alcalase (7.92 mg GAE/ g algae) and Alkalyne degradation (7.66 mg GAE/ g algae). Regarding the antioxidant activity, a similar trend was observed. Nevertheless, it was found that the extraction without previous hydrolysis presented the highest extraction yield, TFC and antioxidant activity among all extractions, suggesting that the PLE procedure without any pre-treatment is capable of extracting most phenolic compounds present in the algae.

PS1-31

INFLUENCE OF NITROGEN ON THE FORMATION OF AROMA COMPOUNDS IN *MERLOT* (*Vitis vinifera*) WINE

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Wine aroma depends mainly on the alcoholic fermentation, stage of vinification that occurs the production of compounds such as alcohols and esters. The lack of nitrogen in musts can result in a slow fermentation, with the formation of biomass insufficient and the low synthesis of aromatic compounds [1]. This study aimed to spray different concentrations of ammonia in the soil of *Merlot* in order to supplement the nitrogen in the must and evaluate the influence of this compound on the alcohols and esters formed during the vinification. The vineyard was divided into plots, some of them were kept in their natural form (control) and others received different concentrations of ammonia in the soil (10, 60, 90, 120 and 180 kgN.Hectare^{-1}). At the time of harvest, the grapes were harvested and vinified. The musts were analyzed for content of assimilable nitrogen (IRTF) and amino acids (UHPL) and wines were analyzed for content of the volatile compounds (GC/MS)[2]. The results showed that the applied technique was satisfactory, because the musts coming from the spraying process had higher concentrations of assimilable nitrogen (136 to 167 mg.L^{-1}) and amino acids (534 to 752 mg.L^{-1}) compared to control (128 mg.L^{-1} of assimilable nitrogen and 525 mg.L^{-1} of amino acids). The presence of these nitrogen compounds in musts was dependent on the ability of assimilation of ammonia in the soil for vines. The musts with higher concentrations of these compounds were related to plots that received 120 kgN.Hectare^{-1}. The addition of ammonia in the soil contributed positively to the formation of volatile compounds, which were in higher concentrations in wines from the treatments containing the highest concentrations of nitrogen compounds in musts, indicating that the synthesis of volatile compounds was benefited by the presence of high concentrations of assimilable nitrogen and amino acids in the musts. The results obtained for wine from vines that received ammonia in the soil reached values between 176 to 190 mg.L^{-1} to the higher alcohols and 4586 to 6505 µg.L^{-1} to the esters, having a composition aromatic more intense compared to wines made from control treatments (175 mg.L^{-1} of higher alcohols and 4538 µg.L^{-1} of esters). The highest concentration of aroma compounds was found in wines coming from the plots that received 120 kgN.Hectare^{-1}, whose vines have assimilated higher concentrations of ammonia and musts showed higher concentrations of assimilable nitrogen and amino acids.

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PS1-32

FLAVONOL COMPOSITION BY HPLC-DAD-ESI-MS^n IN COMMERCIAL BLUEBERRIES PRODUCED IN BRAZIL

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Blueberries contain several health-promoting factors, including flavonols. These compounds are phytochemicals that, although not essential for survival, may over the long term be one of the factors that contribute to the protective effects of a fruit- and vegetable- rich diet [1]. The aim of the present study was to determine the flavonols of sixteen samples, belonging to nine cultivars of blueberries produced in 2010/2011 in three different cities of Brazil. Quantitative extraction of flavonols was achieved using a mixture of methanol-water-formic acid (50:48.5:1.5 v/v/v) (0.5 g of freeze dried blueberries; 3 x 25 mL; 2 min in ultrasonic bar and centrifugation each batch) and further analyzed by HPLC-DAD-ESIMS/MS [2]. A total of 34 flavonols could be assigned on the basis of, first, their characteristic UVvis spectra and, second, their MS and MS/MS spectra. Three free aglycons were detected, quercetin, syringetin and laricitrin. Six myricetin-3-glycosides were detected: the 3-glucosides, 3-glucuronides, 3-galactosides, 3-rhamnosides and two myricetin pentosides; Quercetin-type flavonols dominated the flavonol profiles, twelve quercetin-3-glycosides were detected: galactosides, glucuronides, glucosides, rhamnosides, two kinds of rutinosides, three pentosides and three quercetin acetyl glucosides. A co-elution of Kaempferol was found (Kaempferol 3-galactoside and Kaempferol 3-glucoside), the same occur with laricitrin 3-glucuronide + laricitrin 3-glucoside. Even though other three laricitrin glycosides were detected: laricitrin 3-galactoside, laricitrin 3-pentoside and laricitrin 3-rhamnoside. Galactoside, glucoside, glucuronide, rhamnoside and one pentoside of syringetin were detected and three isorhamnetin 3-glycosides were still found. The large number of flavonols found in Brazilian blueberries, concludes that the fruits are presented as a potential source of bioactive compounds.

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PS1-33

PHENOLIC COMPOUNDS DETERMINATION IN AQUEOUS EXTRACTS OF YERBA MATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: AN OPTIMIZATION STUDY

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Yerba mate (*Ilex paraguariensis*), a plant originated from South America, is employed for preparing "chimarrao" and the mate tea beverage. Many biological assays have postulated the beneficial consequences from the consumption of these beverages for the human health, which have been associated with its high contents of phenolic compounds. In this study, a new method for determination of phenolic compounds present in beverages obtained from yerba mate (gallic acid, syringic acid, caffeic acid, ferulic acid, *p*-coumaric acid, 5-caffeoyilquinic acid, 3,4-dicaffeoyilquinic acid, 3,5- dicaffeoyilquinic acid, 4,5-dicaffeoyilquinc acid and rutin) by High Performance Liquid Chromatography has been developed. For method optimization, a central composite design was used to evaluate the effect of initial and final concentration of methanol in the mobile phase and the gradient time on the resolution and symmetry value of the peaks. The design was undertaken with quadruplicate in central point, totalizing 18 experiments conducted in aleatory order. The mobile phase consisted of methanol and aqueous formic acid(0,1%). The separation was carried out in a reversed phase column (C18) and the chromatography equipment was coupled to a photodiode array. The optimization was conducted using a food matrix added of pure standard. The dates were adjusted for linear and quadratic models and the better fitting models were validated through analysis of variance (ANOVA), at the level of confidence of 95%. From the adjusted math models acquired, the responses were simultaneously optimized employing the Derringer and Suich desirability function and following validated.

Through using the multivariate statistical techniques was possible to minimize the time of chromatography analysis, maximize the symmetry and promote good resolutions between peaks. According to results obtained, the increase of initial concentration of methanol decreased the resolution between peaks. Similarly, it was verified that the elevation of the final concentration of methanol reduced this parameter, as well as the time of analysis. In addition, the dates showed that as higher the initial concentration of methanol lesser is the peak symmetry. The optimized chromatography conditions were: 13.9% and 38,9%of initial and final concentration of methanol, respectively, in a gradient time of 39.4 minutes. According to the validation parameters evaluated, the developed method is able to accomplish quantitative and qualitative analysis in aqueous extracts of yerba-mate, by the fact of presenting good linearity in the studied range of compounds concentrations, as well as good repeatability and reproducibility.

PS1-34

PESTICIDE RESIDUE MONITORING IN BANANA FROM THE CANARY ISLANDS

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A seven year pesticide residue monitoring program (2006-2012) has been carried out for bananas grown in the Canary Islands which are the main producer among the outermost European regions, including Madeira, Martinique and Guadeloupe [1]. The total banana production in the Canaries is larger than the other three outermost regions together. The major destination of the Canary banana is the mainland Spain. The monitoring program described in the present effort has been executed at two different sampling steps: samples taken at the fields and samples taken at the processing and packaging banana buildings, where two different kind of samples were also taken: at the door of the processing buildings (samples from field) and just after the post-harvest fungicide application. A total of 794 samples have been processed, 389 from field and 405 from the packaging buildings, fruit that is ready to be sent to the markets of mainland Spain. A total of 1058 pesticide residue identifications have been found for the total of samples, a 1.33 pesticide residue/sample ratio. Pesticide residue identifications were 357 for field samples, 0.92 ratio, and 701, 1.73 ratio, for post-harvest sample. Post-harvest fungicide application to the fruit lead to a higher pesticide residue/sample ratio.

The pesticide most frequently identified was, by far, Chlorpyriphos (69% of samples) an insecticide used to fight against the cochineal, one of the major threats for Banana in the Canary Islands. 28 different pesticide residues were identified in this monitoring program. Among them, 11 had never been authorized in banana during the period 2006-2012. Cipermethryn, Dicofol and Tetradifon (active ingredients formulated together in the commercial pesticide formulation used in the Islands), as well as Dimethoate were the most relevant findings. The other 17 pesticides identified are or have been authorized to be used in banana cultivations any time between 2006 and 2012. For these pesticides, 18 residues were found above the MRLs; while 39 findings were detected for these pesticides on dates after their prohibition of use. Taking into account all the adverse results, 77 cases of residues have been found for non authorized pesticides (9,6% samples) and 18 cases (2.3% samples) for residues above their MRLs, corresponding to Bifenthrin (MRL 0.10 mg/kg) 10 times -2008, 2009(9)- Carbaryl (MRL 0.05 mg/kg) 1 time -2007- Chlorpyriphos (MRL 3.00 mg/kg) 4 times -2007, 2010(2), 2012- Indoxacarb (MRL 0.20 mg/kg) 3 times -2006(2), 2012-.

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PS1-35

LONG TERM PESTICIDE RESIDUE MONITORING IN TOMATO FROM THE CANARY ISLANDS: 2006-2012

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A long term pesticide residue monitoring in Tomatoes grown in the Canary Islands has been carried out during the last seven years, 2006-2012. A total of 443 samples have been analyzed until now, finding as far as 550 pesticide residues, 1,24 pesticide residue /sample ratio, for 56 different pesticides. A total of 203 samples (46%) show no pesticide residues. For the 240 samples containing pesticide residues, the averaged ratio was 2,29 residues /sample. For those samples with pesticide residues, the 40% has residues of one pesticide and 29% residues of two pesticides. Samples containing more than 5 different pesticide residues are scarce, 13 cases (5,4% samples). Two multirresidue methods, MRM, and a single residue method, SRM, have been run for the pesticide residue determinations: ethyl-acetate extraction for GC(IT)MSMS (gas chromatography - ion trap mass spectrometry) determination, Quechers for LC(TQ)MSMS (liquid chromatography – triple quad mass spectrometry) determination and Dithiocarbamates, expressed as carbon disulfide, based upon sample digestion using hydrochloride acid and stannous chloride, followed by gas chromatography with pulsed flame phosphorus detector, GC-PFPD, determination. Dithiocarbamates has been the most frequently detected pesticide residue, 106 times (24%), in the tomato samples. On behalf of EU Regulation 396/2005, the results of the pesticide residue monitoring for tomato samples grown in the Canary Islands between 2006 and 2012, a total of 12 results, 2,7% of samples, above the corresponding Maximum Residue Limits, MRLs, have been found. Pesticides with MRLs violations were: Bromopropylate 5 times -2009(3), 2012(2)-; Carbendazina twice -2006 and 2007-; Dithiocarbamates once -2008-; Endosulfan once -2007-; Oxamyl twice -2007 and 2008- and Procymidone one -2011-. For these 12 findings above the MRLs, 6 of them come from applications after their expiring date of authorization in the EU: Bromopropylate and Procymidone. The other 6 results above the MRLs come from authorized pesticides, with approved status in the Regulation 1107/2009 and authorized to be applied to tomato crops in Spain. Taking into account the uncertainty of 50% for the pesticide residue analysis results, we found three significant results, legislation violations for authorized pesticides, for the whole monitoring program: Endosulfan (2007) and Oxamyl (2007 and 2008). Finally, adding the 6 results for no authorized pesticides, for the seven years monitoring program the 2% of samples show MRL violations.

PS1-36

DISSIPATION CURVES OF TWO STROBILURIN FUNGICIDES IN PAPAYA PLANTS OF GRAN CANARIA (CANARY ISLANDS)

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Tomato has been the traditional exportable crop in the Canary Islands. However, diseases, pests and low prices due to aggressive competitors have led to a decline in its production. Several alternatives have been studied, such as papaya (*Carica papaya L.*), which cultivation area has increased in the last years since it seems a good business option with respect to tomato.

There is still no national legislation in Spain regarding a list of authorized pesticides to be used in the treatment of papaya plants. However, the papaya farmers together with the Plant Health Canary Public Administration officers have requested to the Spanish Ministry of Agriculture for exceptional authorizations. The authorized pesticides must meet the following requirements: they must have a long term approval status in the Regulation (EC) 1107/2009 of the European Union, they should be authorized for other crops in Spain, and studies of the dissipation of their residues in papaya cultivation carried out in the islands must be developed.

Following this indications, a field assay to determine the dissipation curves of two strobilurin fungicides, *i.e.* pyraclostrobin and trifloxystrobin, has been carried out in the north of Gran Canaria (Canary Islands). The study has been restricted to a delimitated area inside a large greenhouse of papaya, where the target pesticides had never been applied. This space was divided in three plots: one for each assayed pesticide and a blank segment. Two applications of each pesticide were done, with an interval of two weeks between them. One sample (5 units of papaya) was taken before the first application (blank sample) and then 1, 3, 7, 12 and 19 days after the first application and 1, 3, 7, 10, 14 and 21 days after the second one. The fruits were characterized (size, weight, ripening status -°Brix-), homogenized and frozen until they were analyzed.

For extraction, identification and quantification of the fungicide residues, the acetate-buffered QuEChERS method followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) was employed. The limit of quantification was 0.001 mg/kg for both fungicides and the maximum residues found were 0.013 and 0.031 mg/kg for pyraclostrobin and 0.017 and 0.040 mg/kg for trifloxystrobin, after the first and the second application, respectively, showing a cumulative effect. The monitoring data permitted to estimate the exponential first order decay dissipation curve for each pesticide/application combination. The dissipation time needed to lose 50% of the initial applied pesticide was estimated in 6 days for pyraclostrobin and 18 days for trifloxystrobin, so the first fungicide would result in a better option to be used for papaya plant treatment.

PS1-37

PESTICIDE RESIDUES FROM VINE TO WINE

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The pesticide residues detected in wine samples have their origin, always, in the grapes used to elaborate them. No pesticides are used during the fermentation process. Therefore, a net pesticide residue transfer is occurring from the grapes to the final product, the wine. The present effort is a first approach to study the pesticide residue transference from vine to wine in the Canary Islands. A local vine and a familiar winery, from the north of Tenerife Island, have been selected to study the pesticide residues. Samples of grapes, initial grape must, must from the tumultuous fermentation, the residue of pressed grapes (skins, seeds) and young wine, have been analyzed for pesticides residues. The study has been conducted for different years, 2009 and 2010. The Quechers method (acetate buffered) and gas chromatography tandem mass spectrometry determination has been used for the pesticide residue analysis. Nine different pesticide residues have been identified in the samples: Chlorpyrifos, Chlorpyriphos-methyl, Kresoxim-methyl, Boscalide, Iprodione, Penconazol, Tetraconazol, Tiophanate-methyl (Carbendazima) and Dithiocarbamate (expressed as carbon disulphide). These pesticide residues has been detected all in pressed grapes (skins and seeds) samples, both from 2009 and 2010 assays, except Penconazol and Tetraconazol (belonging to the conazole family of systemic fungicides used against Powdery mildew in vine), that have been found in 2009 and 2010 respectively. The pesticides identified are all of those applied during the year to the vine. The pesticide residues found at a higher concentration level were Iprodione and Boscalide, applied to the grapes the last two times to prevent against Botrytis during the ripening period. Last application usually dates 45 days before the harvest. Residue concentrations levels were close for the both years monitored, except for Dithiocarbamates, showing higher concentration in 2009 samples. Despite the initial concentration, no residues of Dithiocarbamates were found in the young wine. Residues of both insecticides, Chlorpyriphos and Chlorpyriphos-methyl, were not detected in the initial grape must. For the conazole fungicides, low concentration residues were identified in the samples, with no residue in the final product. At the end of the fermentation, in the young new wine produced, the profile of pesticide residues shows residues of Boscalide, Iprodione, and Kresoxim-methyl. The remaining concentration of these fungicides moves about the 10% of the initial residue in 2009 and the 30% of the initial residue in 2010. For Tiophanate-methyl and Carbendazime, residues were found in the young wine.

PS1-38

PESTICIDE RESIDUE MONITORING IN WINE GRAPES FROM THE NORTH OF TENERIFE (CANARY ISLANDS, SPAIN)

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The incidence of diseases and pests over the vine in the north of Tenerife is very severe because of the influence of the dominant and humid trade winds from the north-east, mostly at summer, during the grapes ripening. Despite the inherent benefits of trade winds in the north of Tenerife they propitiate grape diseases (powdery and downy mildew and grey rot), a hard challenge to keep the vine healthy. An intense use of pesticides is needed to conserve the grapes until the harvest; grapes as healthy as possible are needed to make a good wine.

In this work, pesticide residue monitoring has been carried out over seven years for wine grapes from the north of Tenerife Island. A total of 419 samples of grapes, taken at the harvest moment, have been analyzed for pesticide residues using three different methods: two multirresidue methods, an ethyl acetate extraction method with no clean-up step followed by gas chromatography tandem mass spectrometry (ion trap MS/MS) and the QuEChERS (acetate buffered) method followed by liquid chromatography MS/MS (triple quad MS/MS), plus one single residue method for the determination of the dithiocarbamate fungicide residues, based upon carbon disulphide detection by gas chromatography with pulsed flame photometric detector. A high ratio of pesticide residues has been found during the seven years, reaching values greater than 3 residues per sample, 1309 residue in 419 samples. The number of residue free samples was very low, only 45 of the total 419 samples (11% average). It should to be pointed that the ratio has been decreasing slowly in the last years as the percent of residue free samples increases, with the lowest and highest values, 2.7 and 26% respectively in 2012. A large list of 55 different pesticides has been identified for the 419 samples, reaching 1309 pesticide residue findings. However, 27 of these residues correspond to 19 different pesticides all of them are pesticides without authorized uses in vine crops. The maximum residue limits (MRLs) have been exceed 54 times (12.9% of samples) in the present effort, identifying 19 of these violations linked to those pesticides with no authorized uses in vine crops. Taking into account the uncertainty of the pesticide residue analysis, using the accepted value of 50% uncertainty in the European Union, the number of violations of the MRLs for purposes of punishment (Regulation 396/2005) goes down to 36, 8.6% of samples, during the seven year program. For the last two years 3 samples with residues over the MRLs have been found: chlorpyrifos at 0.90 mg/kg (incorrect use of an authorized one) and procymidone at 0.18 mg/kg (unauthorized use) in 2011, and carbendazim at 0.95 mg/kg (incorrect use of an authorized fungicide) but thiophanate-methyl was not identified in the sample (unauthorized use) in 2012.

PS1-39

ETHYL ACETATE BASED METHOD FOR THE DETERMINATION OF DIFFICULT PESTICIDES. APPLICATIONS FOR CAPTAN, FOLPET AND DICOFOL

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Multiclass-Multiresidue methods are a powerful tool in the pesticide residue analysis for monitoring purposes, where a large list of different pesticides must be determined. However, these multiresidue methods are not capable to cover all the pesticides included in the EU monitoring programs, EU Regulation Nº 788/2012, where a large list of pesticides should be analysed by single residue methods. This is the known case for pesticides like Glyphosate, Fosetyl-Al or Paraquat, among others. Between the pesticides suitable to be analysed using multiresidue methods on one hand or by single residue methods in the other one, we can find a group of pesticides suitable to be identified in multiresidue methods without an accurate quantification because they are unstable pesticides showing degradation to known metabolites, not included in the residue definition, in the multirresidue method steps, even in the chromatographic analysis. This is the case scenario for pesticides like the phtalimide fungicides Captan and Folpet or the organochlorine acaricide Dicofol. These pesticides show a degradation to tetrahydro-phtalimide, phtalimide and 4,4' dichloronezophenone, respectively, metabolites not included in the residue definitions for each one. The determination of these pesticides should be conducted using a method that guarantee a minimum degradation of the parental compound. In the present effort a method based upon acidified ethyl acetate (using acetic acid) has been applied to the determination of Captan, Folpet and Dicofol residues, with satisfactory results, regarding the stability of these pesticides. The standard addition approach has been carried out, with recoveries of 80% for phtalimide fungicides, Captan and Folpet, and 96% for Dicofol. A very good linearity, ($R^2 > 0.995$) is found for the standard addition curves in all cases. For routine monitoring purposes, Captan, Folpet and Dicofol could be included in the multiresidue methods, joined to their metabolites. The positive residue identification of the parental compound and/or the corresponding metabolites leads to the quantitative determination using the acidified ethyl acetate approach with standard addition. A detection limit of 0.02 mg/kg has been found for Captan and Folpet, and 0,01 mg/kg for Dicofol following this approach.

