SECyTARRAGONA 2012

XII SCIENTIFIC MEETING OF THE SPANISH SOCIETY OF CHROMATOGRAPHY AND RELATED TECHNIQUES

Book of abstracts

Universitat Rovira i Virgili

SECyTA Sociedad Española de Cromatografía y Técnicas Afines

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of the Spanish Society of Chromatography and Related Techniques

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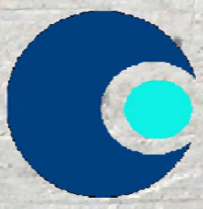

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FOREWORD

We are pleased to welcome you to the XII Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA), which is held in Tarragona from the 14th to 16th November 2012. This would be the 41st edition of the Group of Chromatography and Related Techniques, as it was formerly known, covering a period in which huge advances have been made in the field of chromatography.

On this occasion, three plenary lectures and four invited lectures are included in the program, which covers a diverse range of aspects of both theoretical and applied chromatography. The program also includes 38 oral communications and over 140 poster communications, a selection of which will be discussed in two poster discussion sessions, five vendor seminars and a round table discussing the role of the chromatography in the industry. In addition, there is a vendor exhibition of chromatographic instruments and products.

The papers presented in this Meeting may be submitted to the 'Journal of Chromatography A', the highest impact factor journal in the field.

In the Meeting, the VIII edition of the José Antonio García Domínguez Awards of SECyTA will take place to honour the scientific value of communications in the field of Chromatography and Related Techniques. The Special Recognition Prize in the 6th SPME Users Club Awards will also be presented to the most relevant communication that involves the SPME technique.

We would like to thank all of the participants for their contributions and also for attending the Meeting, especially taking into account the current economic situation and, in particular, the repercussions for research centres and industries. With this in mind, we would also like to express our gratitude to the companies for their participation and financial support to the Meeting, as well as the organisations and industries that have contributed financially to the Meeting.

The organisation of a Meeting on this scale involves the time and effort of a lot of people. We would like to thank the Organising and Scientific Committees as well as the Governing Body of SECyTA for their assistance throughout the organisation process and the Meeting itself.

Finally, we would like to welcome all of the participants to Tarragona. We hope you all enjoy this Meeting and our city. We have tried our very best to ensure that your stay is scientifically successful and socially enjoyable.

Rosa Maria Marcé
Chairwoman of the Organising Committee

Francesc Borrull
Chairman of the Scientific Committee

PL

Plenary Lectures

PLO1

MINITURISING CHROMATOGRAPHY FOR FIELD APPLICATIONS

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Chromatographic methods are a key tool for environmental monitoring, but they are used almost always in a laboratory setting. Typically the sample is brought to the lab for analysis rather than the other way around. There are many applications and target chemicals that would be better measured *in situ* including reactive and labile species and semi-volatile compounds. For many applications an *in situ* observation allowS for a more rapid response to changing conditions. Achieving this this can be challenging using standard commercial chromatography instrumentation. Size, power consumption and speed of analysis are all important factors that require optimization for field analysis, along with sample preparation steps, such as thermal desorption and data analysis. This presentation will discuss a number of approaches that can be used to take difficult environmental measurements out the laboratory and in to the field.

An evolutionary approach can be taken to this problem; there is great potential to modify existing laboratory instrument to increase sample throughput and to improve field robustness. An example approach modifying a bench-top thermal desorption - GC-MS to allow for very fast response volatile organic compound measurements to be made from an aircraft in flight will be presented, along with the new insight this field data brings [1]. This application shows how sample throughput can be greatly enhanced using low thermal mass nickel clad GC columns which are resistively heated, in place of column heating via standard turbulent fan oven.