PS1-40

APPROACHES TO CHARACTERISE COLUMN PERFORMANCE RELATED TO PEAK SHAPE

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The study of column performance is of great interest to characterize stationary phases, develop new materials and increase the resolution of complex samples. A particular concern is the analysis of compounds that interact with the silica support on the alkyl type silica-based columns, giving rise to significant peak broadening and asymmetry. These effects are produced by a slow adsorption-desorption equilibria, and saturation of the adsorption effect on the silanol groups.

Traditionally, this study has been performed through the elution of probe compounds at different flow rates and drawing of the Van Deemter plots [1] , which relate the plate height, H (variance per unit length) of the column to the linear mobile phase velocity, u_0. The interest of these plots is that they can reveal the different contributions to band broadening. A more recent approach, developed by Desmet et al., are kinetic plots where H^2/K_v is represented versus $K_v/(Hu_0)$ (K_v being column permeability) [2]. Multiplying the values on both axes by the ratio of a reference pressure drop and mobile-phase viscosity, the plots inform about the kinetic performance limits of the column. The disadvantage of these approaches is that they require manipulation of the peak parameters (position and variance), which can lead to data affected by significant uncertainty due to the propagation of random errors.

A more recent and simple approach to characterize column performance, developed in our laboratory, is the representation of half-widths versus the retention times (half-widths plots) [3,4]. This approach reduces the uncertainty of the data, since the experimental peak parameters are directly used. In this work, the three approaches are compared using several sulfonamides and β-blockers as probe compounds, and Spherisorb and Chromolith columns at 25 and 50^{o}C.

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PS1-41

MODELLING OF RETENTION AND PEAK SHAPE IN COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

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The interest in developing approaches for separating highly challenging samples, such as biopharmaceuticals, peptide maps, plant extracts, food matrices, polymers, *etc*. is growing. In this regard, comprehensive two-dimensional liquid chromatography (LC×LC) offers the possibility of substantially increasing the peak capacity in comparison with conventional LC, by combining orthogonal separations. Contemporary instrumentation provides many new opportunities for changing the mobile phase composition in the second dimension, applying gradients, varying the initial and final compositions of the second-dimension gradient, *etc*. A recent example of such instrumentation is the Agilent 1290 Infinity 2D-LC solution.

However, method development in LC×LC is a bottleneck. The ability to reliably predict chromatograms after a few initial runs may help overcome the most important obstacles to the proliferation of LC×LC. Generic retention models can be generated and validated efficiently using LC×LC experiments. The resulting models may be used to formulate guidelines and strategies for method development in LC×LC.

In this work, the separation of a set of polyphenols is studied with RPLC and HILIC, using acetonitrile-water mixtures in the gradient-elution mode. These systems show “orthogonal” (complementary) behavior, while using similar eluents. Gradient times and peak profiles were modeled for both dimensions versus the gradient parameters (initial and final concentration of organic solvent and gradient slope) for HILIC×RPLC separations. The models were used to predict the retention time of all solutes at any condition, and optimize the gradients. The parameters that affect the gradient modeling (e.g. dwell time, dead volume, sample injection volume and equilibration time) were considered.

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PS1-42

PERFORMANCE OF DIFFERENT C18 COLUMNS WITH MICELLAR MOBILE PHASES

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Theoretically, in reversed-phase liquid chromatography (RPLC) with hydro-organic mixtures as mobile phases (conventional RPLC), the retention is only due to adsorption on the alkyl-bonded phase; consequently, it is related to compound hydrophobicity. However, underivatized silanols in silica-based columns, which remain on the stationary phase in a non-negligible amount, when ionized interact with cationic analytes by ion exchange, increasing the retention. Also, the sorption-desorption kinetics on silanols is a slow process that yields tailed and broad peaks. The concentration of free silanols in a C18 stationary phase varies with the brand and manufacturer, but the information provided when purchasing the column is frequently limited.

The difficulties related to column selection could become a challenge, especially if the target compounds have a basic character, which yield high retention and poor peak shape with silica-based columns. The addition of several reagents to the mobile phase (amines, surfactants or ionic liquids) to mask the residual silanols has revealed as an efficient strategy to minimize the deleterious effects found in the analysis of basic compounds. Masking of silanols by these additives can be produced by the direct interaction of a cation in the reagent with the negatively-charged silanols (case of amines and ionic liquids), or by covering the stationary phase surface with the additive thus avoiding the access of analytes to the silanols (case of SDS).

Published studies on the effect of additives are usually carried out with a single column, and do not focus on finding out if the impact of the additives is similar, independently of the selected C18 material. In this work, the chromatographic behavior of five basic compounds separated with a set of eight conventional C18 columns of different brands and similar dimensions was examined under different conditions: aqueous-organic mobile phases in the absence and presence of a selected additive, the surfactant sodium dodecyl sulphate (SDS) and two alkyl-imidazolium ionic liquids. The results were analyzed in terms of retention, selectivity, peak shape and total analysis time, and were extended to a wide range of mobile phase compositions. It has been found that SDS should cover the stationary phases in diverse degree, since remarkable differences in chromatographic behavior are still kept among different columns as is the case in conventional RPLC with the same columns.

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PS1-43

OPTIMIZATION OF GRADIENT ELUTION WITH SERIALLY-COUPLED COLUMNS

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Recent advances in column technology have allowed the development of functional hybrid columns, built up by serial coupling of stationary phases. In the approach originally formulated by Nyiredy *et al.* (Chromatographia 63 (2006) S3–S9), selectivity optimization was based on changes in the nature of the coupled stationary phases and their lengths. This separation mode was called "stationary phase optimized selectivity liquid chromatography" (SOSLC) by the authors. Bischoff Chromatography marketed a system based on this idea (POPLink kits), where hybrid columns of five different stationary phases with lengths differing in one cm are possible. An optimization software is also provided by the company to facilitate the routine application of SOSLC. In all instances, either an eluent composition in isocratic elution selected attending the analysis time, or a gradient program giving rise to acceptable retention, is fixed. This represents a limitation in practice. Another limitation is the small number of stationary phases included in the Bischoff kits.

Recently, we published a work where, instead of dedicated columns, conventional short columns were assembled through zero dead volume junctions, which give access to any already existing or newly branded column available in the market (Journal of Chromatography A 1281 (2013) 94–105). In addition, the mobile phase was optimized in cooperation with the stationary phase assembly, considering peak width and symmetry in the treatment (unpublished results). This allowed predicted results virtually matching the experimental separations. Interestingly, by modifying the mobile phase composition, a small number of column lengths (e.g., 2 and 5 cm) for each stationary phase allows resolution vs. analysis time maps with Pareto fronts matching the results observed with column length increments of one cm. That is, the solvent modification gives rise to an impressive reduction in the number of different column lengths needed in the serially-coupled columns approach without loss of performance, and reduces drastically the cost.

In this work, a further step in the systematic method development for serially-coupled columns is reported, by optimizing multi-step linear gradient programs along column assemblies. The application of gradients give access to the separation levels associated to slow isocratic eluents (upper solutions in the Pareto front), with significantly reduces analysis times.

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PS1-44

CONSIDERATIONS ABOUT THE SEPARATION OF PHENOLS BY ISOCRATIC RPLC USING TIME AND SPECTRAL INFORMATION

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Current HPLC instruments are able to yield second-order signals, where full spectra are collected at different elution times. Since such rich signals are readily available, highly complex samples have become now accessible at routine level without the need of highly specific columns. Hence, the usual practice of neglecting spectra (giving prevalence to reach chromatographic separation focusing in the time order) results at least contradictory. Several reasons justify this, being likely the tradition the most important: retention time has been used along decades for identification purposes, and in a further step the spectra are checked to correspond to a pure analyte. If the peak is found pure, the area at a given wavelength can be used for quantifying the analyte.

Taking full advantage of second-order signals requires multivariate objective functions, and implies accepting deconvolution as a valid tool, since often full resolution cannot be achieved in any data order. Several second-order figures of merit (FOM) have been considered. The simplest one is the Net Analyte Signal (NAS), defined as the fraction of the analyte signal that cannot be explained as a linear combination of the signals of the remaining compounds. NAS can be outlined in an unfolded first-order way, or as a full second-order measurement. Another second-order FOM is the Multivariate Selectivity, calculated through several mathematical expressions. Multivariate Selectivity has been told that correlates particularly well with deconvolution results, and this suggests that it is an excellent complementary measurement to appraise the difficulty of peak configurations. Naturally, all these expressions are more or less correlated to each other. One of the objectives of this work is assaying critically their performance. One and two-way peak purities are used as reference.

The application of resolution criteria based on time-spectral comparisons is not free of difficulties. In this communication, we show the problems associated to different time scales, data dimensions, normalizations and not-straightforward meaning of the second-order FOMs, which altogether give rise to deceiving results, or not comparable to each other. Some solutions are assayed. The data set used for the study consists of a mixture of phenols of significant impact in the environmental and toxicological fields.

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PS1-45

SIMPLIFICATIONS ON SEVERAL CHROMATOGRAPHIC MODELS THAT PREDICT GRADIENT ELUTION OF ACID-BASE ANALYTES

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Gradient elution is a chromatographic mode that changes composition during the elution process and thus, when measuring retention of acid-base compounds in buffered mobile phases, both the pK_a of the analytes and the pH of the mobile phase also change throughout the process because both parameters depend on the mobile phase composition. Hence, the variation of these variables has to be taken into account if accurate predictions of the retention of acid-base analytes are desired.

Following our previous work in acetonitrile-water [1], a new study has been carried out using previously studied substances but also some other compounds with more structural complexity and pharmaceutical interest with methanol as the organic modifier.

The main experimental simplification is that instead of measuring them experimentally, pK_a values of the different compounds have been estimated through the following equation:

$$ {}^{s}_{w}pK_a = a_s \, {}^{w}_{w}pK_a + b_s + \delta $$

where ${}^{s}_{w}$pK_a is the pK_a in the mobile phase referred to water calibration, ${}^{w}_{w}$pK_a is the pK_a in pure water, δ is a parameter that depends on the organic fraction of the mobile phase and a_s and b_s are parameters that depend on both the functional group of the analyte and the organic fraction of the mobile phase [2].

Since no experimental determination of the pK_a values is performed, retention factors of the neutral and ionized forms of the analytes are found by measuring isocratically their retention at a very acid and a very basic pH value (pH 2 and pH 11) instead of being determined by measurements at different pH values.

Several initial mobile phase pH values have been tested in order to check different ionization degrees of the analytes, their variation through the elution process has been modeled by fitting experimental values to a second grade equation. Acceptable results have been obtained in most cases. Thus, taking into account that little accuracy is lost, this simplification has been found very useful in these predicting models since it decreases a lot the experimental work to be done.

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PS1-46

MICELLIZATION PARAMETERS OF DICATIONIC AND TRICATIONIC IONIC LIQUID-BASED SURFACTANTS BY CONDUCTIMETRIC AND SPECTROFLUORIMETRIC STUDIES

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Ionic liquids (ILs) are non-molecular solvents characterized for presenting melting points below 100 ºC, high thermal stabilities, and negligible vapor pressures at room temperature. These properties, altogether with their impressive solvation abilities, permit to propose ILs as alternative to conventional organic solvents. In addition to this, it results quite simple to chemically modify the structure of ILs by incorporating specific groups. This way, the properties of the resulting ILs are easily modified, leading to tuneable solvents.

A new group of ILs, able to aggregate in water, has been recently described. It results of high interest the study of these new ILs which resemble to cationic surfactants, as a new area of surfactant development if the limited number of conventional cationic surfactants is taking into account. Furthermore, the chemical tuneability of ILs is also observed with IL-based surfactants.

IL-based surfactants present lower critical micelle concentration (CMC) values than those of structurally analogues of cationic surfactants. This is quite advantageous because it is possible to form micellar aggregates using lower amounts of surfactants and so the generation of wastes in the laboratory is minimized.

The present work reports the characterization of a novel group of dicationic (Gemini) and tricationic IL-based surfactants utilizing conductimetric and fluorimetric studies. Thus, CMC and aggregation number values have been obtained for these IL-based surfactants. Moreover, the influence that a common solvent in analytical applications such acetonitrile exerts in the aggregation behavior has also been considered.

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PS1-47

USE OF MULTIVARIATE STATISTICS TECHNIQUES TO DEVELOPMENT OF A CHROMATOGRAPHY METHOD TO ANALYSIS OF CAPSAICINOIDS

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Fruits of chili pepper plants belong to the family Solanaceae and genus Capsicum which are one of the most consumed spices throughout the world [1] and are very important commercially. The consumption of chili peppers is due mainly to their very pungent flavour. The pungency is caused by capsaicinoids and is proportional to the combined concentrations of various vanillyl amides that are collectively referred to as capsaicinoids [2]. Among the most abundant of these components are capsaicin and dihydrocapsaicin. Besides these two major capsaicinoids, other minor capsaicinoids have been shown to occur in peppers [1, 3], including nordihydrocapsaicin, norcapsaicin, homocapsaicin, homodihydrocapsaicin, nornorcapsaicin, nornornorcapsaicin, and nonivamide, among others [3, 4].

This work shows the use of multivariate statistics techniques to development of a separation method of capsaicinoids in a HPLC. Capsaicin and dihydrocapsaicin were purchased and other 15 minor capsaicinoids were synthesized. Their structures were confirmed by NMR^{H1}. The study of separation on HPLC was initiated with the standards of capsaicinoids. A HPLC (Hitachi) equipped with a column Halo C18 (100 x 3.0mm, 2.7µm) and a mobile phase composed by water and methanol, both solvents with 0,1% of acetic acid, was used. A central composite design and response surface as design of this experiment, with three variables was used: First: Initial percentage of methanol; Second: Gradient time change until 100% of methanol, and third: the flow rate. The response chosen was the resolution into each pair of peaks and the time analysis. A multi-criteria response technique of Derringer and Suich was used, and the desirability values were established for each individual response and they were combined into their recommended global desirability function [5], with the objective of minimize the analysis time keeping a good compounds separation. The better condition found was composed by an initial mobile phase with 26,43% of methanol, change to 100% of methanol with a linear gradient of 11.25 min and a flow rate of 0.934mL.min^{-1}, time analysis of 10.6 min and a desirability of 0.275. The results showed a rapid method separating a large number of compounds, and the efficiency of the multivariate techniques in the optimization process.

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PS1-48

USE OF MULTIVARIATE STATISTICS TECHNIQUES TO DEVELOPMENT OF A CHROMATOGRAPHY METHOD TO ANALYSIS OF CAPSINOIDS

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Fruits of chili pepper plants belong to the family Solanaceae and genus Capsicum which are one of the most consumed spices throughout the world [1] and are very important commercially. The consumption of chili peppers is due mainly to their very pungent flavour. The pungency is caused by capsaicinoids, but in the last years a new family of non-pungent compounds similar to capsaicinoids were found, the capsinoides. This family has the organic function ester in place of amide and this difference cause the pungence absence, however, the capsinoids have similar biological activities to capsisaicinoids such as antioxidant, anti-inflamatory, antimicrobial, antimutagenic and promote the weight loss and decrease the fat body accumulation.

This work shows the use of multivariate statistics techniques to development of a separation method of capsinoids in a HPLC. Capsiate, dihydrocapsiate and other 15 minor capsinoids were synthesized and their structures were confirmed by NMR^{H1}. The study of separation on HPLC was initiated with the standards of capsaicinoids. A HPLC (Hitachi) equipped with a column Halo C18 (100 x 3.0mm, 2.7µm) and a mobile phase composed by water and methanol, both solvents with 0,1% of acetic acid, was used. A central composite design and response surface as design of this experiment, with three variables was used: First: Initial percentage of methanol; Second: Gradient time change until 100% of methanol, and third: the flow rate. The response chosen was the resolution into each pair of peaks and the time analysis. A multi-criteria response technique of Derringer and Suich was used, and the desirability value were established for each individual response and they were combined into their recommended global desirability function [5], with the objective of minimize the analysis time keeping a good separation of the compounds. The better condition found was composed by an initial mobile phase with 50.0% of methanol, change to 100% of methanol with a linear gradient of 15 min and a flow rate of 0.542 $mL.min^{-1}$, time analysis of 14.9 min and a desirability of 0.146. The results showed a rapid method separating a large number of compounds, and the efficiency of the multivariate techniques in the optimization process.

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PS1-49

ENANTIOMERIC RESOLUTION OF FMOC-AMINO ACIDS WITH VANCOMYCIN BY CAPILLARY ELECTROPHORESIS COUPLED TO ION-TRAP MASS SPECTROMETRY

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L-Amino acids are biomolecules playing an essential role in nature. Their determination has originated a high number of applications in the pharmaceutical, environmental or food analysis fields. Nevertheless, the physiological role of many D-amino acids is still unknown and hence, the development of chiral methodologies for the simultaneous separation and unequivocal identification of L- and D-amino acids is required. Capillary Electrophoresis (CE) has shown great usefulness and applicability in the study and chiral separation of amino acids. Combining chiral CE separation methods with MS detection promises high selectivity and sensitivity in amino acids analysis.

In this work, the simultaneous separation and identification of L- and D-amino acids has been carried out by CE coupled to electrospray ionization tandem mass spectrometry (CE-ESI-MS^2). 9-Fluorenylmethoxycarbonyl (FMOC) was employed as derivatizing reagent to allow the interaction of amino acids with the chiral selector, vancomycin, and the formation of the major precursor ions for each amino acid. The electrophoretic and interface parameters affecting enantiomeric resolution and sensitivity were optimized. To prevent the non-volatile chiral selector entering in the MS detector avoiding possible source contamination and ion suppression, two electrophoretic strategies were combined as the use of a coated capillary and a partial filling procedure. In addition, the use of MS^2 experiments enabled an increase on selectivity and sensitivity (approximately 10 times in comparison with the MS experiments). Under the optimized conditions, the developed CE-ESI-MS^2 methodology enabled to obtain the chiral separation and identification of 17 amino acids (two of them being from non-protein origin) in about 20 min with good precision and LODs in the µM range.

PS1-50

ANALYSIS OF HYDROXYLATED POLYCHLORINATED BIPHENYLS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Polychlorinated biphenyls (PCBs) are toxic and persistent pollutants that have been widely dispersed in the environment before they were banned by most countries in the 1970s. PCBs exert various detrimental effects on wildlife and humans, and they are classified by several agencies as suspected carcinogens. In living organisms, PCBs are metabolised leading to polar metabolites which are supposed to be more easily excreted, such as their corresponding hydroxylated metabolites (OH-PCBs). However, some studies have shown that OH-PCB metabolites can be bound to specific plasma proteins and interact with hormone receptors. In fact, it is still not clear if PCBs' toxicity is only due to PCB concentrations or is also due to the presence of PCB metabolites in the same individual.

In the present work, a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method has been developed, for the quantification of eight OH-PCBs, and characterised in terms of its repeatability, intermediate precision, linear calibration ranges, detection and quantification limits and ionisation matrix effects in human serum samples. The separation process was carried out on a Thermo Hypersil amide column (100 mm x 2.1 mm, 3 µm) by a chromatographic method previously optimised [1] that allows all isobaric compounds tested in the mixture to be separated in a short analysis time. A triple quadrupole Xevo-TQS (Waters) working in MRM mode was used for the detection.