For some applications, modification of commercial technologies is not possible and in these cases completely new separation and preparation approaches are required which deliver the required analytical standards but with greater device efficiency. Some recent examples of micro-fabricated lab-on-a-chip GC [2] and GCxGC devices [3] will be discussed along with potential detectors such as micro-PID for field analysers. For some applications sample preparation is the most complex and costly element of the analytical procedure and this forms a major barrier to field measurements. A miniaturized approach to sample preparation using a microfabricated on-chip technology for the measurement of carbonyl compounds in urban air has been demonstrated. Using pentafluorophenylhydrazine reagent and elevated temperatures a 0.5 mm i.d. microlitre multicomponent mixing unit is used to produce derivative analyte in near real-time [4]. This methodology has the potential to greatly increase the time resolution of measurements, reduces consumable costs and allows measurement system to operate unattended.

[1] A.C Lewis et al. Atmospheric Chemistry and Physics Discussions. 12, (2012) 23433-23469.
[2] A.C Lewis, J: Chromatogr. A 1217 (2010) 768-774.
[3] R.T Lidster, J.F Hamilton, and A.C Lewis. J. Sep. Science. 34, (2011) 812-821.
[4] X Pang and A.C Lewis. Analytical Methods. 4, (2012) 2013-2020.

PL02

METABOLOMICS OF BODYFLUIDS: FROM A METABOLITE TO A PHENOTYPE

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Metabolomics is one of the most dynamic and rapidly developing fields in the “postgenomic” research. It is a key part of the system biology and as such it contributes enormously to our understanding of the complexity of regulatory mechanisms of an organism. The totality of the metabolites of a given biological system – “metabolome” is the closest approximation physiological phenotype expressed in the strict terms of the chemical sciences. In the context of the clinical metabolomics, where the main goal is to establish a causal link between the metabolic composition of the body fluids and human physiology/pathophysiology, the link between metabolome and phenotype acquires a clear practical significance. To this end, using clinical studies curried out in our lab as an example we are going to give an overview of the challenges and obstacles needed to be managed to turn metabolomics of the bodyfluids into the real "clinical chemistry of the future”.

PLO3

HYDROPHILIC INTERACTION CHROMATOGRAPHY-RENAISSANCE OF AN OLD SEPARATION TECHNIQUE

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Hydrophilic interaction chromatography is a technique that may have its origins in the earliest developments of liquid chromatography by Martin and James in the 1940s. The technique uses a polar stationary phase in conjunction with eluents that are more similar to those used in reversed-phase LC such as acetonitrile and water. The water content of the mobile phase (usually at least 2.5% v/v) distinguishes the method from normal phase separations, where measures are often taken to exclude water completely, as it is such a strong solvent and is disruptive of the separations. HILIC has received considerable attention in the literature in the last 10 years, which can be attributed to its advantages over RP techniques. The elution order of solutes is broadly the opposite of that found in RP, but the separations can be regarded as orthogonal, giving different selectivity. Low back pressures are obtained due to the low viscosity of the mobile phase, which results from the high concentrations of organic solvents (such as acetonitrile) which are typically used. Mass spectrometer sensitivity is increased due to the ease of spraying and desolvation in electrospray techniques. It may be possible to inject directly in solvents that result from the elution of RP solid phase extraction cartridges, thus also providing a different separation mechanism in the purification and analysis steps. Van Deemter curves can be flatter at high flow velocity due to the higher solute diffusion that results in low viscosity mobile phases. Finally the loading properties of some HILIC phases may be superior for the same solutes used in the RP mode. Examples of these advantages will be given. Nevertheless, HILIC is a niche technique, as it does not have the wide applicability of RP methods. Sufficient retention for separation is gained mostly for compounds that have log D values < 0. Even compounds such as phenol have very poor retention in HILIC.

The mechanism of HILIC is complex. It is thought to be largely partition between a water layer held on the surface of the polar stationary phase and the bulk mobile phase. Our work has given physical evidence for the presence of this water layer, and other researchers have indicated its presence through molecular simulation dynamics. Our attempts to correlate retention with the log of the octanol-water distribution coefficient will be discussed, which indicate only moderate agreement with the partition theory. It is also clear from the different elution order of solutes on different columns that the stationary phase cannot act merely as an inert support for the water layer. Ion exchange retention of strong bases and repulsion of strong acids is a feature particular of bare silica columns, but is shown to occur also to some extent on all silica based materials. However, the retention of weak acids and bases may increase as they become more ionized by change of mobile phase pH and therefore more hydrophilic. We have also attempted to deduce whether the mechanism of HILIC contains elements of adsorption as well as partition, although these studies have in general proved rather inconclusive. We have further demonstrated that HILIC columns can exhibit RP properties, providing that organic solvent concentration in the mobile phase is very low.

More recently, we have investigated the relative magnitude of the effect of changing various parameters, such as the stationary phase, organic solvent, buffer pH/strength and temperature on the selectivity of HILIC. The objective of this work has been to develop a series of methods yielding appreciably different selectivity that could be attempted in sequence, using an empirical approach to solve separation problems. The nature of the column was found to have the greatest influence on separation selectivity.

[1] D.V. McCalley, J: Chromatogr. A 1217 (2010) 3408.
[2] D.V. McCalley, J. Chromatogr. A 1171 (2007) 46.

11

Invited Lectures

ILO1

HOW ANALYTICAL CHEMISTRY CAN DESCRIBE CLIMATE CHANGE?

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In marine sediments climate changes are recognized by changes in $\delta^{18}O$ in foraminifera and sea surface temperature measurements (SSTs), generally based on the C_{37} alkenone composition or sometimes on Mg/Ca ratios in foraminifera. Obviously, these climate changes involve many other variations such as marine productivity, wind speed, vegetation type, marine surface currents, intensity of deep water currents, rain and snow regimes, and others that should be characterized for a complete understanding of their origin and impact.

Biomarkers take advantage of the wealth of information contained in the complexities of sedimentary organic constituents and may be useful for the description of both SST and these additional climate parameters. In this paper, several applications in the use of biomarkers for this purpose are summarized based on our on-going studies in the north Atlantic Ocean. The usefulness and range of application of C_{37} alkenones for the description of SST and displacements of the arctic front are considered [1-4]. This proxy is compared to the SST measurements obtained with isoprenoid glycerol dialkyl glycerol tetraethers (TEX_{H86}) in sequences recording abrupt changes [5]. The usefulness of odd carbon numbered C_{23}-C_{31} n-alkanes and even carbon numbered C_{22}-C_{30} *n*-alkan-1-ols for the measurement of wind intensity is also considered [6]. Ratios between n-hexacosan-1-ol and n-nonadecane are also used for the measurement of the intensities of deep water currents [3, 7]. Examples of applications of all these proxies are shown. These records are compared with those obtained from the analysis of foraminifera, pollen and other proxies.

[1]. J.O. Grimalt et al. (2001) Sea surface paleotemperature errors in UK'37 estimation due to alkenone measurements near the limit of detection. *Paleoceanography* **16,** 226-232.
[2] B. Martrat et al. (2004) Abrupt temperature changes in the western Mediterranean over the past 250,000 years. *Science* 306, 1762-1765.
[3] B. Martrat et al. (2007) Four climate cycles of recurring deep and surface water destabilizations on the Iberian Margin. *Science* 317, 502-507.
[4] M.J. Vautravers et al. (2004) Gulf stream variability during marine isotope stage 3. *Paleoceanography* **19,** PA2011, doi:10.1029/2003PA000966.
[5] C. Huguet et al. (2011) Coherent millennial-scale patterns in Uk'37 and TEXH86 temperature records during the penultimate interglacial-to-glacial cycle in the western Mediterranean. *Paleoceanography* **26,** PA2218, doi:10.1029/2010PA002048.
[6] C. López-Martínez et al. (2006) Abrupt wind regime changes in the North Atlantic Ocean during the past 30,000-60,000 years. *Paleoceanography* 21, PA4215, doi:10.1029/2006PA001275.
[7] I. Cacho et al. (2000) Evidence for enhanced Mediterranean thermohaline circulation during rapid climatic coolings. *Earth and Planetary Science Letters* **183,** 417-429.