A comprehensive optimisation of the negative electrospray ionisation process was carried out, being the desolvation gas temperature the most critical variable. Two MS/MS transitions were selected for quantifying each compound and the corresponding fragmentation energies were optimised as well. Limits of detection were found to be between 4 and 292 fg on column, and the average intermediate precision was 6% for an amount of 1 pg of each congener on column. Ionisation matrix effects were evaluated on human serum samples by a dilution process, finding that the matrix effect almost disappeared with a 10-25 times dilution. Nevertheless, a good and reliable quantification method was obtained when labelled internal standards were used.

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PS1-51

CONCURRENT SOLVENT RECONDENSATION – LARGE VOLUME SPLITLESS INJECTION – GAS CHROMATOGRAPHY – MASS SPECTROMETRY FOR THE ANALYSIS OF LINEAR AND CYCLIC VOLATILE METHYLSILOXANES IN ENVIRONMENTAL SAMPLES

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Volatile methylsiloxanes (VMSs) are a new group of emerging contaminants that represent a potential risk to the environment due to their toxicity, persistence and bioaccumulation capacity [1]. The analysis of these compounds is not easy due to both, their high volatility and the potential sources of background contamination. To ensure reliable results, there must be a thorough control of the blanks and a prevention of losses by eliminating concentration steps, although this results in a decrease on sensitivity. The use of large volume injection (LVI) for sample introduction in gas chromatography (GC) can be a good way to overcome this problem. On-column (OC) and programmed temperature vaporization (PTV) are the most widely used LVI techniques for GC analysis. Nevertheless, OC-LVI is not sufficiently rugged for analysing dirty samples and PTV-LVI is not suitable for highly volatile and thermally labile compounds. Concurrent solvent recondensation (CSR) is an alternative LVI technique applicable to the analysis of volatile compounds that allows the splitless injection of up to 50 µL of sample in a conventional split/splitless injector [2]. Despite its advantages, the CSR-LVI technique has been used for a limited number of applications [3,4], so there is interest in increasing the use of this technique for the analysis of environmental volatile pollutants.

The aim of this work is to evaluate the performance of CSR-LVI combined with gas chromatography-mass spectrometry (GC-MS) for the analysis of linear and cyclic volatile methylsiloxanes (VMS) in several environmental matrices. For this purpose, CSR-LVI injection parameters (liner internal diameter, retention gap length, type and volume of injection solvent, injection temperature and initial oven temperature) that affect the sensitivity of the method were optimized. Low limits of detection ranging from 0.004 to 0.14 ng g^{-1} (wet weight) for soil and sludges, between 0.03 and 0.7 ng m^{-3} for ambient air, and between 0.8 and 1.5 ng L^{-1} for wastewaters, were obtained. In addition, recoveries higher than 85% and good precisions (RSD, <15%) were achieved for all matrices. Examples of the application of the proposed method for the analysis of linear and cyclic methylsiloxanes in soils, sludges, ambient air and wastewaters collected in Barcelona metropolitan area will be presented.

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PS1-52

STUDY AND CONTROL OF MORPHOLOGY IN GLYCIDYL AND HYDROXYETHYL METHACRYLATE-BASED MONOLITHS POLYMERIZED IN SILICA CAPILLARIES FOR FUTURE SEPARATION METHODS

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Monolithic materials have quickly become a well-established stationary phase format in the field of capillary electrochromatography and capillary liquid chromatography. Both the simplicity of their *in situ* preparation method and the large variety of readily available chemistries make the monolithic separation media an attractive alternative to capillary columns packed with particulate materials. In the last years, the post-modification of monolithic columns with interactive functionalities for dedicated chromatography modes, in contrast to the single step copolymerization of functional monomer with crosslinker and other comonomers, has become more popular allowing the independent tuning of the macroporous structure with its major effect on mechanical and flow-through properties and of the surface chemistry via derivatization of the parent monoliths. Glycidyl and hydroxyethyl methacrylate monomers are mainly used as carriers for post-modification of the monolithic columns. The mechanism of pore formation during the heterogeneous polymerization of monomers in the presence of porogens does not yet allow an accurate and reliable prediction of the resultant pore sizes. The current knowledge of factors that control pore size in macroporous polymers is mostly empirical. The necessity to have a control on the pore size, that had a significant effect on the retention and efficiency of the capillary columns, is an important field of study for trying to predict this parameter. In this work, the pore size in macroporous polymers was examined and empirical correlations allowing predictions of the pore size are presented over a wide range of experimental parameters. Using the OriginPro software (v. 9.1) we herein study systematically the data obtained from mercury intrusion porosimetry and nitrogen adsorption-desorption isotherm measurements. Different plots of the porous volume, the porous diameter and the surface area in dependence of different variables (concentration of the monomers and the porogens) clearly reveal the influential factors and allow targeted preparation of monoliths with specific properties. In conclusion, the modeled 3D surface plots can provide an alternative method to select the best morphology for a given column application instead of the classical trial and error approach.

PS1-53

FIELD AMPLIFIED SAMPLE INJECTION-CAPILLARY ZONE ELECTROPHORESIS (FASI-CZE) FOR THE ANALYSIS OF BENZOPHENONE UV-FILTERS IN SUNSCREENS FORMULATIONS

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Nowadays it has been well established that excessive UV radiation is clearly detrimental and may cause sunburn, premature aging of the skin, development of skin cancers and cataracts, immune suppression, and even activation of latent viruses. Inorganic UV filters (such as titanium dioxide or zinc oxide) and organic UV filters (synthetic organic chemicals) which can reflect or absorb harmful UV radiation, are commonly added to various sunscreens in order to reduce the harmful influence of solar light on human skin, but also to prevent damage to cosmetics by sunlight during storage. Among the last ones, benzophenones (BPs) are widely used as organic UV filters because of their excellent absorbing abilities for the UV-A component (320-400 nm wavelengths) of the solar radiation. However, some dermatological reactions as well as photoallergies have been described related to the presence of some BPs [1]. The European Union has established a list of allowed European cosmetic UV filters which include several BPs [2]. The analytical control of sunscreen cosmetics is therefore necessary to ensure that the concentration levels are lower than those permitted by legislation and to prevent misuses in a globalized marked.

The aim of this work is to develop a capillary zone electrophoresis (CZE) method for the analysis of eight benzophenone UV-filters in sunscreens formulations. Uncoated fused-silica capillaries (40 cm effective length x 75 µm I.D.) and a 35 mM sodium tetraborate buffer were used for the electrophoretic separation carried out in counter-electroosmotic flow conditions by applying a capillary voltage of +30 kV. In order to enhance sensitivity, the applicability of the in-line preconcentration method field amplified sample injection was evaluated. A 2.5 mM sodium tetraborate buffer solution was proposed as sample matrix in order to achieve an effective FASI application when benzophenones were analyzed. Then, a water plug (20 s, 3.5 kPa) was hydrodynamically introduced previous to the electrokinetic sample injection (25 s, -10 kV) to obtain sensitive enhancements between 9 and 25 compared to conventional CZE under hydrodynamic injection, and achieving instrumental limits of detection (LODs) down to 20-50 µg L^{-1} for some BPs. Quality parameters such as LODs, limits of quantitation (LOQs), linearity, and run-to-run and day-to-day precisions at two concentration levels were obtained for both CZE and FASI-CZE methods. Finally, the applicability of the developed methods was evaluated by analyzing benzophenones in sunscreens formulations.

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PS1-54

IDENTIFICATION OF AROMA COMPOUNDS RESPONSIBLE FOR BAD ODOURS IN DISHWASHERS

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The use of home appliances has inherent problems like the apparition of bad odours. In the case of dishwashers, odours can be generated in two different situations: on the one hand the degradation of leftover food inner of the dishwasher due to oxidation of food or to apparition of different microorganisms, on the other hand when a dishwasher has been washed but it remains closed during a long period of time some unpleasant odors could appear. Two different situations were studied: dirty dishes with standard dirt were into the dishwasher during one week and then they were washed and the dishwasher remained closed during two weeks.

A study with gas chromatography-olfactometry (GCO) was made to know the most important odour zones in the characterization of bad odours in dishwashers. This study was made with two ways of sample preparation: using a fiber of SPME which was introduced in the dishwasher during 24 hours and using a Petri dish with 400 mg of Lichrolut EN which remained into the dirty dishwasher during one week or two weeks in the case of the clean dishwasher (the resins were eluted with 3.2 mL of dichloromethane- 5% methanol). Analyses were carried out by a panel composed of six expert sniffers. Detection frequency and intensity were evaluated to obtain percentage of modified frequency.

Only odour zones with a modified frequency higher or equal to 50% were taken in account to be identified. 21 different compounds were identified. More compounds were identified thanks to Lichrolut EN resins however, some compounds (dimethyl sulfide –DMS-, 2,3,5-trimethylpyrazine, methional, furaneol) appeared only with SPME. There were less compounds when the dishwasher was empty and closed during two weeks than when the dishwasher was loaded, but 2 compounds (2, 4, 6-trichloroanisole and geosmin) appeared in the first situation and not in the second situation. Twelve compounds appeared in both situations: sulphur compounds (DMS, dimethyl trisulfide, benzylmercaptan), carbonyl compounds (diacetyl, hexanal, 1-octen-3-one, Z-2-heptenal, nonanal, E-2-nonenal, isovaleric acid) and b-pinene and p-cresol.

In conclusion, the combination of two strategies of sampling has allowed the identification of compounds responsible for bad odours in two different situations in a dishwasher.

Separación y detección

Cromatografía Líquida

Lider en LC, UHPLC y sistemas nanoLC

Cromatografía de Gases

Multiples soluciones en GC

Cromatografía Iónica

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Espectrometría de Masas

Sistemas precisos de identificación y cuantificación

Software Cromatográfico

Rápida interpretación de datos y flujo de información

Prep. Muestras

Mejores extracciones en menos tiempo

Columnas y Consumibles

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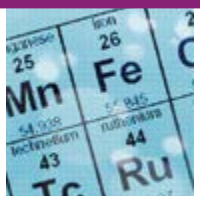

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PS2-01

TRACE ANALYSIS OF SPINOSAD IN BEE POLLEN AND BEESWAX BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY MASS SPECTROMETRY

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Spinosad is a relatively new insecticide, which is derived through the fermentation of a naturally occurring bacteria, Saccharopolyspora spinosa, consists in two macrolides tetracyclic compounds as active ingredients, spinosyns A and D. This insecticide is an alternative to traditional pesticides because is a natural product with a favorable environmental profile But there are a lot of controversies, because it has been postulated in some works that spinosad does not affect to pollinator insects as adult honeybees, broods and queens. On the other hand, it has been also found in other studies that spinosad possesses sub lethal effects on bumblebees and bees. According to the application procedure, when this insecticide is sprayed over the crops, residues of its active ingredients (spinosyns) would be found in flowers pollen and consequently in different bee products as beeswax and corbicular bee pollen. For these reasons, the analysis of spinosyns presents a great interest when monitoring the contamination of bee products as well as to explain their potential relation with the honey bee decline known as Colony Collapse Disorder.

So, it has been developed a new analytical method based on liquid chromatography coupled to electrospray ionization mass spectrometry (LC -ESI-MS) to determine spinosyns A and D in corbicular bee pollen and beeswax. The results obtained were also compared with earlier data and to existing maximum residue levels (MRLs) European legislation to check the suitability of our new method. The extraction procedure consisted of a solid-liquid extraction of both spinosyns with acetone (pollen) or acetonitrile (beeswax).It has been used a reverse phase (RP) analytical column, Kinetex, and isocratic elution mode of the mobile phase, which was composed by 0.1% formic acid in water and acetonitrile applied at a flow rate of 0.5 mL/min. Finally, the method was validated and applied to analyze several corbicular pollen and beeswax samples taken from apiaries located close to fruit orchards of two Spanish regions in order to check the presence of spinosyn A and D residues.

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PS2-02

OPTIMIZATION OF A METHODOLOGY TO ANALYZE GEOSMIN AND 2-METHYLISOBORNEOL BY PURGE AND TRAP WITH GC/MS

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Geosmin and 2-methylisoborneol are known by the scientific community by the musty - earthy odour and taste that they confer to water, as well as their capability to change its organoleptic properties. These compounds are one of the main source of odour problems that water utilities have to cope with.

Geosmin (t-1, 10-dimethyl-t-9-decalol) is produced by the growth of several blue-green algae and actinomycetes. 2-methylisoborneol (1,6,7,7-Tetramethylbicyclo[2.2.1]heptan-6-ol) is produced by some cyanobacteria species. These algae metabolites are perceived by the human nose in very low concentration levels, from 5 to 10 ng/L^{-1} for 2-MIB and from 1 to 10 ng/L^{-1} for Geosmin [1].

The Grob Closed-Loop Stripping Analysis method [2] has been traditionally used in laboratories for the determination of Geosmin and 2-MIB. The extract obtained after the stripping process, is analysed by gas chromatography coupled to mass spectrometry. This is a highly recommended technique, used for the detection of a wide range of volatile and semi-volatile compounds, and especially useful in case of odour and taste problems episodes, but its disadvantage arises from the long time required for the analysis of each sample. In order to overcome this problem and being able to reduce the analysis time - an especially important factor in case of routine analysis- a method by Purge and Trap extraction has been carried out .

The Purge and Trap technique consists of extracting volatile compounds from the sample using a Helium current purge. The compounds remain kept on a trap, which is then warmed to high temperatures, allowing the compounds to be desorbed from the trap and directed to the gas chromatograph, where they will be separated and then detected by mass spectrometry. Different parameters have been optimized, such as purge temperature, purge time and sample volume, among others.

This paper reports a method for the detection and quantification of Geosmin and 2-methylisoborneol by Purge and Trap extraction. A calibration curve has been done, obtaining correlation coefficients greater than 0.99 for both compounds. Limits of detection and quantification calculated were around 10 ng/L-1, being similar to those obtained with the internal accredited procedure by the Grob CLSA methodology. Several concentration levels from the calibration curve have been tested, obtaining recoveries greater than 75%.

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PS2-03

IDENTIFICATION AND QUANTIFICATION OF ALCOHOL ETHOXYSULFATES IN RIVER SEDIMENTS FROM GRANADA BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Surfactants are active ingredients in detergent formulations, cleaning and personal care products, emulsifiers, pesticides, adjuvants and wetting agents. These compounds are produced and consumed in large quantities. In 2010, total consumption (not including soaps) in Europe was 2.94 million tons [1]. Over the last few years, due to the increasing public concern over environmental safety, laws regarding the use of these compounds have become stricter because of their potential to produce adverse effects on ecosystems and the wildlife that live in them.

Although some studies have been conducted to understand the distribution of major anionic surfactants in different environments [2,3] however, there are few papers on the determination of AES in river sediments. The main reason for this is the limitations of existing analytical techniques available over the last decade. The analysis of these compounds is complicated due to their structure, the complexity of the matrices and also because these compounds are generally found in very low concentrations. Therefore, it is necessary to develop new analytical methods to improve the isolation and extraction of these compounds.

A method for the identification and quantification of alcohol ethoxysulfates (AES) ethoxymers in river sediment samples is proposed. The method involves the extraction of 5 g of dry sample with methanol using pressurized liquid extraction (PLE) and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). 2–Octylbenzene sulfonic acid sodium salt (2ØC_8–LAS) was used as internal standard. The analytical method was applied to river sediments collected from the Monachil river (Granada, South–East Spain). Quality parameters were determined and satisfactory results were obtained.

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PS2-04

NEW FIELD STUDY OF ALCOHOL ETHOXYSULFATES IN AGRICULTURAL SOILS FROM GRANADA BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Surfactants are the active ingredients in household detergents, industrial cleaning agents and personal care products. These compounds are manufactured in large quantities and after use, they usually are disposed to wastewater treatment plants (WWTP). Depending on the chemical structure of the surfactant molecule and on the operating conditions of the WWTP, they are removed by biodegradation processes or adsorption onto the sludge particles from wastewaters. These compounds may be introduced into the terrestrial compartment by irrigation with wastewater, by sewage sludge application, when they are used as emulsifying, dispersing and spreading agents in agriculture [1], horticulture and in the remediation of contaminated soil by mobilizing heavy metals, PAHs, etc.[2].

We focus on the commercial mixture of AES, which contains homologues of up to 14 carbon atoms in their alkyl chain and of up to 12 ethoxymers. The aims of this work are: 1) to develop a new methodology to improve the extraction and determination of AES in agricultural soil samples. Prior to instrumental analysis, an extraction procedure using pressurized liquid extraction with methanol (PLE) was carried out followed by direct identification and quantification by means of reversed-phase Liquid Chromatography - Mass Spectrometry (LC-MS/MS) with electrospray ionization (ESI) operating in negative mode and 2) to study the role of the alkyl chain length and ethoxymers in their adsorption in soil. This study was carried out for the seasons: Summer, Autumn, Winter and Spring, to evaluate their adsorption in agricultural soils.

The method was satisfactorily applied in a field study designed to evaluate the environmental behavior of these compounds in agricultural soil. Validation parameters such as linear dynamic range detection and quantification limits, inter-day and intra-day repeatability and accuracy were established.

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PS2-05

FATE AND OCCURRENCE OF ALCOHOL ETHOXYSULFATES ETHOXYMERS IN MARINE SEDIMENTS FROM SPAIN

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Surfactants are chemicals that typically contain hydrophobic and hydrophilic groups. The hydrophobic domain is usually a hydrocarbon whereas the hydrophilic group can be non-ionic, positively or negatively charged, or amphoteric. These characteristics give them specific physical and chemical properties. Because of the low solubility and great ability to associate with particles, surfactants are always present in sediments. Marine sediments act both as reservoirs and as potential sources of these chemicals and can adversely affect sediment–dwelling organisms by causing direct toxicity or altering benthic invertebrate community structure [1, 2]. Some of these contaminants are persistent in the environment, and the cumulative effects in coastal environments are expected to be considerable, for this reason is very important to develop new analytical methods to improve the isolation and extraction of these compounds.

The aim of the present work was to develop and validate an accurate and sensitive analytical method for the determination of AES ethoxymers in marine sediments based on a pressurized liquid extraction (PLE) procedure, followed by a liquid chromatography–tandem mass spectrometric (LC–MS/MS) analysis. After validation, the method was successfully applied to the analysis of sediment samples collected from three different marine matrices, which are located in three different geographical areas of Spain: the Almería coast, the Mediterranean coast and the Tenerife coast. Quality parameters were determined and satisfactory results were obtained.

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PS2-06

ANALYSIS OF ORGANIC POLLUTANTS IN SURFACE WATERS BY ON-LINE SOLID PHASE EXTRACTION-LIQUID CHROMATOGRAPHY-ULTRAVIOLET DETECTION

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Nowadays, there is an increasing public concern for environmental safety and for this reason it is necessary to study the presence of organic pollutants in different environmental compartments [1,2]. The use of large quantities of insecticides (carbamates and pyrethroids) and pesticides (pyrethroids) in agriculture activities is one of the main causes of pollution of surface and grounwater [3].

This work presents an accurate and reproducible method for the determination of the following compounds: oxamyl, aldicarb, carbaryl, pirimicarb, carbofuran, kadethrin, flumethrin, fenpropathrin, fenoxycarb, acrinathrin, tau-fluvalinate, tefluthrin.

The proposed method was performed using large volume injection of sample followed by an on-line solid phase extraction (SPE) procedure and finally the compounds were identificated and quantificated by liquid chromatography with ultraviolet detection, the separation was carried out on C18 reversed phase column based on fused-core particle technology.

This new analytical procedure was satisfactorily applied for the determination of these organic pollutants in surface water samples from Hradec Králové (Czech Republic).

Analytical and statistical parameters such as linear dynamic range detection and quantification limits, inter-day and intra-day repeatability and accuracy were established.