IL02

ME-ED A PORTABLE ANALYTICAL TOOL

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Among all miniaturized systems described, the most developed devices, since they were proposed in the early 1990s are microchips capillary electrophoresis (MCE).

The main advantages of these microdevices are: miniaturization and portability, automatization, speed, low cost, performance of parallel assays, negligible consumption of reagents/sample and waste generation as well as integration of several steps (pretreatments, mixing and reaction, separation, detection, etc…).

Many of the potential benefits associated with miniaturization will be negated if microchips must be used in conjunction with bulky and expensive equipment. Thus, other detection systems more compatible with these microdevices have been developed.

Electrochemical detection has shown several advantages for using in combination with microchips such as inherent miniaturization, portability, high sensitivity and selectivity, low cost and compatibility with microfabrication technology.

In the present presentation will be addressed:

1. Aspects related to the portability of the instrumentation;
2. How different materials and chemical modifications of the microchannels can contribute to an improvement on the electroforetic separation.
3. The paths that are been followed to improve the sensitivity of the ME-ED system.

IL03

SCREENING OF HERBAL BLENDS FOR LEGAL HIGHS BY UHPLC-QTOFMS

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"Legal highs" are novel psychoactive substances which are either synthetic chemicals, plant or fungal material. They are intended to elicit a psychoactive response, being either stimulant, hallucinogenic, sedative or a combination of the three phenomena. "Legal highs" are sold from "head shops", the internet and from street suppliers and may be possessed without legal restriction. An increase in the marketing of these materials has resulted in a corresponding increase in published reports of their adverse effects.

Europe has seen an explosion in the number of substances containing new compounds that are appearing on the internet and display high and close structural similarity with existing and controlled drugs of abuse. These substances are invariably marketed as "not for human consumption" or even as "plant food", "bath salts" or "pond cleaner".

Smokable herbal blends under the brand name 'Spice' are known to have been sold on the Internet and in various specialised shops since at least 2006. Although advertised as an "exotic incense blend which releases a rich aroma" and "not for human consumption", when smoked, 'Spice' products have been reported by some users to have effects similar to those of cannabis.

The aim of this study was to identify active ingredients of herbal highs and to compare their chemical composition. Six different blend products were analysed. Herbal extracts (0.1 g) were prepared by ultrasonic-assisted extraction with 3.0 mL of acetone for 15 min. Supernatants were ten-fold diluted with water. The diluted extracts were analysed by ultra performance liquid chromatography coupled to hybrid quadrupole-time of flight mass spectrometry (UHPLC-QTOF MS) working under MS^E mode. Data were processed using specialised software ChromaLynx XS.

A customised home-made database containing more than 300 designer drugs as well as 70 natural ones was used, comprising synthetic cannabinoids, amphetamines, cathinones, piperazines, tryptamines but also cocaine, natural cannabinoids, hallucinogenic mushrooms or khat. Although initially few reference standards were available at our laboratory, information about fragmentation was obtained from the literature, and included in the exact-mass database for several compounds.
Initially, the presence of the (de)protonated molecule measured at its accurate mass was evaluated in the samples. When a peak was detected, collision induced dissociation (CID) fragments (in any of the two functions acquired, at low or high collision energy) or characteristic isotopic ions were also evaluated. In this sense, UHPLC turned valuable for choosing perfectly co-eluting ions trying to avoid spectrum interferences that would complicate the identification process.
Under these circumstances, the most usual situation was the proposal of more than one possible candidate for each detected peak. After that, the accurate mass of the fragment ions for the different candidates was justified using MassFragment software, trying to reduce the number of possible candidates. In some cases, compatible structures for the fragment ions were proposed by the software for all candidates. In this case, the tentative identification of the analyte was supported by the MS/MS product ions reported in the literature for this compound (both accurate or nominal mass), if available. After this careful evaluation process, the reference standard/s of the remaining candidates (if commercially available) was/were finally acquired and injected, to discard between the different possibilities and/or unequivocally confirm the identity of the compound.
The main active compounds of the herbal mixtures analysed were synthetic cannabinoids: JWH-081, JWH-250, JWH-203 and JWH-019. Their content differed between the products; some contained two cannabinoids whereas others contained even four.