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PS2-07

TRACE ANALYSIS OF ACIDIC EMERGING POLLUNTANTS IN FRESHWATERS BY HPLC-MS/MS

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This study is focused on determining 3 families of acidic emerging pollutants – licit and illicit drugs and personal care products – on freshwaters. Selected contaminants include 13 pharmaceuticals (ibuprofen, naproxen, gemfibrozil, triclosan, thiamphenicol, chloramphenicol, diclofenac, salicylic acid, indomethacin, triclocarban, flufenamic acid, bezafibrate and warfarin), 5 personal care products (methylparaben, ethylparaben, propylparaben and buthylparaben) and two illicit drugs (tetrahidrocannabinol and 11-nor-9-carboxy-Δ9-tetrahidrocannabinol). These substances were determined with an Agilent Technologies HPLC linked with a Triple Quad LC/MS in negative ion mode using for compound separation a Sunfire C_{18} analytical column of 2.1 x 50mm and 3.5 µm particle diameter. The optimal mobile phase used was a gradient of 5mM ammonium formate in water and 5mM ammonium formate in methanol with a flow rate of 0.2mL/min in a gradient that starts with a 30% of methanol and ends with 95 % in 12 min, which is maintained 8 min more.

The analytes were extracted from 250 mL of water by solid-phase extraction using Strata-X cartridges. Analytes were eluted with 6 mL of methanol, evaporated to dryness using a Stuart air evaporator and dissolved in 1 mL of methanol.

This procedure provides acceptable recoveries (>70%) and relative standard deviation (RSDs <20%) at the limits of quantification, which are in the low ppb range ensuring sensitivity enough to determine them in environmental waters.

Turia River has been selected since is a representative river in Mediterranean area heavily affected by drought. This River Turia is a 280-km Mediterranean river with an average flow rate of 10.43 m3/s that has its source in Teruel province (Spain) and discharges near from Valencia city (Spain). Some of these compounds were detected in low concentrations in these waters samples. Sources, routes, ecological effects and environment persistence will be ascertained throughout the study process.

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PS2-08

ANALYSIS OF EMERGING ILLICIT DRUGS IN WASTEWATER BY LIQUID CHROMATOGRAPHY TANDEM MASS

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A total of 24 new psychoactive substances were officially notified for the first time in the EU in 2009 through the EWS (Early Warning System), up from 41 in 2010, 49 in 2011 and 73 in 2012 [1]. Illicit drugs are continuously discharged into wastewaters due to human excretion after consumption, occasional direct disposal or clandestine laboratory wastes into sewage systems [2]. Therefore, the analysis of wastewater has the potential to be used as a tool to detect the use of emerging illicit drug in societies.

The main objective of this study was to optimize and validate an analytical procedure based on solid phase extraction (SPE) and liquid chromatography triple quadruple mass spectrometry (LC-QqQ-MS/MS), for the simultaneous analysis of 9 emerging illicit drugs and to apply the validated procedure to influent wastewater samples collected from the wastewater treatment plants (WWTPs) in Valencia.

The 9 emerging illicit drugs selected were α- pyrrolidinopropiophenone (PVP), α- pyrrolidinopentiophenone (PPP), 4'-methyl- α-pyrrolidinohexanophenone (MPHP), 4'-methyl- α- pyrrolidinobutiophenone (MPBP), belong to pyrrolidinophenone group, Mephedrone (4MMC), Dibutylone (bk-MMBDB), α-Butyrolactone (GBL), 4-Methoxyphencyclidine (4-MeO-PCP) and Bufotenine (BUF).

Illicit drugs were extracted from 250 ml of water by SPE using Strata-X cartridges as sorbent. The extract was evaporated to dryness and reconstituted with water-methanol (9:1). Illicit drugs were determined by LC-QqQ-MS/MS using an electrospray ionization source (ESI) in positive ionization mode. Specific MS parameters as ionization mode, collision energy and fragmentor voltage, were optimized for each compound. The fragmentation of the pseudo-molecular ion of each compound was performed in multiple reaction monitoring (MRM). The method detection limits ranged from 0.01 to 1.54 ng l^{-1} and the recoveries from 70 to 120 %, which are appropriate values to identify their presence in wastewater.

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PS2-09

DETERMINATION OF PESTICIDE RESIDUES AND OTHER ORGANIC POLLUTANTS IN WATER BY UHPLC-QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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Quadrupole time of flight (QqTOF) technology has evolved over years into a stable, sensitive tool that allows multiresidue analysis of a wide range of pollutants with varying physico-chemical parameters in a single run. The advantage of using QqTOF is high selectivity due to good mass accuracy and product ion scan that yield greater confidence in identification [1,2].

In this study, a UHPLC system coupled to a hybrid quadrupole time-of-flight ABSciex Triple TOF™ 5600 has been used as an efficient tool for systematic analysis of a mixture of 43 pesticides. Water samples from different rivers and wastewater treatment plants were extracted by solid-phase extraction (SPE): 200 mL were extracted using Oasis HLB cartridges previously conditioned with methanol/dichloromethane (50:50) and water. Analytes were eluted with 4 mL of the methanol-dichloromethane mixture.

A Poroshell 12 D EC-C18 column 50 × 30 mm internal diameter, 2.7 µm (Agilent) was used to carry out the separation with a flow rate of 0.4 ml/min and an injection volume of 5 µl. The mobile phase employed was a gradient of Milli-Q-water and methanol, both with 10 mM ammonium formate.

The QqTOF was calibrated as recommended by the manufacturer in MS and MS/MS in high sensitivity mode. The MS acquisition was performed in positive ionisation using information-dependent acquisition (IDA) between m/z 100–950.

This method has been a suitable way for routine screening of pesticides and control of other organic contaminants in water. The results shows the presence of pharmaceuticals and drugs of abuse in the samples analyzed, in addition to the 41 selected pesticides. Moreover, good reproducibility and sensitivity was shown across the calibration range (four orders of magnitude) and better sensitivity than expected has been achieved in quantitative applications. The use of databases including accurate mass information for the compounds and fragments represents a useful tool for identification of additional compounds and transformation products in environmental surface water samples.

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PS2-10

ANALYSIS OF VOLATILE POLYFLUORINATED COMPOUNDS IN WATER BY SOLID-PHASE EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Polyfluorinated compounds (PFCs) are man-made chemicals ubiquitously found in the environment and in both humans and wildlife [1]. Owing to their unique physicochemical properties such as chemical stability, photostability, thermostability, and ability to repel both water and oil, they have been widely used for more than 60 years in a great variety of industrial and consumer applications. Among PFCs, several ionic compounds, including perfluorocarboxylates (e.g., perfluorooctanoate, PFOA) and perfluoroalkyl sulfonates (e.g., perfluorooctane sulfonate, PFOS), have drawn a great attention because they were reported to be persistent in the environment and can cause adverse effects on living organisms [2]. The long-range atmospheric transport and subsequent oxidation of the volatile precursors, including fluorotelomer alcohols (FTOHs) and perfluorooctane sulfonamides/ethanols (FOSAs/FOSEs), is the major contributor to the ubiquitous environmental occurrence of ionic PFCs. Nevertheless, volatile PFCs have received a limited attention despite they contribute to the presence of PFOA and PFOS in the environment. FTOHs, FOSAs/FOSEs are released into the environment through volatilisation and can contaminate surface waters either via precipitation in the form of wet deposition from air or via disposal from industrial products and wastewaters [3]. Until now, very few papers have been published regarding their presence in water samples and the methods proposed only cover the analysis of some of these volatile compounds [4,5]. Therefore, there is a great interest to develop sensitive and selective methods for the determination of FTOHs and FOSAs/FOSEs in water samples.

In this work, we have developed a new method for the analysis of neutral volatile PFCs, including FTOHs, FOSAs and FOSEs, in water samples at low concentration levels (ng L^{-1}) using solid-phase extraction (SPE) combined with gas chromatography-mass spectrometry (GC-MS). Among the SPE sorbents tested (ENVI-C18, ENVI-Carb, Silica, and Oasis HLB), Oasis HLB was the stationary phase that provided the highest recoveries (> 85%) for all the compounds. Parameters affecting the retention and elution of the target compounds were optimised to achieve maximum sensitivity and selectivity. In addition, different MS ionization modes (EI, PCI and NICI), were evaluated to the detection of these compounds. Finally, quality parameters of the method developed were established and it was successfully applied to the analysis of FTOHs, FOSAs and FOSEs in water samples collected in the area of Barcelona.

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PS2-11

NEW METHODOLOGY BASED ON GC-MS AND GC×GC-ToF MS FOR THE CHARACTERIZATION OF EXTRACTABLE ORGANIC MATTER IN SOILS

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Quali- and quantitative composition of extractable organic matter in soils is both cause and effect of the biogeochemical functionality of terrestrial ecosystems, and is related to topics of current interest such as carbon sequestration, as well as of economic importance, such as ecosystem productivity [1]. Recently, the study of the relationships between the chemical composition of soils and their physical and agroecological properties by -omic techniques has been proposed. Such an approach is limited by two problems: the low number of samples available after time-consuming protocols and the difficulty of selecting the relevant characterization variables, on the contrary in a very high number, which reduces its practical significance.

In this work, a protocol based in gas chromatography coupled to mass spectrometry (GC-MS) is proposed for obtaining rapid and reliable qualitative and quantitative data on the evolution of the organic matter in leaf, litter and soil samples in forest ecosystems.

Samples (leaves, litter and soils) from *Quercus pyrenaica* (oak), *Juniperus communis (*juniper) and *Pinus sylvestris* (pine) were collected at El Espinar (Segovia) and extracted with methanol and dichloromethane. GC-MS analyses of both extracts were performed using a capillary column coated with methylsilicone as stationary phase. Previous to their analysis, methanolic extracts were submitted to derivatization. Internal standards were used in order to afford quantitative information.

Compounds determined by GC-MS ranged from about 60 for oak to more than 100 for juniper and pine, in concentrations in leaves from 150 □g g^{-1} to 1500 mg g^{-1}. These values can be used for an assessment of intrasystem dispersion, which is not well known. Then, they will allow characterizing samples from the different ecosystems through the tracking of relevant compounds in litter and soils, to unravel possible trends in the humification process and to relate environmental impacts with quantitative changes in organic matter.

Concentration of many of these compounds is very low in litter and soils, and their determination requires of a technique of high sensitivity and resolution. Due to the presence of many coeluting compounds in 1D GC, samples were also analyzed by comprehensive two-dimensional gas chromatography coupled to time-of flight mass spectrometry (GC×GC-ToF MS), using as stationary phases HT-8 for ^{1}D and BPX-50 for ^{2}D.

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PS2-12

MULTIRESIDUE ANALYSIS OF INSECTICIDES AND SELECTED ENVIRONMENTAL CONTAMINANTS IN POULTRY MANURE BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Spreading poultry manure as fertilizer on agricultural fields has been actively promoted by national authorities as an economic way of recycling. However, poultry manure may contain contaminants and other toxic substances, which could be incorporated into crops or be distributed in the environment. Among the different types of contaminants, pyrethroids, widely used in animal husbandry and persistent organic pollutants, an important group of compounds due their high potential of bioaccumulation and resistance to degradation, are expected to be found in poultry manure.

In this work, an analytical method was developed for the simultaneous determination in poultry manure of 41 organic contaminants belonging to different chemical classes: insecticides, polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Poultry manure was extracted with acetonitrile-water and the extract purified by d-SPE with C18 and PSA, applying a modified QuECheERS method. The compounds were determined by isotope dilution gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC-MS-SIM), using labeled compounds as surrogate standards. In the present work, the use of labeled internal standards in fortified blank extracts yielded relative responses similar to those in neat solvent standards, which indicates that the matrix effect was counteracted.

Recoveries from spiked samples were between 84.3 % and 113 % with relative standard deviations ≤ 11 %. The limits of detection ranged from 0.8 to 9.6 ng/g, being deltamethrin and PBDE 183 the compounds with the highest limits. The response obtained with this method was linear over the range assayed, 5 to 100 ng/mL, with correlation coefficients equal or higher than 0.998.

The validated method was used to investigate the levels of these compounds in poultry manure collected from different farms located in Spain. Pyrethroids and PAHs were the main contaminants detected, DDT and its metabolite DDE were also found but at relatively low concentrations, whereas PBDEs and PCBs were not found.

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PS2-13

DETERMINATION OF SELECTED PHARMACEUTICAL COMPOUNDS IN BIOSOLIDS BY GAS CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Human and veterinary pharmaceuticals are chemical compounds widely used to treat or prevent diseases. The determination of pharmaceuticals in the environment has focused on the aquatic compartment since the main route to enter the environment is through the discharge of wastewater effluents into surface water; although, most pharmaceutical drugs are designed to be hydrosoluble and biodegradable many compounds have high log Kow and, therefore, present a high affinity to sludge or soil.

In this work, an analytical method was developed for the simultaneous determination in biosolids of pharmaceuticals belonging to different therapeutic classes such as non-steroidal anti-inflammatory drugs (NSAIDs), lipid regulators and tension regulators. Biosolid samples were extracted with mixtures of water with acetone or acetonitrile at acidic pH with formic acid. The clean-up of extracts was evaluated by using supported liquid extraction (SLE) and dispersive solid-phase extraction. The compounds were determined by gas chromatography–tandem mass spectrometry using matrix-match calibration after silylation to form their dimethyltertbutylsilyl derivatives. This method presents various advantages, such as a fairly simple operation, the use of inexpensive glassware and a low consumption of solvents. In general, good recoveries from spiked samples were obtained for most of the compounds.

The validated method was used to investigate the levels of these compounds in biosolids collected from different wastewater treatment plants (WWTPs) located in Spain. Among the pharmaceutical compounds studied, NSAIDs were the predominant compounds found in the biosolids analyzed.

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PS2-14

PRESSURE-ASSITED ELECTROKINETIC INJECTION FOR ON-LINE ENRICHMENT IN CE IN THE DETERMINATION OF UV-FILTERS IN SLUDGE SAMPLES

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UV-filters are a family of compounds used in personal hygiene products such as toothpaste, soaps and sunscreen cosmetics. They are included in the group of emerging contaminants known as personal care products (PCPs). The analysis of these UV-filters in environmental samples requires a determination at very low concentration levels [1, 2].

Capillary Electrophoresis (CE) has been used to determine UV-filters compounds in PCPs, since they are found in concentration levels that could be measured by CE without any previous preconcentration step. However, in the case of environmental samples a preconcentration step is needed to increase the injected amount and in this sense an improvement of sensitivity is achieved [3, 4].

Among the different sampling enhancement techniques, pressure-assisted electrokinetic injection (PAEKI) is an example that uses principles of both countercurrent electroconcentration and stacking. In PAEKI, the sample consists in ionized analytes with an ionic strength much lower than the BGE solution. During injection, the speed of electroosmotic flow (EOF) is balanced with an external hydrodynamic pressure in order to create a stationary boundary at the inlet end of the capillary where the analytes accumulate according to stacking principles [5, 6].

In this study the benefits of using PAEKI as a preconcentration mechanism in the determination of a group of UV-filters in sludge samples are investigated. For that purpose, different parameters that can affect the total amount of analytes injected as the injection voltage, pressure and injection time have been evaluated. Finally, the optimized method has been applied to measure trace levels of UV-filters in sludge samples from different water treatment plants.

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PS2-15

SORBENT-PACKED NEEDLE MICROEXTRACTION TRAP FOR MUSK FRAGRANCES DETERMINATION IN ENVIRONMENTAL WATER SAMPLES

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A sorbent-packed microextraction trap method was developed to determine a mixture of musk fragrances (polycyclic musk, nitro musk and macrocyclic musk) extensively used in personal care products from wastewater sample prior to analysis by gas chromatography ion trap mass spectrometry (GC-IT-MS).

According with previous bibliography [1, 2] the needle trap (NT) device was performed by using 22g needles with side holes and was filled with a C18 (120 µm) sorbent [3, 4]. Different parameters affecting the adsorption capacity of the sorbent, such as sampling time and temperature, headspace/sample volume, salting-out and stirring rate were optimized in both 10 µL and 100 µL syringes. Furthermore desorption and transferring of the target compounds into the GC column were studied.

Validation parameters like method detection limits, method quantification limits, linear range, intra-day repeatability and inter-day repeatability were evaluated under optimal conditions. Then, the applicability of the method was tested with wastewater samples from three urban wastewater treatment plants (WWTPs) located in the area of Tarragona with population around 140,000 inhabitants. The WWTPs receive urban sewages and some industrial discharges and use a secondary treatment based on activated sludge biological treatment. Moreover, at one of the WWTPs, and additional sample was taken from the effluent of the tertiary treatment based on reverse osmosis.

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PS2-16

INCIDENCE AND DISTRIBUTION OF ABUSE DRUGS AND HEAVY METALS IN WATERS OF A MEDITERRANEAN MARSHLAND OF THE TURIA RIVER CATCHMENT

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Anomalous levels of heavy metals in the environmental media have been considered during decades a result of human activities, mainly, of the industrial development. Recently, a set of organic compounds of very different chemical families has been assumed as strict indicator of the human fingerprint, they are called “emerging contaminants”. Among them, illicit or abuse drugs are of special concern nowadays.

In this work, the levels and spatial distribution of 14 illicit drugs belonging to different families (opiates, cannabinoids, cocainics and amphetaminics) and 7 heavy metals have been studied, with the possible interactions among them and the main water quality parameters. The study has been carried out in surface waters of La Albufera Natural Park (Valencia, Spain). Samples were taken in 16 zones covering the marshland area, from irrigation channels and springs, and under different land uses. The possible influence of these last and their spatial distribution were also studied.

Illicit drugs were extracted using solid phase extraction (SPE), and determined by liquid chromatography–triple quadrupole-tandem mass spectrometry (LC-QqQ MS/MS). The method proved to be sensitive enough to surface waters and wastewaters, providing high recoveries for all the analytes (71–104%) and achieving low LODs and LOQs (0.01–1.54 ng/L and 0.03–5.13 ng/L, respectively). Seven heavy metals (Cd, Co, Cr, Cu, Ni, Pb and Zn) were also determined in the samples by atomic absorption spectrometry (AAS), with detectors of flame (AAS-F) and graphite furnace (AAS-GF).

All samples were contaminated by, at least, one illicit drug, ranging from levels <LOQ to 78.71 ng/L. Benzoylecgonine was found in all zones and cocaine in 12 of them. Regarding heavy metals, Zn were not detected in the water samples, being Ni is the one that presents higher values, even surpassing those established by legislation. This metal showed significant interactions with codeine.

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PS2-17

STUDY OF THE PRESENCE OF EMERGING POLLUTANTS IN COASTAL WATERS OF GRAN CANARIA ISLAND (SPAIN) BY USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Continuous exposure to hazardous chemicals has led to many environmental problems that directly or indirectly affect the water cycle [1]. Some of these compounds are being controlled in different regulations, but many others have not yet been included in laws, they are considered emerging contaminants. Control of these pollutants is particularly difficult and they are very widespread in rivers, lakes and sea. Because they are dispersed through wastewater, whose customary purification systems are not designed to remove it, so that their levels in the medium can easily achieve high values [2, 3].

The principal aim of this study is to evaluate the presence of a specific group of emerging pollutants from urban areas and productive activities in the coastal waters of the Gran Canaria Island (Spain). A solid-phase extraction (SPE) coupled to liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been developed for determination the presence of three antifouling (diuron, irgarol and tributyltin) in seawater samples from three different ports, and seven pharmaceutical compounds (atenolol, acetaminophen, norfloxacin, ciprofloxacin, carbamazepine, ketoprofen and diclofenac) in seawater samples near four outfalls from urban wastewater in Gran Canaria Island (Spain), for one year. During the monitoring time, in most port samples two antifoulings (diuron and irgarol) were found in a concentration range of 24.5-138.2 $ng \cdot L^{-1}$. Moreover, all pharmaceuticals compounds under study were found in variable concentrations ranging from 4.4 to 3,551.7 $ng \cdot L^{-1}$ except atenolol and carbamazepine wich were not detected in any of the collected samples. The fluoroquinolones (norfloxacin and ciprofloxacin) compounds were detected more often and at the highest concentrations in the analysed samples.