IL04

MAJOR ANALYTICAL CHALLENGES IN THE INTERPRETATION OF AROMA AND FLAVOUR

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The basic principles about the aromatic and gustative properties of the chemical components and about the nature of the olfaction process, pose several logic restrictions to the way in which the analysis seeking the understanding of the aroma-flavor perception phenomena must be conducted. In this sense, it is possible to set forth some big challenges that this branch of analytical chemistry has to face. Some of these challenges have found satisfactory experimental responses; some others are yet far from being solved.

The first challenge is related to the need of using sensory directed screening techniques at the beginning of nearly all analytical processes. This is the simple consequence of the huge selectivity of our chemical senses. In the case of olfaction it is possible to find more than 12 magnitude orders in the differences in the sensory responses elicited by equivalent amounts of different molecules. This causes that there is no correlation between the instrumental profiles (GC-MS or HPLC-MS) and the sensory profiles. In the case of olfaction, GC-Olfactometry (GC-O) is a well established technique, while in taste and chemostesic studies similar strategies have been proposed more recently. In the case of GC-O the difficulties nowadays are more related to the preparation of representative extracts. A quite mysterious challenge derives from those molecules that not having per se clear sensory properties (odor, taste or chemoestesis) can impair the perception of others.

A major second challenge is related to the need of designing complete systematic quantitative strategies in order to monitor all the molecules that are potentially relevant in the aroma and flavor of a complex product. In the case of wine, for instance, and only in reference to aroma components, up to 8 different analytical strategies can be required, since the quantitative analysis of nearly 80 different molecules belonging to 10 different chemical families and ranging 10 magnitude orders in concentration may be necessary for understanding completely wine aroma. The integration of this chemical information in order to provide a satisfactory explanation for the sensory perception is a great challenge in which the advances have been really scarce and in which most researchers are still using quite primitive sensory concepts.

The third challenge has specifically to do with odorants (and tastants) present in liquid products (such as wine, cologne, beer, coffee, natural juices…) and in particular with the interactions that some of those molecules can establish with the surrounding chemical structures. Some of those interactions, varying in the degree of reversibility, can deeply affect to the proportion and rate at which the odorant can be transferred to the vapor phases which will be directly perceived during the ortho or retronasal olfaction of the product. The challenge is how to gather reliable information not only about the chemical content of extremely diluted odorants, but also about their availability and potential concentration in the headspaces.

Yet more challenging is the understanding of the dynamism of the sensory properties of solid foodstuffs. In this case, there are several phenomena causing that the numbers and profiles of aroma compounds reaching the olfactory receptors during the processes of mastication and swallowing change with time. This poses a huge challenge for the flavor industry and the general food industry and several analytical strategies (APCI-MS, PTR-MS) and many funny devices for the continuous monitoring of the composition of the vapors exhaled during have been proposed in recent times.