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PS2-18

ASSESSMENT OF HORMONAL COMPOUNDS BY SPE-UHPLC-MS/MS PROCEDURE IN SAMPLES OF WASTEWATER TREATMENT PLANTS OF GRAN CANARIA (SPAIN)

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Determination and control of emerging pollutants have been important topics in environmental analytical chemistry in the last decades. One important group of these contaminants is the hormonal residues, which are considered Endocrine Disrupting Compounds (EDCs). These compounds are defined as chemical substances capable of altering the natural hormonal equilibrium producing harmful effects in animals, humans and their progeny [1]. The consumption of hormones has been increased exponentially in the last decades due to the use of hormones in human and veterinary medicine. Since 1990s some studies established that the main pathway of these compounds to go into the environment is through effluent of wastewater treatment plants (WWTPs). Because of that, many studies in the last years determine the concentrations of sexual hormones in water and wastewater samples [2, 3].

In this study, the determination of a group of several natural and synthetic hormones (estrogens, androgens, progestogens and corticosteroids), is presented. The extraction and preconcentration method chosen has been Solid Phase Extraction (SPE) which has been combined with ultra-high performance liquid chromatography coupled to mass spectrometry detection (UHPLC-MS/MS).

The hormonal compounds have been studied in wastewater samples from wastewater treatment plants around Gran Canaria island (Spain), with different water treatment methods (conventional and modern methods), to evaluate the presence of this type of compounds in different settlements of the island.

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PS2-19

OCCURRENCE OF BENZOTRIAZOLE UV STABILIZERS IN LIQUID AND SOLID ENVIRONMENTAL SAMPLES FROM GRAN CANARIA ISLAND USING ON-LINE SPE-UHPLC-MS/MS

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Benzotriazole ultra-violet stabilizers (BUVSs) are emerging compounds that reflect and absorb the solar radiation [1]. They are added in a wide variety of personal care products, due to the growing concern about the link between sunlight exposure and skin cancer.

After be used, these compounds can reach the environment through recreational activities such as swimming and bathing in oceans, lakes or rivers (direct inputs) [2] or after passing throughout wastewater treatment plants without be removed. Then, it has been demonstrated that their derivatives can present negative effects over aquatic systems. For example they are mutagenic in bacterial systems and toxic in plants [3], and can exert adverse effects on the fecundity and reproduction of fish [4].

We evaluated the presence of seven BUVSs in liquid and solid environmental samples using on-line solid phase extraction (On-line SPE) and microwave-assisted extraction followed by On-line SPE (MAE-On-line SPE), respectively, both coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS).

The limits of detection achieved for liquid samples were in the range of 0.73-4.18 $ng \cdot L^{-1}$ [5], while they were from 53.3 to 146 $ng \cdot kg^{-1}$ for solid samples [6]. The repeatability was between 8.1 and 13% for liquid samples [5] and between 8.2 and 17% for solid samples [6].

The studied BUVSs were detected in samples from wastewater treatment plants and seawater samples and also in marine sediments and sewage sludges from Gran Canaria Island (Spain).

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PS2-20

SURVEY OF BPA, NP AND OP IN DRINKING WATER SUPPLY RESERVOIRS BY SPE-AND UPLC-MS-MS

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Evidence on endocrine disruptive properties of alkylphenols (AP) and bisphenol A (BPA) has caused much concern about their use as additives of plastics and epoxy resins in contact with products intended for human consumption. Research has shown that these compounds can be transferred into the foods and beverages packaged with these materials, such as water bottles or precooked meals. These migrations are thought to be a significant source of human exposure [1]. The same concern applies to the materials used in the construction of drinking water supply systems. It is known that some materials such as internal lining used in pipes or storage tanks contain these compounds, and a few studies have reported migration to the water [2]. BPA may react with residual chlorine usually added as disinfectant, which would prevent its presence in the tap water. However, little is known about the pathways it follows, and some authors suggest that the by-products could also show estrogenic activity [3].

The aim of this study was to assess the presence of BPA, nonylphenol (NP) and octylphenol (OP) in the reservoirs of Barcelona's drinking water supply system and –if that were the case– its possible origin in migration from epoxy paints used in the internal coatings of the tanks and the subsequent significance in the delivered tap water. Chlorinated by-products of BPA were also monitored.

For this purpose, solid-phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) were applied. Recoveries of the target compounds (60-95%) were calculated after pre-concentration of the samples in styrene-divinilbenzene (SDB) cartridges and elution with ethyl acetate and methylene chloride. Chromatography was carried out with a gradient flow of water and methanol in a C18 column and electrospray ionization under the negative-ion mode was used for detection. Under these conditions limits of quantification between 80 and 120 ng/L were achieved.

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PS2-21

DETERMINATION OF PERSONAL CARE PRODUCTS IN ENVIRONMENTAL WATERS USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND A LIQUIDPHASE MICROEXTRACTION METHOD

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Personal care products (PCPs) are a wide group of compounds utilized in different cosmetic products. Recently, they have been categorized as emerging contaminants due to their probable toxicity. PCPs are normally classified in six groups, such as UV filters (like benzophenones), preservatives (like parabens), disinfectants (like triclosan), musk, insect repellents, and siloxanes.

Once PCPs are applied onto the human body, they can directly or indirectly (by means of wastewater treatment plants) reach the environment. Therefore, humans are exposed to higher amounts of PCPs than those coming from personal direct use.

Given their low levels in aqueous samples, extraction and preconcentration methods are normally utilized for their determination. With the purpose of utilizing a green extraction method, in terms of minimization of solvent consumption and therefore minimization of wastes, liquid-phase microextraction has been selected as the analytical tool to accomplish extraction and preconcentration of PCPs present in waters.

This work presents the determination of 10 PCPs, including 7 parabens, 2 bezophenones, and triclosan, in waters using dispersive liquid-liquid microextraction (DLLME) and vortex-assisted emulsification microextraction (VAEME). These techniques are compared, in all cases using tetrachloroethylene as extraction solvent. The extraction method is used in combination with ultra-high performance liquid chromatography and UV-Vis detection.

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PS2-22

IDENTIFICATION OF HALOGENATED BY-PRODUCTS AFTER UV-IRRADIATION OF PARABENS AND BENZOATES IN WATER USING SPME-GC/MS

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Parabens (esters of 4-hydroxybenzoic acid), and benzoates are commomly employed as antimicrobial preservatives in a wide range of cosmetics and personal care products (PCPs). Although no harmful effect on consumer's health has been attributed to benzoates use in cosmetics [1], parabens have been reported to have estrogenic- and antiandrogenic-like properties. A potential relationship between breast cancer and application of paraben-containing products on skin is speculated [2]. These preservatives are continuously released in bath water and recreational waters, reaching relatively high levels in urban wastewater. The reaction of parabens with free chlorine in chlorinated tape water has been well investigated [3], yielding halogenated derivatives that have been detected in aquatic environments. This fact should be taken into account, bearing in mind that the resulting chlorinated by-products showed acute toxicity responses in the *Daphnia magna* test [4]. In this work halogenated by-products after UV-irradiation of parabens and benzoates in water were identified during the performance of photodegradation studies of the aforementioned preservatives. Aliquots of aqueous solution in quartz cuvettes were irradiated using a selectable power UV-photorreactor for the required time. SPME was selected as the extraction procedure and different SPME fiber-coatings were tested. Gas chromatography coupled with mass spectrometry (GC-MS) has been employed to monitor the degradation kinetics of the investigated preservatives. Similar photodegradation kinetics was observed within each family of preservatives. Using UV light (254 nm, 18 W), parabens exhibited first order kinetics; whereas benzoates showed a much slower degradation. Several chlorinated/brominated photoproducts were detected and some of them were tentatively identified by means of their mass spectra and using the information found in the literature. Considering that under intensive solar irradiation certain degree of photochemical degradation may occur in surface water, the results obtained in this study, to some extent, might be extrapolated to recreational waters, very often crowded with PCPs-smeared bathers, where significantly high levels of chlorine are present.

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PS2-23

PHOTODEGRADATION STUDY OF COSMETIC PRESERVATIVES USING SPME-GC/MS: FIRST APPROACH IN COSMETIC MATRICES

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The addition of preservatives is absolutely essential to ensure that cosmetics are safe to use for a long time. The widespread use of preservatives in cosmetic formulations has risen scientific and social concern, as some of these compounds have been shown to have negative side effects on consumers health. However, apart from this primary harmful effect, in the case of direct exposure to the radiation coming from the sun, the photodegradation of the aforementioned preservatives may occur. The degradation induced by UV solar radiation, not only may cause the inactivation of the cosmetic ingredient, but also may produce potentially hazardous photoproducts, even directly on the skin. Although the photochemical behavior in water under different oxidative conditions has been reported for some preservatives, photodegradation studies in cosmetics are almost non-existent. Therefore, the research about the photodegradation kinetics of cosmetic preservatives, as well as the identification of their by-products chemical structures is needed. Besides, in the case of cosmetic products, the interaction between the photodegradation by-products and other formulation components, may led to the formation of new molecules with unknown toxicological properties. The photochemical behaviour of several cosmetic preservatives, both in ultrapure water and 'on-fiber' Photo-SPME (Photo-Solid-Phase Microextraction) [1] was carried out. Preservatives such as parabens, benzoates, iodopropynyl butylcarbamate (IPBC), 2-*tert*-Butyl-4- methoxyphenol (BHA) and 2,6-bis(1,1-dimethylethyl)- 4-methylphenol (BHT) has been investigated. The photodegradation of these compounds in aqueous-based cosmetics was also carried out and compared with the photodegradation in ultrapure water and in Photo-SPME. A selectable power UV-photorreactor was used in order to perform the trials for the required time. Gas chromatography coupled with mass spectrometry (GC-MS) has been employed to monitor the degradation kinetics of the investigated preservatives. Different photodegradation kinetics were observed depending on the type of preservative. The photodegradation in ultrapure water and in Photo-SPME was faster than in the cosmetic matrix. Several photoproducts were detected and some of them were tentatively identified by means of their mass spectra and using the information found in the literature. Finally, photodegradation pathways were tentatively proposed for some of the preservatives under study.

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PS2-24

RECYCLED TIRE RUBBER SURFACES. CASE STUDY: RESTAURANT PLAYGROUND IN AN INDOOR SHOPPING CENTER

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Disposal of used tires has been a major problem in soil waste management. The indestructible nature of discarded tires makes them persist in the environment indefinitely, creating long-lasting piles of toxic, synthetic waste. Today, one of the most valuable applications of used tires is the transformation in recycling products such as rubber mulch and recycled rubber pavers that are used for sidewalks, animal flooring, fitness centre flooring, playground surface, sport fields, etc… Nevertheless, rubber tire debris contains toxic chemicals such as heavy metals, highly aromatic oils and other reactive additives. The present of hazardous organic chemicals, including high PAH levels, in recycled tire outdoors playground surfaces have been recently demonstrated [1].

Our approach aims at strengthening the study of this kind of playground surfaces, the organic hazardous substances content and the possible migration of the compounds from the material though the air and the water. In this case, we chose indoor playground samples from a restaurant of a shopping center. Direct material analyses using solvent extraction and HS-SPME analysis of the vapour phase above the sample were carried out.

The analytes were extracted from the sample to the organic solvent using ultrasonic energy followed by GC-MS analysis. Fourteen up to sixteen target polycyclic aromatic hydrocarbons (PAHs) were identified in the extract. The analysis also confirmed the presence of a high number of harmful compounds including phthalates and adipates, antioxidants, benzothiazole and derivatives among other chemicals. The samples were also analyzed by HS-SPME exposing the fiber (PDMS and PDMS-DVB) to the headspace over the sample for 30 min. Regarding PAHs, most compounds found in the playground solvent extract were also identified in the vapor phase, excluding the less volatile ones. Other hazardous organic chemicals (phthalates, antioxidants, phenols, vulcanisation additives, preservatives) were confirmed as well. In addition, some additional studies demonstrate the partial transfer of contaminants from the playground tire surface through the run off water put in contact with the sample.

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PS2-25

ANALYSIS OF DECHLORANE PLUS AND RELATED COMPOUNDS (DECHLORANE 602, 603 AND 604) IN VEGETABLE AND MARINE OILS

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Dechlorane Plus® (DP, $C_{18}H_{12}Cl_{12}$) is a chemical product used as flame retardant in substitution of others such as polybrominated diphenylethers, because of its properties (chemical and photochemical stability and lower cost) [1]. DP was first detected in the environment in 2006 [2]. Since then, concern about it has grown quickly and several studies have been carried out that show the capacity of accumulation and transport of this compound [3-5]. Due to its lipophilia, DP can be accumulated in lipid material. Therefore, in this work, we have developed a method for the analysis of DP (syn and anty isomers) and other related compounds, such as Dechlorane 602, Dechlorane 603 and Dechlorane 603, in vegetable and marine oils.

The methodology consists of the following steps: (1) solution of the sample in hexane, (2) clean-up, (3) concentration and (4) instrumental analysis by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) (5) quantification by the isotopic dilution method.

Clean-up: Several open chromatographic columns have been tested to clean up the sample: (1) sulfuric silica column (2) multilayer silica column (sulfuric silica, sodium hydroxide silica and silver nitrate silica) (3) pre-packed alumina SPE cartridges and (4) Florisil (activated at 500ºC) column.

Instrumental analysis: The instrumental analysis has been carried out by GC-HRMS, operating at 10,000 resolving power. The separation has been performed in 5% phenyl 95% dimethylpolysiloxane columns. Different lengths have been tested: 15 m and 60 m. Both columns allow the separation of different Dechlorane. However, sensitivity is lower in 60 m column and Dechlorane 604, the only brominated one, shows a significant decrease of signal. Different injection temperatures have been tested: 260ºC, 280ºC and 300ºC. The best results were achieved at 280ºC. Mass spectra of each compound were studied to select masses for SIR mode. The possibility to acquire the signals in three monitoring windows or in a single one was also considered, especially regarding the signal of Dechlorane 604.

Several samples of vegetable and marine oils were analyzed following the developed method. Some Dechlorane compounds were detected in several samples.

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PS2-26

STUDY OF THE ORGANIC SEMI-VOLATILE FRACTION OF PRIMARY AND SECONDARY SUBMICRON AEROSOLS IN MADRID AND BARCELONA

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Atmospheric aerosol particles originate from a wide variety of natural and anthropogenic sources. Primary particles are directly emitted as liquids or solids into the atmosphere. Secondary particles are formed by oxidation of biogenic emissions or by the formation of their precursors already present in the aerosol phase (1). Fine (<1 µm) and ultrafine particles (<0.1 µm) mainly stem from traffic emissions and are suspected to be hazardous to human health. A comprehensive study on the composition of the submicron organic matter (PM1) in urban aerosols has been conducted and some preliminary results are presented here. A Digitel High-Volume Autosampler DHA-80 was used to obtain PM1 aerosol samples in the cities of Madrid and Barcelona (Spain). 150 mm ∅ quartz microfiber filters QM-A (Whatmann) and QF-20 (Schleischer & Schuell) previously conditioned at 400ºC (8 h) were employed for sampling and kept frozen at -20ºC until their analytical treatment. The procedure consisted in a Soxhlet extraction for 6 hours in 100 ml DCM:MeOH (2:1 v/v). The extracts were filtered through a membrane and then concentrated to 1 mL. The screening analysis was carried out in an Agilent 6520 Accurate Mass Q-TOF coupled to an Agilent 1200 Series Liquid Chromatograph. The extracts were analyzed in a flow of 100 µL of isocratic gradient with MeOH/H_2O (50/50: v/v) as a mobile phase in ESI. The temperature of the source gas (N_2) was set at 325ºC, the pressure and flow of the nebulizer gas were 5 psi and 11 L/min, respectively. The voltages of fragmentation and skimmer were set at 90 and 65 volts. The acquisition was done in a wide mass range (25-1,700 m/z) in order to obtain a broad screening of the aerosol samples.

A preliminary fingerprint of the main organic components of primary and secondary aerosol has been obtained. Two main sources can be observed: anthropogenic and biogenic (2,3). *n*-alkanals, alkanols, alkanoic and alkenoic acids come mainly from heaters and/or petroleum based sources like gasoline or diesel vehicles exhaust. Resin acids and triterpenoids likely from biogenic sources were also identified.

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PS2-27

COMPREHENSIVE STUDY OF POLYPHENOLS IN CORK BOLING WATER

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Cork is the outer surface of the cork oak tree and it is a versatile material that can be used for a variety of products, mainly stoppers. One of the first stages of industrial preparation of cork consists of its immersion for approximately one hour in boiling water. This process improves cork textural and plastic properties. Waste streams are very complex mixtures with high COD (3,7-7,2 g / L), BOD (0,5-0,9 g/L), total solids (2,5-6,4 mg/L) and particularly high loads of phenolic compounds (0,2-0,9 g / L. Recovery of these polyphenols (1) can reduce pollution of these spills and, besides, it is a good opportunity to obtain useful by-products.

Previously studies carried out by ICMC in cork wastewater from several factories shown four low molecular weight polyphenols detected and quantified (gallic, ellagic, vanillic and protocatechiuc acids). This work characterized samples of cork boiling water from other factories, with an improved methodology with HPLC-DAD and confirming with HPLC-QTOF.

Boiling cycles	**Factory 1**				**Factory 2**			
Days	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total polyphenols mg/L (gallic acid)	320,3	672,46	869,91	883,53	577,69	810,70	891,65	953,77
Gallic acid mg/L	--	13,58	21,59	21,73	16,2	19,8	22,63	25,76
Ellagic acid mg/L	4,77	8,77	11,78	10,66	6,93	5,28	5,67	7,69
Vanillic acid mg/L	1,08	3,74	4,87	5,20	4,65	4,87	5,08	5,59
Protocatechiuc acid mg/L	--	9,33	14,31	14,1	4,59	14,38	16,34	19,26
Syringic acid mg/L	--	2,43	3,42	3,72	3,23	4,09	4,42	5,05
Vainillin mg/L	--	1,52	2,56	1,68	--	2,70	3,38	3,94
Ferulic acid mg/L	--	1,10	1,38	1,08	1,05	0,95	1,04	1,24
Sinapaldehyde mg/L	--	--	--	--	1,06	1,31	1,36	1,55
Syringaldehyde mg/L	--	1,13	1,56	1,64	1,27	1,65	1,67	1,94
Coniferaldehyde mg/L	--	--	--	--	0,60	1,08	1,35	1,56

Six new polyphenols have been recovered and quantified and their recovery can generate added-value to cork factories due to their potential interest for several industrial sectors.

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PS2-28

STUDY OF THE IN SITU ACETYLATION CONDITIONS TO DETERMINE PHENOLIC COMPOUNDS BY PDMS ROD-HPLC-DAD IN WATER SAMPLES

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Endocrine disrupting compounds (EDC) have received significant attention in the last ten years, with respect to their fate and removal in water samples. Compounds identified as EDC are members of different groups of chemicals, including drugs, pesticides, industrial-by-products, alkylphenols, and so on. Endocrine-disrupting phenolic family includes bisphenol A (BPA), trichlorophenol (TCP), pentachlorophenol (PCP), 4-nonylphenol (NP) and 4-octylphenol (OP), among others. Because of the incomplete removal of these EDCs from wastewater treatment plants they reach the aquatic media and are found in environmental samples. The potencial effects of EDCs on human health and wildlife make it necessary to develop simple, rapid and efficient methods for monitoring these compounds.

The polarity of these compounds and their low concentration in environmental samples makes it difficult their extraction from water samples. The most used techniques are solid phase extraction (SPE) and solid phase microextraction (SPME). Stir-bar sorptive extraction (SBSE) devices have been used as an effective alternative [1]; however it represents the use of an expensive process. The application of PDMS rods permits the extraction and preconcentration of the compounds in a single step, avoiding the carryover problems related with SBSE and reducing costs [2]. Moreover, in the case of high polar compounds, the introduction of an in situ derivatisation step could help to increase the extraction rate and therefore the sensitivity of the method. Silylation and acetylation are the most used derivatisation reactions, allowing the determination of polar compounds.