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OP

Oral Presentations

OPO1

DEVELOPMENT OF A FAST METHODOLOGY FOR THE DETERMINATION OF NONYLPHENOL IN WATER SAMPLES BY MINIMAL LABELING ISOTOPE PATTERN DECONVOLUTION AND UHPLC-MS/MS

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Nonylphenols (NPs) are degradation products of non-ionic surfactants, NP polyethoxylates, which are widely used in plastics, textiles, paper and agricultural chemical products. These compounds mimic the structure of natural hormones which confers on them endocrine disrupting capabilities. The widespread use of NP polyethoxylates coupled with the harmful effects of alkylphenols had led to include NPs in the list of the priority substances of the Water Framework Directive. The new Proposal for a Directive amending the WFD (COM(2011)876) includes in the list of priority substances branched nonylphenol, a complex mixture of nonylphenol isomers which is known as "technical grade nonylphenol". Moreover NPs are usually found as contaminants in the laboratory material so their identification and quantification present considerable challenges.

In this work, a new method for determining 4-nonylphenol (technical grade) from water samples based on Isotope Dilution Mass Spectrometry (IDMS) and minimal labeling has been developed. To this end, 4-(3,6-dimethyl-3-hepthyl)-phenol (Ring $^{13}C_1$) was synthesized. The quantification was carried out by using isotope pattern deconvolution (IPD), which allows obtaining the molar fractions of the natural and labelled compound without methodological calibration. Since the ratio of molar fractions is equal to the ratio of the number of moles of the natural (N_{nat}) and enriched compound (N_{lab}), N_{nat} can be obtained directly, reducing the total analysis time [1]. Matrix-effects are corrected due to the methodology is based on IDMS analysis. Besides the calculation method, IPD allows overlapping in mass spectra and isotopic effects are avoided because there is only one labelled carbon atom in the molecule. This methodology was compared with the classical IDMS procedure employed in nonylphenol analysis based on the use of linear isomer 4-n-nonylphenol as internal standard and a calibration curve.

Analysis of the compounds were performed under isocratic conditions with 95% MeOH (0,05% NH_4OH/0,1mM NH_4Ac)/water in a reverse phase C18 column, and then detected by an ESI ion source working in negative mode with selected reaction monitoring (SRM). These conditions resulted in a run time of only 3 min with no column equilibration step required between injections. The combination of UHPLC-MS/MS in isocratic conditions with IPD calculations results in very short times for the determination of nonylphenol in water samples taking advance of the selectivity of MS/MS transitions to obtain an accurate quantification.

[1] González-Antuña, A., Rodríguez-González, P., Alonso, J. I. G. Anal Bioanal Chem (2012) 402, 1879–1888

OP02

OCCURRENCE OF POLYETHER IONOPHORES IN URBAN SEWAGE SLUDGE. AN ANALYTICAL METHOD BASED ON PRESSURISED LIQUID EXTRACTION FOLLOWED BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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A broad spectrum of organic chemicals is essential to modern society. Once discharged from industrial, domestic and urban sources into the sewer system they can be transferred to the residual solids during sewage treatment. Recycling treated sewage sludge on land is recognised internationally as the most sustainable option for managing the residual sludge from urban sewage treatment plants (STPs) and most risk assessment demonstrate that this practice does not place human health at risk from the majority of organic contaminants worldwide studied. However, continued vigilance in assessing the significance and implications of emerging organic contaminants in sludge is necessary to support and ensure the long-term sustainability and security of the beneficial agricultural route for treated sludge management [1].

In this study, we present the investigation about the presence of polyether ionophores (also known as ionophores) in sewage sludge. They are antibiotics mainly used to treat coccidiosis disease in livestock farms. Also, they have been used as grown promoters. These compounds are considered as emerging organic contaminants because their use has been on the rise in many developed countries in last years. Previous studies demonstrate their presence both in influent and effluent sewage [2, 3]. However, information about their presence in sludge is very limited.