In this study, an in situ acetylation of the analytes, as it can be carried out in aqueous media, was selected to increase the extractability of the compounds by PDMS rod. Parameters affecting the extraction and desorption steps, such as sample volume, extraction and desorption times, desorption solvent and desorption volume were studied, as well as the parameters affecting the derivatisation reaction (amounts of acetic anhydride and potassium carbonate). The effect of the addition of modifiers such as NaCl and MeOH to the aqueous phase was also studied. The determination was carried out by liquid chromatography with diode array detection (LC-DAD), using a reverse phase column, with acetonitrile and MilliQ water with acetic acid as mobile phases in gradient mode. The validation of the method was done by analysing spiked water samples at different concentration levels. Finally, the method was applied to the analysis of water samples.

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PS2-29

CHROMATOGRAPHIC SIGNATURE OF SOIL LIPID ASSEMBLAGES RESPONSIVE FOR AGRICULTURAL DISTURBANCE (CANARY ISLANDS, SPAIN)

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Identification of descriptors sensitive to the effect of land use is not a trivial matter in the case of soils subjected to recent anthropogenic perturbations. In biogeochemical terms, the quantitative assessment of soil diversity could be approached by determining the extent to which soil molecular assemblages reflect the changing structure of the trophic system. In this sense, the *soil free lipid fraction* is expected to inform on the most recent changes in the structure of the ecosystems [1]. In fact, soil lipids differ in the distribution patterns of their alkyl series as well as in the characteristics pattern of diagnostic biomarker compounds suitable for monitoring the impact of agricultural use. In addition, changes in soil diversity following transformation of secondary forests into cultivated plots were assessed by analyzing the chromatographic signature of soil lipids in a total of 30 soil samples collected in Tenerife (Canary Islands; Spain).

The lipids were extracted from soil samples of *ca.* 5 g using a mixture of dicloromethane-methanol 3:1. For gas chromatographic analyses, the samples were methylated with trimethylsilyldiazomethane [2] The lipid compounds were separated and identified by GC/MS using an HP 5890 chromatograph connected to an HP 5971 mass detector (EI, 70 eV) and equipped with an 25-m 0.22 mm i.d, cross-linked OV-1 column. Helium flow was adjusted to 1 cm^3 min^{-1}; the oven temperature was programmed from 70 to 220 ºC at 4 °C min^{-1} during the chromatographic run. The identity of the compounds was assessed by their EI mass spectra and using digital spectral databases.

The main results showed as the chromatographic signature of free soil lipid quantitatively reflected the impact level of agricultural practices. After clearing original forests, an enhanced even-to-odd C-number preference of alkanes was observed. Concerning the chain length, both in the case of alkanes and fatty acids, some significant trend to short-chain length were observed in cleared and cultivated sites. The lipid fractions displayed a strong decrease in the total amount of cyclic constituents (mainly terpenoids), which suggested a loss in biododiversity associated with the monoculture. In pine soils there was a conspicuous series of typical diterpene resin acids whereas, in cultivated sites, series of steroids and root-derived friedelan-type triterpenoids were found as diagnostic compounds.

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PS2-30

CHARACTERIZATION OF ETHYLENE OXIDE BASED SURFACTANTS AND BLOCK COPOLYMERS BY SINGLE-PUMPED BI-DIMENSIONAL LIQUID CHROMATOGRAPHY

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Using heart-cut bidimensional liquid chromatography (2D-HPLC), highly selective separations of complex mixtures are possible. In a previous work, a 6-port 2-position injection valve (V1) and a 6-way column selection valve (V2) were combined to implement 2D-HPLC in a system driven by a single pump. This setup had the capability to automatically introduce selected segments of the eluate of a first column into another column [1]. In this work, a simpler and more flexible configuration, based on the same devices, is presented.

The 6-port 2-position valve (V1) was used to switch between two alternative paths: the column, to perform the 1st dimension elution, and the by-pass, which directs the flow through a column selection valve (V2). Up to five 2nd dimension columns were inserted in 5 channels of V2. The sixth channel of V2 was used to by-pass the 2nd dimension. With V2 in bypass it was possible to optimize the 1st dimension separations. Using any one of the other 5 channels of V2, selected segments of the eluate of the 1st dimension column were introduced into a 2nd dimension column. With both V1 and V2 in bypass, the system was flushed with the new mobile phase required to start gradient elution on the 2nd dimension column. Finally, with V1 in bypass, gradient elution along the 2nd dimension was carried out. Independent temperature control of the two dimensions was also implemented.

Fatty alcohol ethoxylates (FAE) are non-ionic surfactants industrially obtained as complex mixtures of oligomers. The 2D-HPLC system here described, allowed the separation of the derivatized oligomers of FAE using RP-HPLC in both dimensions (C8 columns). Orthogonality was also achieved using complementary mobile phases Thus, separation according to the hydrocarbon chain was achieved in the 1st dimension using MeOH/water gradients, while separation according to the number of ethylene oxide (EO) chains was accomplished along the 2nd dimension using ACN/water gradients. The eluent strength at the beginning of the 2nd dimension separations was adjusted to reduce the total analysis time to a minimum. The system was also applied to the separation of block copolymers also having EO chains.

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PS2-31

IMPROVED SAMPLE PRETREATMENT FOR THE ANALYSIS OF LOW MOLECULAR WEIGHT PEPTIDES IN HUMAN PLASMA BY CAPILLARY ELECTROPHORESIS MASS SPECTROMETRY AND SOLID-PHASE EXTRACTION COUPLED ONLINE TO CAPILLARY ELECTROPHORESIS MASS SPECTROMETRY

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The detection and identification of low molecular weight (LMW) peptides in human plasma continues generating a great interest in many fields of proteomic research, such as biomarker discovery [1]. However, the complexity of human plasma, especially with regard to the wide dynamic range of protein concentrations, is an inconvenient for direct analysis of LMW peptides using liquid chromatography mass spectrometry (LC-MS), capillary electrophoresis mass spectrometry (CE-MS) or many other separation techniques [2].

There are different strategies to remove salts and major high molecular weight (HMW) proteins from plasma before the analysis of the LMW peptide fraction [3]. Solvent precipitation and centrifugal filtration are widely used because of the good efficiency, simplicity, high throughput and low cost. Furthermore, they are often combined together because the solubility and size-exclusion mechanisms that govern both methods can be regarded as complementary [4].

In this paper, we evaluate acetonitrile precipitation followed by a range of centrifugal filtration conditions for the analysis of LMW peptides in human plasma before using capillary electrophoresis mass spectrometry (CE-MS) and solid-phase extraction coupled online to capillary electrophoresis mass spectrometry (SPE-CE-MS). The first step of protein precipitation was useful before CE-MS, but not before C18-SPE-CE-MS that required the subsequent centrifugal filtration step for an improved sample matrix clean-up.

Three opioid peptides were used as model compounds i.e. dynorphyn A 1-7 (Dyn A), endomorphin 1 (End 1) and methionine encephalin (Met). 3, 10 and 30 kDa molecular weight cut-off (MWCO) cellulose acetate filters (Amicon Ultra-0.5) and 10 kDa MWCO polyethersulfone filters (Vivaspin 500) were studied. Recoveries and reproducibility were only optimum after passivating the 10 kDa MWCO cellulose acetate filters with polyethylene glycol to avoid peptide adsorption on the inner walls of the sample plastic reservoir.

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PS2-32

ANALYSIS OF GLYCOPEPTIDE GLYCOFORMS OF HUMAN TRANSFERRIN BY CAPILLARY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY. APPLICATION TO CONGENITAL DISORDERS OF GLYCOSYLATION AND TRANSFERRIN POLYMORPHISM DIAGNOSTIC

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Congenital disorders of glycosylation (CDG), also known as carbohydrate-deficient glycoprotein, are a steadily increasing group of genetic defects caused by mutations in the genes coding for enzymes involved in the biosynthesis or transport of the oligosaccharide moieties of glycoconjugates [1]. The clinical characteristics differ depending on the CDG type but they often consist in psychomotor, growth and mental retardation from early childhood. CDGs are the result of defects in the assembly and/or transfer of the glycan moieties onto the nascent glycoprotein (CDG-I) or the processing of the glycan moieties (CDG-II). Therefore, the carbohydrates chains are missing (CDG-I) or structural incomplete (CDG-II) [2, 3]. Transferrin (Tf) is an iron-binding serum transport glycoprotein whose N-glycan composition is altered according to the CDG type. Hence, Tf is usually considered a reliable biomarker for medical diagnosis of CDG. Analysis of intact Tf by isoelectric focusing (IEF) or anion exchange liquid chromatography (LC) with UV detection is commonly used as routine method for the detection of CDGs [3]. However, the use of these techniques does not allow the reliable characterization of Tf glycoforms, which is important to differentiate between different CDG subtypes. Additionally, over 30 genetic Tf variants, differing in the amino acid backbone structure of the protein, have been described, from which transferrin C is the most predominant form in all races [4]. Heterozygous combinations of Tf variants, being the most common the BC and CD variants, may lead to misinterpretation of the results owing to the co-focusing or co-elution of the shifted Tf isoforms.

In this study, capillary liquid chromatography coupled to time-of-flight mass spectrometry (µLC-TOF-MS) was used to analyze Tf glycopeptide glycoforms. After immunoaffinity purification, Tf was digested with trypsin and µLC-TOF-MS permitted to detect the N_{413} and N_{611} glycopeptide glycoforms. The proposed methodology was applied to serum samples of individuals with CDG type I, CDG type II and Tf BC and CD variants. The use of this methodology for the characterization of glycoforms from both N-glycopeptides allowed the confirmation of each type of CDG and was proved useful for the diagnostic of Tf alterations in patients with heterozygous Tf variants without leading to misinterpreted conclusions.

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PS2-33

POLYAMINE METABOLITE PROFILING OF HUMAN COLORECTAL CANCER CELLS BY UHPLC-MS

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Polyamines are organic cations derived from amino acids that are involved in diverse functions related to cell growth and differentiation. It has been observed that polyamine biosynthesis is up-regulated in actively growing cells, including cancer cells [1]. As polyamines are apparently essential for tumor growth, for a deeper understanding of this pathogenic process, new robust analytical methodologies are of critical importance for their analysis [2]. Hyphenation of high resolution separation techniques and MS offers the best combination of sensitivity and selectivity in metabolite profiling approaches. Thus, in this work, a UHPLC-MS-based method has been optimized for the analysis of polyamine-related compounds. Since highly polar metabolites, such as polyamines, are not retained by conventional reversed-phase LC (RP/LC) columns, hydrophilic interaction liquid chromatography (HILIC) was studied in this work as complementary analytical mode to the more common RP/LC. Optimization of analytical parameters included mobile phases composition, flow and elution gradient. The HILIC/UHPLC-MS method optimized in this work provided high peak resolution and reproducibility of the polyamines and related compounds in a short analysis time (10 min). After extensive metabolite extraction optimization procedure, the HILIC/UHPLC-MS method was applied to the analysis of intracellular polyamines and related compounds in the cell line HT-29 (derived from human colon adenocarcinoma).

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PS2-34

A NON-TARGETED METABOLOMICS APPROACH TO STUDY *Crepis vesicaria* L. EDIBLE PLANT

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Crepis vesicaria L. is an edible wild plant used as food-medicine on the basis of the popular Italian tradition. It presents dark green rosettes of bitter taste with toothed leaves that characteristically fold back on itself when picked. Their basal leaves are eaten raw in salads or stewed, both for pleasure and for health reasons [1,2]. As this plant continues to be consumed as traditional food in many Italian regions, a better understanding of its therapeutic benefits/risks is needed. For this purpose, metabolic content of *Crepis vesicaria* L. plants from Bologna region (Italy) was studied through a MS-based non-targeted metabolomic approach in this work. An UPLC-ESI-TOF MS method was optimized using a reversed phase C8-bonded silica column, operating in negative ion mode. Several extraction procedures were compared in terms of metabolite peak number and intensity. The most effective extraction solvent was observed to be methanol-water (75:25, v/v) with 0.1% formic acid. More than 800 different metabolite species could be detected in these conditions in less than 18 min. Accurate m/z value and retention time for each metabolite peak was annotated, and tentative identification, based on the obtained theoretical molecular formula and several metabolites databases, was carried out.

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PS2-35

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATERS AND FRUIT-TEA INFUSIONS USING AN *IN SITU* IONIC LIQUID DISPERSIVE LIQUID-LIQUID MICROEXTRACTION METHOD

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Polycyclic aromatic hydrocarbons (PAHs) are well-known ubiquitous environmental contaminants, which present mutagenic and carcinogenic effects. PAHs can also be present in foods by environmental contamination issues of crops or as a consequence of certain foods industrial processing methods (such as smoking, heating or drying). The United States Environmental Protection Agency (US-EPA) regulates the monitoring of sixteen PAHs in environmental samples, whereas the European Food Safety Authority (EFSA) recommends the monitoring of sixteen PAHs in foods. Both priority lists only have eight PAHs in common.

In addition to water, tea is probably one of the most widely consumed drinks in the world. PAHs have been detected in fresh tea leaves and in tea infusions. In any case, the reported PAHs levels are relatively low.

Thus, sensitive analytical methods are needed for the determination of PAHs in tea infusions and drinking waters. Conventional extraction and preconcentration techniques used to achieve low limits of detection are tedious, and require a relatively large number of analytical steps in which large amounts of organic solvents are consumed. Miniaturization or elimination of organic solvents during the extraction step, or the employment of novel reagents can be cited among new trends to overcome environmental issues of conventional extraction approaches.

This work reports the monitoring of PAHs in drinking waters and fruit-tea infusions using ionic liquid (ILs) as novel reagents, and an *in situ* dispersive liquid-liquid microextraction (DLLME) as the miniaturization extraction technique. The *in situ* DLLME method uses a hydrophilic IL that positively interacts with PAHs present in waters or in fruit-tea infusions. Afterwards, an anion exchange reagent is added to promote a metathesis reaction, and so the IL is transformed *in situ* into a hydrophobic IL that settles down containing the extracted PAHs.

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PS2-36

POLYMERIC IONIC LIQUIDS (PILs) *VERSUS* SOLID-PHASE MICROEXTRACTION COMERCIAL COATINGS IN THE DETERMINATION OF VOLATILE COMPOUNDS OF CHEESES

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Cheese aroma is a quite important quality, and one the main reasons in which consumers based their selection when acquiring these products. Volatile free fatty acids, aldehydes, ketones and several phenolic compounds can be included among volatile compounds responsible of cheese organoleptic properties.

Headspace solid-phase microextraction (HS-SPME) is an environmental-friendly technique that has been successfully employed for the monitoring of volatile compounds responsible of food aroma in recent years, mainly in combination with gas-chromatography (GC). The technique is characterized for high preconcentration factors and reproducibility. One important limitation of the technique is related to the limited number of commercial SPME coatings currently available. This has led to an increasing interest in developing novel coating materials valid for SPME.

Ionic liquids (ILs) are non-molecular solvents exclusively formed by ions. Their outstanding characteristics, such as negligible vapor pressure at room temperature, high viscosity, tuneability in terms of chemical structure, and hydrophobic or hydrophilic properties depending on the IL composition, justify the high interest raised on them. Polymers which use ILs as monomers are named as polymeric ionic liquids (PILs). PILs normally present higher thermal stability and mechanic resistance than ILs, but they still retain some of their interesting properties. Thus, they can be easily supported to silica and so be utilized as reusable SPME coatings. PILs used as SPME coatings are characterized by their selectivity, stability, and versatility.

A group of different volatile compounds commonly present in cheese aroma has been analyzed in this work by HS-SPME in combination with GC and flame ionization detection (FID), using different PILs materials as SPME coatings. A comparative has been also established with the commercial carboxen-polydimethylsyloxane (CAR-PDMS) coating, which is the SPME coating commonly used for the analysis of volatile polar compounds. Moreover, it has been obtained the partition coefficient values for the analytes selected with respect to the SPME coatings studied.

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PS2-37

IN SITU DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING A GLUCAMINIUM-BASED IONIC LIQUID TO EXTRACT BORON FROM SEAWATERS

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Boron is a naturally occurring element in seawater, with an average concentration of 5 $mg \cdot L^{-1}$. The removal of boron using reverse osmosis (RO) in seawater treatment plants is challenging as it possesses very low rejection rate for uncharged boric acid which is primarily present in water at neutral pH. Several studies have suggested that higher levels of boron in drinking water can lead to teratogenic effects in humans. Therefore, the World Health Organization (WHO) has set a threshold of 0.5 $mg \cdot L^{-1}$ for boron in drinking water, and of 0.3–0.5 $mg \cdot L^{-1}$ in irrigation water. The European Union has established a limit of 1 $mg \cdot L^{-1}$. Thus, it results essential to regularly monitor the boron content in drinking waters, especially in those waters obtained through seawater via RO. Moreover, it is of high interest the development of techniques able to efficiently remove boron from seawater.

Ionic liquids (ILs) are a class of ionic, nonmolecular solvents with melting points below 100 ºC. The most notable properties include their negligible vapor pressure at room temperature, high thermal stability, and variable viscosity. Their miscibility in water and organic solvents can be controlled by selecting the cation/anion combination or by incorporating certain functional groups in the IL molecule. They have been successfully used in multitude of extraction and preconcentration schemes in analytical chemistry, being particularly successful in liquid-phase microextraction procedures such as dispersive liquid-liquid microextraction.

A group of glucaminium-based ILs has demonstrated promising results for the efficient removal of boron species in simulated studies with deionized water using an *in situ* dispersive liquid-liquid microextraction method. These ILs were also proven to be easily regenerated after extraction.

The present work reports the removal of boron from real seawaters using an *in situ* IL dispersive liquid-liquid microextraction method, utilizing N,N-didecyl-N-methyl-D-glucaminium bromide as the efficient glucaminium-based IL to perform the task.

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PS2-38

DETERMINATION OF TRIHALOMETHANES IN DRINKING WATERS USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND GAS-CHROMATOGRAPHY COUPLED WITH MASS-SPECTROMETRY

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Trihalomethanes (THMs) are a family of compounds belonging to water disinfection by-products (DBPs), characterized for presenting methane as the basis structure, in which three out of four hydrogen atoms have been substituted by halogens. They are formed when waters intended for use in public supplies, with high contents in organic matter, are subjected to chlorination under certain operational conditions.

Although the chlorination of drinking water provides many advantages, it has been shown that several THMs provoke harmful effects in human health. Indeed, some THMs have been classified as possibly carcinogenic and other are not classifiable as to its carcinogenicity to humans (depending on the THM) by the International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA). In this sense, it results of enormous interest the development of sensitive extraction methods to monitor THMs in waters intended to human consumption.

THMs are highly volatile, and so their determination is normally accomplished using gas-chromatography. Given their low levels in aqueous samples, extraction and preconcentration methods are normally required in combination with gas-chromatography. Among them, headspace [1], purge-and-trap [2], and liquid-liquid extraction [1], can be cited. However, novel trends in analytical chemistry seek the development of green methods in terms of minimization of solvent consumption during the extraction step and also minimization of wastes.

This work presents the determination of four THMs in tap waters using dispersive liquid-liquid microextraction. This novel extraction technique uses few microliters of decanol (as extraction solvent), and also low amounts of dispersive solvent (acetone). In this sense, the extraction method can be included among the green chemistry requirements. The method is used in combination with gas-chromatography coupled to mass-spectrometry.

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PS2-39

STUDY OF PRECISION IN THE SOLID-PHASE MICROEXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY ANALYSIS OF BLACKBERRY (*Rubus ulmifolius*) VOLATILES

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Solid-Phase Microextraction (SPME) is a widely used technique for fractionation / preconcentration of food volatiles prior to their gas chromatographic analysis [1]. Although many papers have been devoted to the use of this technique for qualitative purposes, the changeable precision when multicomponent mixtures are to be analysed has frequently questioned its quantitative performance, particularly for studies on food characterisation where precision is the most important analytical parameter.

Chemometrics has been applied for the first time to the study of the dispersion of quantitative data in the multicomponent analysis by SPME followed by GC-MS of blackberry volatiles. Experimental and randomly simulated data were compared by using different statistical parameters (correlation coefficients, Principal Component Analysis loadings and eigenvalues). Non-random factors were shown to significantly contribute to total dispersion, and groups of volatile compounds could be associated with these factors. Model systems simulating blackberry composition were used to assess for the first time the importance of matrix effect on precision of SPME data.

A significant improvement of precision was achieved when considering percent concentration ratios, rather than single percent values, calculated among those blackberry volatiles with a similar dispersion behavior as pointed out by different statistical parameters.

The utility of this approach, previously applied by the authors to the study of dispersion of data from other food samples and obtained by other fractionation techniques [2, 3], is now fully confirmed by its successful application to the precise characterisation of fourteen Italian blackberries from different harvesting year. This approach, of general application, can also be used to improve data precision from other food samples and analytical techniques.

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PS2-40

IONIC LIQUIDS AS AN ALTERNATIVE TO THE SEPARATION OF BIOACTIVE KETOSES FROM ALDOSES IN ISOMERIZATION MIXTURES

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Lactulose is obtained from lactose by isomerization in basic media using different catalytic systems, and yield ranges from 20 to 80%of dry extract, including variable amounts of lactose and small contents of other sugars [1]. On the other hand, tagatose is a stereoisomer of D-fructose and normally obtained from galactose by an isomerization process. In US is considered a GRAS (Generally Recognized As Safe) product and its use in foods and beverages has been approved in several countries [2]. Both carbohydrates are considered as prebiotics and their purification is of especial interest for the food industry taking into account that these processes represent the90% of costs of food production [3]. Current commercial processes for purification of carbohydrates are usually based on the use of chromatographic techniques involving ion-exchange resins or activated charcoal [4].

Room temperature ionic liquids (RTILs) are non-molecular ionic solvents and classified as environmentally friendly and provide a safe alternative to the use of traditional organic solvents which produce volatile compounds [5]. In a previous study, we have evaluated the solubilities of aldoses (glucose, galactose, and lactose) and ketoses (fructose, tagatose, and lactulose) in different RTILs at 26 and 45 ºC, detecting significant differences depending on the carbohydrate structure [6]. Therefore,the possibility of using RTILs for the selective separation of binary aldose/ketose mixtures and mixture of synthesis of lactulose from lactose has been evaluated.

The best results for the selective separation of the binary mixtures of lactulose - lactose and tagatose - galactose were achieved using [EMIM][DCA] and [BMIM][$MeSO_4$], respectively. The ketose/aldose molar ratios were enriched from 1to 2.9 in the pair lactulose/lactose and to 2.53 in tagatose/galactose. When these results were applied to the mixture of synthesis, concentration of lactose was notably reduced and the ratio lactulose/lactose raisedfrom 0.5 to1.47. Consequently, these results are the first evidence of the ILs selectivity which can beusefulfor the efficient separation of bioactive ketoses from their corresponding aldoses.

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PS2-41

MICROEXTRACTION TECHNIQUES COUPLED TO CAPILLARY ELECTROPHORESIS FOR THE ANALYSIS OF BIOLOGICAL SAMPLES

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Among the analytical techniques currently used, capillary electrophoresis (CE) represents an attractive alternative to chromatographic approaches for the analysis of different types of compounds in biological samples [1]. However, in this kind of complex matrices, interfering endogenous compounds are normally present in a higher concentration than the target analytes, so an extraction and a preconcentration steps are needed. One of the main current trends in the development of extraction techniques involves the concept of "green chemistry", which is concerned with reducing the large amounts of solvent and sample used in convectional extraction techniques. Taking into account these considerations, an interesting approach is by means of a combination between CE and microextraction techniques (MEs). There are different ME techniques according to their extraction principle [2], and in the present poster we focus on the use of in-line solid phase extraction (SPE)-CE and in single-drop microextraction (SDME)-CE.

Hair analysis is an excellent tool for confirmation of drug abuse. However, the drug concentration in a single hair strand is often very low, and thus a highly sensitive analytical method is required. CE-UV coupled to a preconcentration step such as in-line SPE offers and easy, cheap and green strategy to monitor abused drugs and their metabolites in the hair of subjects undergoing addiction treatment, in order to monitor their compliance to therapy [3]. The in-line coupling SPE-CE allows the detection of the drugs at sub-ng/mg levels in hair samples.

Urine samples can also constitute an example of sample used as biomarker, but as in the case of hair samples, a pretreatment is needed prior to CE analysis. In three-phase SDME, the targeted analytes are first extracted from the aqueous sample (donor) into a water-immiscible organic phase (acceptor I) and then back-extracted into a separate aqueous phase (acceptor II) by simply manipulating the pH in the donor and acceptor phases [2,4]. This configuration can be easily combined in-line to CE, reaching detection of non-steroidal antiinflammatory drugs in urine samples at low ng/mL levels.

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PS2-42

PROPOSAL OF A PROCEDURE FOR THE ANALYSIS OF ATMOSPHERIC PAHS FROM MOSS BIOMONITORS

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Polycyclic aromatic hydrocarbons (PAHs) are highly volatile, carcinogenic and mutagenic compounds, with slow rates of degradation [1]. The most important transport medium of these compounds is the atmosphere, and therefore monitoring of PAH concentrations in ambient air is of great concern for public health. PAHs levels can vary considerably in space, and thus it is of great interest the use of sampling tools that are able to assess spatial deposition of PAHs at a local scale [2]. The use of biomonitors to evaluate environmental contamination has many advantages. They are easy to sample and allow long-term monitoring with a large number of sampling sites. The high cationic exchange capacity and high surface area to volume ratio of mosses make them excellent tools for biomonitoring [3].

In this work we propose a procedure for the analysis of PAHs from mosses using microwave assisted extraction. This extraction technique achieve great extraction efficiencies in less time and using lower solvent volumes than the Soxhlet procedure usually applied for this kind of analysis. The initial conditions for the analysis were selected according previous studies of the group [4]. Different extraction conditions were tested, selecting the extraction at 80ºC with a mixture of hexane:acetone (90:10) as the most adequate. These conditions allow a low coextraction of interfering substances making easier the purification step. The typical clean-up in the analysis of mosses is the use of glass columns with large amounts of sorbents, which is laborious and involves a high volume of solvent consumption. Several sorbents and eluents mixtures were tested. We propose the use of commercial solid phase extraction cartridges containing florisil and silica. The eluates obtained were colourless and with few interfering substances. The determination of the PAHs was performed by gas chromatography coupled to tandem mass spectrometry (PTV-GC-MS/MS). Quantification was performed using deuterated PAHs as surrogates. The proposed method was validated and successfully applied to the analysis of moss samples used for the biomonitoring of atmospheric contamination.

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PS2-43

ARSENIC SPECIES DETERMINATION IN HUMAN SCALP HAIR BY PRESSURIZED HOT WATER EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY – INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

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Forensic and clinical analysis of trace compounds is usually carried out in blood, urine and hair. Although blood and urine are the most common and preferred matrices used for clinical/toxicological studies, hair is gaining in importance as an alternative specimen because of its advantages. Hair can be easily collected; it shows a high stability which facilitates the storage and a high capacity to accumulate different substances during extended periods.

The determination of inorganic and organometallic species in biological samples requires sensitive and selective techniques, using sample preparation strategies addressed to shortening and simplifying the stages previous to the analysis. HPLC in combination with ICP-MS is widely used for analysis of arsenic species in biological samples, offering simplicity and saving time and resources. Although the fast separation and detection methods, many of processes use time-consuming sample pre-treatments, which involve many sample manipulation steps, generate large volumes of waste, and could introduce problems such as sample contamination or analyte losses during filtration/centrifugation steps. Thus, the development of automated and rapid extraction methods remains on interest. Modern extraction techniques including pressurized hot water extraction (PHWE) have demonstrated higher capabilities for trace analyte extraction because extraction and clean-up stages can be performed simultaneously. Under PHW conditions, analyte mass-transfer is enhanced due to viscosity, surface tension and density are reduced (hydrogen bonding becomes weak). PHWE pre-treatment reduces analysis time, sample manipulation (filtration stage is avoided), and the amount of sample required for analysis. In addition, this procedure enhances safety due no toxic solvents or acid are used (linking to the principles of ‘‘Green Chemistry’’).

The aim of this paper is the evaluation of PHW to extract arsenic species from human scalp hair. Optimum condition implies a modifier concentration (acetic acid) of 150 mM and powdered hair samples fully mixed with diatomaceous earth (DE) as a dispersing agent at a DE mass/sample mass ratio of 5. The extraction has been carried out at 100 °C and at 1500 psi for 5 min in four extraction step. Under optimized conditions, limits of quantification of 7.0, 6.3 and 50.3 ng g^{-1} for total As, As(III) and As(V), respectively were achieved. Repeatability of the over–all procedure (4.4, 7.2 and 2.1 % for total As, As(III) and As(V), respectively) was achieved. The analysis of GBW-07601 (human hair) certified reference material was used for validation. The optimized method has been finally applied to several human scalp hair samples.

PS2-44

ANALYSIS OF ORGANOCHLORINE PESTICIDES FROM SEAWATER USING SOLID PHASE EXTRACTION AND DETERMINATION BY GAS CHROMATOGRAPHY- MASS SPECTROMETRY WITH NEGATIVE CHEMICAL IONIZATION

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Organochlorine pesticides (OCPs) have been characterized as carcinogenic, endocrine disruptors, thyroid disrupters and related with liver effects, neurodevelopmental health effects, etc. by the USEPA and European Commission among others. [1] Thus regulations are stricter and stricter for these compounds. The European Directive 2008/105/EC establishes very restrictive environmental quality standards (EQS) for organochlorine pesticides in surface waters. Moreover the marine ecosystem Galician is an important source of wealth, thus it is vital that the seawater is in adequate conditions for the development of marine activities.

A procedure based on a solid phase extraction (SPE) followed by a gas chromatography-mass spectrometry with negative chemical ionization (GC-MS-NCI) is proposed for the analysis of 26 organochlorine pesticides from seawater samples. SPE is cheap, simple, fast, and requires low volume of organic solvents. In spite of the fact that this is not a technique according to the Green Chemistry principles, SPE allows to reach the very low levels required by normative, because allows the percolation of large sample volumes. In addition, this method uses laminar disks Bakerbond Speedisk^{TM} C_{18}, which allow high throughput rates without previous filtration, even when samples contain suspended solids [2].

The main variables affecting the extraction process such as the elution and evaporation conditions were optimized. A mixture of AcOEt:H (20:80) was selected as eluent as supplies the best recoveries. For the evaporation, rotary evaporation and automatic Syncore evaporation were compared, selecting Syncore because provides good results and allows the simultaneous evaporation of 12 samples.

Regarding the determination of OCPs, sensitivity provided by EI-MS is not high enough for many organochorine pesticides. However, NCI provides a limited fragmentation and simple mass spectra, better S/N ratio, higher sensitivity and selectivity [3]. The whole method was validated in terms of accuracy, linearity, precision and sensibility. The technical specifications of the method proposed meet the criteria established by the Directive 2008/105/EC. Finally, the method was successfully applied to the analysis of several samples.

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PS2-45

DEVELOPMENT OF A SELECTIVE METHOD BASED ON IN-TUBE SOLID PHASE MICROEXTRACTION COUPLED TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR ULTRATRACE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN RAINWATER

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Analysis of polycyclic aromatic hydrocarbons (PAHs) at ultratrace level in environmental matrices usually requires several steps of sample treatment. That leads to a significant waste of time and spend of organic solvents. The preparation technique named as in-tube solid phase microextraction (IT-SPME) reduces these disadvantages. IT-SPME can be easily coupled to high performance liquid chromatography (HPLC), combining sample treatment and determination in a single step. The use of IT-SPME-HPLC with fluorescence detection (FLD) allows achieving limits of detection and quantification suitable for analysis of PAHs in rainwater. This work aims to the development of an IT-SPME-HPLC-FLD method for simultaneous determination of 15 priority PAHs at ultratrace level and its application to rainwater samples. The problematic associated with analysis of PAHs in rainwater has been little studied. According to our research, some PAHs present a great lack of stability when the sample is defrosted after storage.

The addition of organic solvents as modifiers has proven to be useful to improve extraction of PAHs by IT-SPME [1]. Lower-molecular weight and higher-molecular weight PAHs have different behaviors, in such a way that each group has a different optimal percentage of modifier. When the differences are so relevant, two options can be proposed: choosing only one compromise percentage, which sacrifices sensitivity; or carrying out two different analysis, one each for group of compounds. The latter means expending twice the reagents and time. In this work, different organic modifiers (acetonitrile, 2-propanol and tetrahydrofuran) are tested to find a compromise for the simultaneous analysis of the 15 PAHs with a minimal loss of sensitivity. Moreover, sample volume has been optimized, and stability of PAHs in the rainwater sample has been studied. The best performance was obtained using 5 mL of sample with a 15% tetrahydrofuran. In these conditions, PAHs were found to be stable during a working day. In addition, a good precision and limits of detection at concentration level of ng L^{-1} were obtained.

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PS2-46

LIMONENE: GREEN NON POLAR SOLVENT FOR SAMPLE PREPARATION

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Traditional non polar solvents like alkanes are obtained from non-renewable sources. The idea of Green Chemistry has its roots in sustainable development. Green Analytical Chemistry emerged from green chemistry in 2000 [1]. The principles of Green Analytical Chemistry emphasize the importance of using reagents obtained from renewable sources, eliminate toxic reagents and increase safety of the operator.

The use of limonene as non-polar extracting agent in order to replace hexane has been slightly studied. Limonene is obtained from citrus peel residues, being the main compound in the terpene fraction of citrus peel oil. This compound possesses a dielectric constant close to hexane [2] and has thus been suggested as a valuable green alternative to n-alkanes and halogenated hydrocarbons.

In the present work a fast method for the isolation of high value lipids is presented involving the use of limonene, a green biodegradable solvent, as an alternative to traditional hexane extraction. The optimized process is based on pressurized liquid extraction (PLE) at 180°C for 15 min using limonene:ethanol (1:1). To prove the method efficacy, the extraction of lipids from marine microorganisms was selected. One green alga (Stigeoclonium) and four cyanobacteria (Spirulina, Nostoc, Anabaena and Phormidium) were tested. After extraction, lipids were analyzed by a Fast-GC-MS method.

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PS2-47

SIMULTANEOUS EXTRACTION AND HPLC DETERMINATION OF XANTHOPHYLLS, β-CAROTENE ISOMERS AND TOCOPHEROLS IN TWO PASTURES (*Trifolium pratense L and Lolium perenne*) AND A WEED *(Plantago lanceolata)* SPECIES: EFFECT OF DRYING PRETREATMENT, SPECIES AND MATURITY STAGE

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Carotenoids (provitamin A compounds) and tocopherols (vitamin E) are fat-soluble micronutrients that play an important role in human health. Those bioactive compounds also act as naturally occurring antioxidants in ruminant diets. Their content in cattle feed can be affected by factors such as plant species, harvest number over season, maturity stage at the harvest time and pre-wilting. In this way, altering the nature of forage fed to ruminants has been identified as an efficient strategy to modulate the carotenoid and vitamin E concentrations in cow's milk [1]. Moreover, for traceability purposes, their concentrations on final dairy products have been evidenced as promising biomarkers of animal diet, making it possible to discriminate the animals´ feeding system.

The aim of present on-farm study was the determination and quantification of neoxanthin, antheraxanthin, violaxanthin, lutein, zeaxanthin, all-E-β-carotene+9Z-β-carotene, 13Z-β-carotene, α-tocopherol and γ-tocopherol in three sward species commonly found in Galicia (NW Spain) pastures: two sown species (*Trifolium pratense L., Lolium perenne L.)* and a weed (*Plantago lanceolata L*.). The effect of pre-treatment was assessed at four drying temperatures (40-100 ºC) and at three maturity stages. The carotenoid content was first evaluated on fresh sample (F) and then on lyophilized (L) or treated under different Tº /time combinations: 40ºC for 72h (TT1), 60ºC for 48h (TT2), 80ºC for 16h (TT3) and 100ºC for 16h (TT4).

The extraction and purification procedures used in the different matrices were conducted on the basis of a procedure first described by Britton *et al* [2] and according to the methodology proposed by Cardinault *et al* [3], mainly separating xanthophylls from chlorophylls. Briefly, the method consists of an extraction step of lipophilic compounds with acetone followed by a partition to diethyl ether (stabilized with BHT) and on a short saponification step [4]. Final extracts were evaporated to dryness and reconstituted in 1 mL of mobile phase before being injected on the HPLC system.

The applicability of pressurized liquid extraction (PLE) for the extraction of the target compounds was also successfully explored [5].

Analyses were carried out on an HPLC system equipped with both, a photodiode array (PDA) and a scanning fluorescence detectors. Simultaneous carotenoid and vitamin separation was performed on a RP dC18, 5μm Atlantis column. The mobile phase consisted of a quaternary gradient of acetonitrile (A), methanol with ammonium acetate (50mM) (B), water (C) and dichloromethane (D). Carotenoids were detected at 450 nm using the PDA, whereas

detection of vitamin E was accomplished by fluorescence with excitation-emission at 295-330 nm. A mix of individual stock solutions was prepared on mobile phase and external standard calibration curves were built to quantify carotenoids and vitamins from each matrix. Concentrations were also adjusted by the recovery factors of the two surrogates, echinenone and δ-tocopherol.

As far as freeze-drying would not be available, drying at 80 ºC during 16 h would to be the best way to minimize losses of both carotenoids and vitamins, irrespective of the species studied.

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PS2-48

DEVELOPMENT OF A MULTIPRESERVATIVE METHOD BASED ON SOLID-PHASE MICROEXTRACTION FOR COSMETIC ANALYSIS

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Preservatives are essential ingredients used in all cosmetic formulations to guaranty product integrity, protecting as well consumer health. The vast family group of cosmetic ingredients are subjected to several restrictions according to the European Cosmetic Products Regulation [1]. Thus, as a part of a quality control procedure, analytical methods for the determination of this diverse group of cosmetic additives in the wide variety of marketed personal care products are required.

In the present work, a simple methodology based on solid-phase microextraction (SPME) followed by gas chromatography mass spectrometry has been developed. In-situ acetylation was successfully applied for the derivatization of target compounds using acetic anhydride and sodium hydrogen phosphate [2]. The major goals were to avoid matrix effects in a representative and broad diversity of cosmetics, as well as to obtain satisfactory sensitivity for a large number of preservatives. Negative matrix effects caused by the complexity of the samples were reduced by sample water dilution as well as by the addition of organic solvent modifiers.

A $3*2^{3-1}$ mix-level fraction factorial design has been carried out to evaluate the influence of experimental parameters affecting SPME efficiency. The most critical factors were the fiber type, the temperature, and the sample mode, as well as the interaction temperature_mode. After optimization, the recommended procedure was established as follows: direct solid-phase microextraction of 10 mL of diluted cosmetic (20 % NaCl), at 40 ºC using DVB/CAR/PDMS fiber for 15 min (magnetic stirring).

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PS2-49

DETERMINATION OF FUNGICIDES IN WHITE GRAPE BAGASSE

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Fungicides are a class of pesticides of widespread use in viticulture to avoid fungi infection of *Vitis* plants. This use is especially extended in rainy regions such as Galicia (Northwestern Spain). Thus, residues of fungicides can be found in the grape bagasse.

There is a lack of methods for the analysis of fungicides in grape bagasse samples. In solid samples such as grapes, more environmentally friendly procedures including pressurized liquid extraction (PLE), solid phase extraction (SPE), QuEChERS, microwave-assisted extraction (MAE) and matrix solid-phase dispersion (MSPD) [1], are substituting traditional methodologies such as Soxhlet extraction [1].

The aim of this work was to develop and validate a method for multiresidue analysis of fungicides selected from different chemical classes in white grape bagasse. Ultrasound Assisted Extraction (UAE) and Pressurized Liquid Extraction (PLE) were evaluated and applied as the sample preparation techniques for the simultaneous extraction of 11 fungicides (Metalaxyl, Cyprodinyl, Procymidone, Iprovalicarb, Myclobutanil, Kresoxim-Methyl, Benalaxyl, Fenhexamide, Tebuconazole, Iprodione and Dimetomorph). The analysis was performed using GC coupled to MS detectors.

Extractions were optimized by means of experimental design and the optimal conditions were selected for validation.

PLE procedure showed higher efficiency than UAE for the target fungicides. Under the selected extraction conditions, PLE showed satisfactory linearity, repeatability and reproducibility. The recoveries for most of the studied fungicides were higher than 80% with relative standard deviations lower than 15%.

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PS2-50

DEVELOPMENT OF A MULTIANALYTE METHOD BASED ON MICRO-MATRIX-SOLID-PHASE DISPERSION FOR THE ANALYSIS OF PERSONAL CARE PRODUCTS

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The Regulation (EC) No 1223/2009 establishes the rules to be complied with by any cosmetic product available on the market, in order to ensure a high level of protection of human health. It includes the prohibited substances, which must not be integrated in the cosmetic formulations, as well as the restrictions applied to other substances [1].

European legislation requires the monitoring of 27 fragrances considered as suspected allergens, frequently used in cosmetics and personal care products. Of these 27 substances, 25 are chemically defined volatile compounds whereas the other two are natural moss extracts. Preservatives are added to cosmetic preparations to inhibiting the development of microorganisms. Parabens are the most frequently used; these substances have restricted use according regulations.

An effective, simple and low cost sample preparation method based on matrix solid-phase dispersion (MSPD) and gas chromatography–mass spectrometry was used for the rapid simultaneous determination of 25 fragrance allergens, and 14 preservatives commonly used in cosmetics and personal care products. Previously, micro-MSPD had been proposed for the analysis of plasticizers and synthetic musks in rinse-off and leave-on cosmetic formulations [2]. The method was optimized by multivariate analysis. The final miniaturized procedure requires the use of only 0.1 g of sample and 1 mL of organic solvent. The micro-MSPD method was extensively validated and it was applied to a broad range of cosmetics and personal care products demonstrating suitability. The use of GC coupled to triple quadrupole mass detection allowed to reach very low detection limits.

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PS2-51

DETERMINATION OF SIX SULFONYLUREA HERBICIDES IN COMMERCIAL GRAPE AND APPLE JUICES BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOLLOWED BY CAPILLARY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Sulfonylurea herbicides (SUHs) are a family of herbicides commercialized worldwide in all major agronomic crops. Due to their increasing use, concerns have been raised by the public and by regulatory authorities regarding potential adverse effects. The European Union (EU) has established maximum residue limits (MRLs) in vegetable foods and fruits due to their toxicity [1] but nevertheless there is an absence of quantitative methods to determine SUHs in fruit juices. Expected concentrations in these samples are supposed to be very low due to the low dosage application of SUHs. Besides, MRLs are in the low nanogram per milliliter concentration range. Therefore, new sample enrichment and purification techniques are needed for monitoring these herbicides in fruit juice samples.

In this study we proposed a simple, rapid and efficient method for the determination of six SUHs: triasulfuron (TS), metsulfuron-methyl (MSM), chlorsulfuron (CS), flazasulfuron (FS), chlorimuron-ethyl (CSE) and primisulfuron-methyl (PSM), in commercial grape and apple juice samples by dispersive liquid-liquid microextraction (DLLME) coupled with capillary high-performance liquid chromatography with diode array detector (capillary HPLC/DAD). Various parameters that influence the extraction efficiency such as, types and volumes of extraction and dispersive solvents, sample pH, salt addition and extraction time, were investigated and optimized. Also optimum chromatographic conditions were established. Limits of detection and quantification of the method were ranging between 2 - 9 and 8 – 29 µg/L, respectively, which are in all cases lower than the MRLs permitted by the EU for raw fruits such as grape and apple. The linear ranges obtained were: 12 - 100 µg/L for TS, 8 - 100 µg/L for MSM, 10 - 100 µg/L for CS, 17 - 100 µg/L for FS, 11 - 200 µg/L for CSE and 29 - 200 µg/L for PSM, with correlation coefficients ranging from 0.995 to 0.997. The intra- and inter-day relative standard deviations (RSDs) varied from 1.0 to 8.2 % and 1.8 – 9.8 %, respectively. The recoveries obtained from fortified commercial grape juice (both white and red) and apple juice samples at three concentration levels were from 72.0 - 109.5 %.

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PS2-52

DETERMINATION OF ALLIUM DERIVATIVE DIPROPYL THIOSULFONATE IN ANIMAL FEED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Two of the main goals of livestock farms nowadays are improving animal health and feed efficiency. Antibiotics have traditionally been used to achieve these goals, but the ban on nutritive antibiotic use in Europe has forced the search of new additives from natural sources, including probiotics, prebiotics, organic acids and plant extracts [1,2]. There are numerous studies showing beneficial effect of plant extracts in animal nutrition, including cinnamon, origanum or clove extracts. But new active ingredients from alliums spp. such as propyl propane thiosulfonate (PTSO) have also proved to be effective in both improving animal health [3] and feed efficiency [4]. Besides they do not show some of the disadvantages associated with antibiotics, and comply with the demand among consumers for more natural and safe products in the human food supply chain. Animal feed containing this additive are now commercially available from DOMCA S.A., that quality control methods are required to ensure concentrations are optimal for an adequate animal production.

In this communication, a new analytical method to determine PTSO in animal feed is presented. First of all, a reversed-phase liquid chromatography methodology was developed to determine PTSO with UV-Vis detection. A C18 column (150 x 4.6 mm) was used in combination with a binary mobile phase containing perchloric acid and acetonitrile. A gradient program was optimized in order to achieve a retention time of just 5 min. Moreover, a sample treatment method was developed and optimized in order to extract PTSO from animal feed. A methodology based on solid-liquid extraction assisted with a Polytron homogenizer was used. Several extraction solvents were tested such as ethyl acetate, dichloromethane or acetone. Acetone was selected based on its extraction efficiency and cleaner extracts. The extraction solvent was evaporated and samples were reconstituted with methanol. Finally the whole analytical method was evaluated in animal feed samples spiked with PTSO. Limits of detection and quantification were 1 and 3 mg/kg, respectively, which are lower than the expected concentrations in samples containing these additives. Linearity was achieved at least up to 60 mg/kg with a correlation coefficient of 0.997. The intra- and inter-day relative standard deviations (RSDs) were 4.4 and 7.3 respectively and recoveries varied from 84 and 93%.

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PS2-53

DEVELOPMENT AND CHARACTERIZATION OF AN UHPLC-MS/MS METHOD BASED ON QUECHERS AND DISPERSIVE LIQUID-LIQUID EXTRACTION FOR THE DETERMINATION OF MULTICLASS MYCOTOXINS IN EDIBLE NUTS AND SEEDS

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Nuts and seeds are highly susceptible to mould growth and consequently to mycotoxin contamination as it has been stated recently in some studies [1]. The presence of mycotoxins in food may affect human and animal health as they may cause many different adverse effects such as induction of cancer and mutagenicity, as well as estrogenic, gastrointestinal and kidney disorders. The most commonly sample treatment used for the determination of mycotoxins in nuts and seeds involves the use of solid-liquid extraction followed by a clean-up step using immunoaffinity columns (IACs) but their inherent selectivity limits the multiclass analysis of these contaminants.

In recent years there has been an increasing interest in the development of methods supporting the so-called green chemistry, being simpler, more efficient and environmentally friendly in terms of reduction of organic solvents, contaminant waste and cost. Moreover, multiresidue methods able to monitor a high number of compounds in a single run according to the established legislation for many different matrices, are also very attractive.

In this work, a sensitive, simple and rapid method for the determination of fifteen mycotoxins in nuts and seeds (including almonds, peanuts, sunflower seeds, pumpkin seeds, walnuts, macadamia nuts, pistachios, hazelnuts and pine nuts) based on ultra high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) has been developed and characterized. The sample treatment comprises a first step based on QuEChERS procedure for the determination of fumonisin B_1, fumonisin B2, deoxynivalenol, fusarenon-X, T-2 and HT-2 toxin, citrinin, sterigmatocystin, zearalenone and ochratoxin A. A subsequent clean-up step based on dispersive liquid-liquid microextraction (DLLME) was necessary for the determination of aflatoxins (B_1, B_2, G_1 and G_2), since their determination was not possible applying only the QuEChERS based extraction. The method was characterized for peanuts as representative matrix and was subsequently evaluated for the other eight matrices. Quantification limits obtained for aflatoxins, the unique mycotoxins legislated on these matrices, were lower than the maximum levels allowed by the current legislation, while quantification limits obtained for the other mycotoxins were lower than the limits usually permitted by the legislation in other food matrices. Precision, expressed as RSD (%), was always lower than 11%, and recoveries ranged between 60.7% and 104.3%.

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PS2-54

TRADITIONAL SOLID PHASE EXTRACTION VERSUS DISPERSIVE LIQUID-LIQUID MICROEXTRACTION PRIOR TO CAPILLARY ELECTROPHORESIS ANALYSIS OF DRUGS OF ABUSE IN HUMAN URINE

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Analytes from three different families of drugs of abuse usually employed as recreational drugs (amphetamines, hallucinogens and anaesthetics) have been considered in the present work.

Amphetamine and its derivatives are powerful stimulants of the central nervous system and they have a notorious reputation in the illicit drug market. Lysergic acid diethylamide (LSD), also known as lysergide and colloquially like “acid”, is considered one of the most powerful hallucinogenic drugs. The low ingested doses and its extensive liver metabolism, leads to very small concentrations in body fluids and tissues. Its determination in biological samples is considered a real challenge for forensic laboratories. Phencyclidine (PCP) was developed in the 1950s by Parke Davis and Company as a dissociative anesthetic but due to its severe side effects, it was withdrawn from the clinical use and was employed in veterinary surgery until 1978. In the 1960s PCP became popular as a recreational drug, known as “angel dust”, synthesized in clandestine laboratories.

We have developed a capillary zone electrophoresis (CZE) method with UV detection for the analysis of 3,4-methylenedioxymethamphetamine (MDMA), LSD and PCP in human urine. The separation of these three analytes has been achieved in less than 8 min. in a 72-cm effective length capillary with 50-µm internal diameter. A 100 mM NaH_2PO_4/Na_2HPO_4 pH 6.0 solution has been employed as running buffer, and the separation has been carried out at temperature of 20 °C and voltage of 25 kV. The three drugs have been detected at a wavelength of 205 nm. Field amplified sample injection (FASI) has been successfully employed for on-line sample preconcentration to achieve the desired limits of detection in biological samples.

In this communication, a comparison between two different sample treatments for urine sample , prior to FASI-CZE-UV, such as solid phase extraction (SPE) and dispersive liquid-liquid microextraction (DLLME), have been carried out in terms of extraction efficiency, throughput, linear dynamic range in matrix-matched calibration curves, detection and quantification limits and accuracy (trueness and precision, by means of recovery assays). In both cases, satisfactory results were achieved, being discussed the characteristics of both methodologies, compatible with the FASI procedure proposed for on-line preconcentration.

PS2-55

SIMULTANEOUS ANALYSIS OF THIRTY-THREE CARBAMATES IN HERBAL PRODUCTS BY UHPLC-MS/MS USING QUECHERS METHODOLOGY

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Carbamates (CRBs) are used worldwide as broad spectrum pesticides against insects, fungi and weeds on a wide variety of foodstuff. In this work, a new multiresidue method for the determination of thirty-three CRB pesticides (including some metabolites) in different teas, chamomile, and other herbal products intended for infusions by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) has been proposed. Parameters affecting the MS/MS detection using triple quadrupole were optimized and the corresponding product ions for quantification and confirmation were selected. The separation of this CRBs was achieved in less than 6 min, using a Zorbax Eclipse plus RRHD C18 column (50 mm × 2.1 mm, 1.8 µm), with a mobile phase of water and methanol, both of them with 0.01% formic acid. The analytes were detected in ESI+ with multiple reaction monitoring mode; fragmentation conditions were optimized in order to obtain the highest sensitivity.

QuEChERS methodology was evaluated as sample treatment; after optimizing the clean-up step (dispersive solid phase extraction), matrix effects were reduced around 30% for most of the considered pesticides, compared with matrix effects obtained with an extraction without additional clean-up. The method allowed recoveries between 74 and 101%, with relative standard deviations lower than 7% at three concentration levels (5, 20 and 50 µg kg^{-1}). Limits of quantification ranged from 1.9 to 4.0 µg kg^{-1}, and were below maximum residue limits established for this type of samples.

The proposed method combines the advantages of the QuEChERS methodology (such as simplicity, minimum steps, and effectiveness for cleaning-up complex samples) and the high sensitivity, selectivity, short analysis time and identification capability of UHPLC-MS/MS, showing its usefulness for the simultaneous monitoring of these residues in a wide range of herbal products.

PS2-56

CHARACTERIZATION OF VISCUMIN FROM MISTLETOE (*VISCUM ALBUM L.*) BY ELECTROPHORETIC TECHNIQUES

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Extracts from plant material of European mistletoe (*Viscum album L.*) have been used for a long time as therapeutic agents, mainly for oncological applications [1]. In particular, the cytotoxic and immune stimulant properties of this plant material have been attributed to lectins, a group of glycoproteins. These proteins belong to the class of type II ribosome-inactivating proteins, which are composed of a catalytically active A-chain ($M_r \sim 29$ kDa) with rRNA N-glycosidase activity and a B-chain ($M_r \sim 32$ kDa) with carbohydrate binging properties [3]. Several variants of mistletoe lectins (ML), which differs in its molecular weight and specificity to monosaccharide, have been identified. The best characterized component is the ML-1 (also known as viscumin), identified as the main pharmacologically active ingredient of the mistletoe extract being largely responsible for its toxicity [2]. Consequently, the capability to rapidly characterize viscumin and its related glycoforms, is very important for monitoring the potential health hazard presented by this plant or its extracts, which is subject of high toxicological and forensic interest.

Due to the chemical complexity of mistletoe extracts, preparative chromatographic techniques such as gel filtration, ion-exchange and affinity chromatography have been applied. However, none of these techniques provided the appropriate combination of speed, resolution, and simplicity needed to accurately characterize many viscumin samples. In this study, we evaluated the feasibility of using several electrophoretic techniques to characterize the mistletoe extracts. To establish the molecular weight of viscumin and its related glycoforms, traditional SDS-PAGE and capillary gel electrophoresis (CGE) were performed and critically compared. In addition, a fast method based on capillary zone electrophoresis (CZE) was optimized for efficiently screening mistletoe samples. To our knowledge, it is the first time that viscumin and other lectin proteins have been analyzed by CE.

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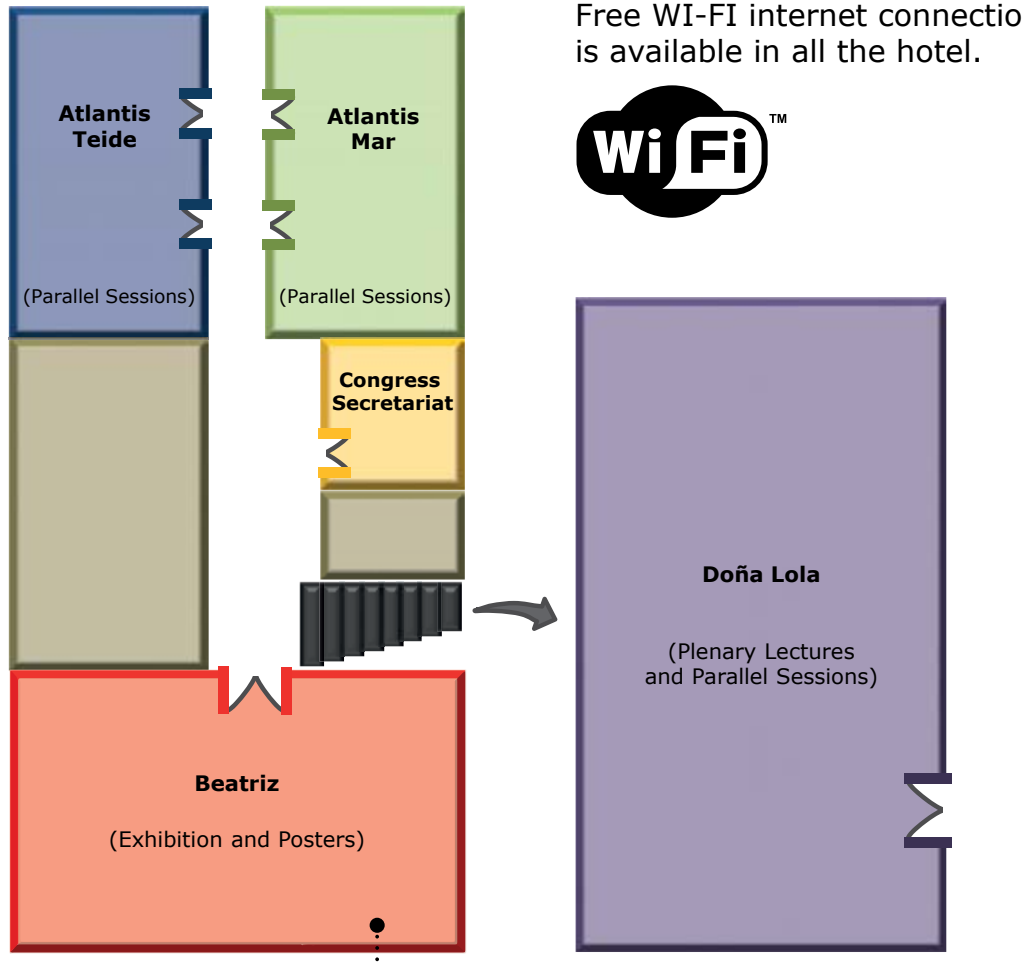

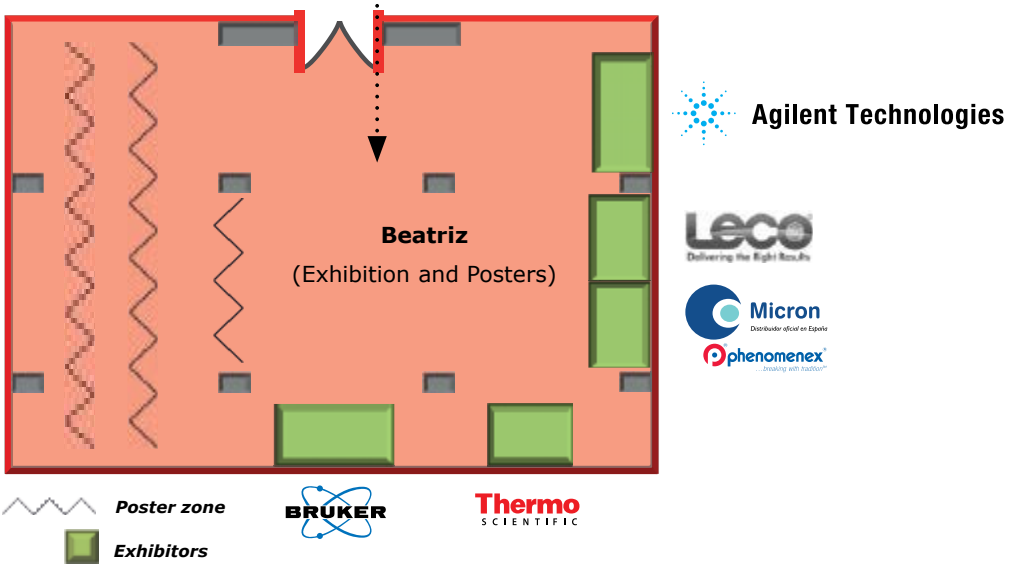

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