Pressurised Liquid Extraction (PLE) has become a versatile tool for the extraction of a wide range of organic compounds in environmental solid matrices with a semi-automation of the extraction process and a low time and solvent consumption [4]. In present study, parameters affecting the extraction performance such as solvent, temperature and time extraction have been optimised. In addition, clean-up strategies (in-cell and solid-phase extraction) have been tested to reduce the matrix effect in the sample extracts. A liquid chromatograph coupled to triple quadrupole mass spectrometer (LC-MS/MS) method has been developed previously by us to determine ionophores in surface and sewage waters [5]. This method was applied for the determination of five ionophores (lasalocid acid, maduramicin, monensin, narasin and salinomycin) in sewage sludge collected in Tarragona's region.

[1] B.O. Clarke and S.R. Smith, Environ. Int. 37 (2011) 226.
[2] M. Scheurer, H. Brauch and F.T. Lange, Anal. Bioanal. Chem. 394 (2009) 1585.
[3] A.J. Watkinson, E.J. Murby, D.W. Kolpin and S.D. Costanzo, Sci. Total Environ. 407 (2009) 2711.
[4] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, Trends Anal. Chem. 29 (2010) 752.
[5] P. Herrero, F. Borrull, E. Pocurull, R.M. Marcé, J. Chromatogr. A (2012) submitted.

OP03

A METHOD FOR A COMPREHENSIVE STUDY OF THE SUBMICRON ORGANIC MATTER IN URBAN AEROSOLS

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Epidemiological and toxicological studies indicate strong adverse health effects of fine and ultrafine aerosol particles. However, the causative relations and mechanisms are not well known [1]. The organic fraction of atmospheric aerosols constitutes an important fraction of these particles (40-90%) [2]. Elucidation of the chemical composition is required for the development of efficient strategies for air-quality control and medical treatment of related diseases. The present work is carried out in the framework of a research project aimed to elucidate the chemical composition of organic matter in submicron aerosols (PM1: aerodynamic diameter <1μm) from urban areas under different situations in terms of pollution and meteorological conditions.

Sampling was conducted in Madrid and Barcelona. These cities represent urban areas with high contamination level from similar emission sources but different climatic conditions. The variability of the organic chemical composition of primary and secondary aerosols was studied at ground level and at 60 m height.

Analytical methodology has been developed to identify and quantify a wide range of organic compounds in PM1. The method includes an accelerated solvent extraction (ASE), followed by a fractionation by normal-phase high performance liquid chromatography (HPLC) based on components polarity [3]. The main organic components of primary and secondary submicron aerosol have been determined by gas chromatography coupled to mass spectrometry (GC/MS) and the non-volatile fraction by time of flight quadrupole coupled to mass spectrometry (QTOF-MS).

[1] J.A. Bernstein et al, J. Allergy Clin. Immunol. 114 (2004) 1116.
[2] J.L. Jimenez et al, Science, 326 (2009) 1525.
[3] I. Tolosa et al, J. Chromatogr. A, 1045 (2004) 71.

OP04

USE OF LIQUID CHROMATOGRAPHY/QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY TO IDENTIFY TRANSFORMATION PRODUCTS OF PROPANOLOL GENERATED DURING WATER TREATMENT BY TiO_2-PHOTOCATALYSIS

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The incomplete removal of pharmaceuticals and other emerging pollutants in conventional wastewater treatment plants (WWTP) has been recognized as the main way by which anthropogenic pollutants reach aqueous environments (Kümmerer, 2004). As a consequence, the presence of pharmaceuticals has become ubiquitous in natural waters, even allowing their entry into drinking water facilities. Some of the adverse effects of these pollutants for ecosystems have been reported but in many cases still remain unknown. The presence of anthropogenic pollutants in treated wastewaters also endangers their reuse, which is becoming a major issue in view of the growing water demand. The occurrence of b-blockers has been repeatedly reported during the last years in the effluents of many WWTP around the World. It has been clearly established that conventional wastewater treatment using activated sludge is not effective in completely removing propanolol and removal efficiencies in the 28-35% range for propanolol have been reported.

Several studies intended to eliminate presence of contaminants in wastewater effluents have pointed out to advanced oxidation processes (AOP) as a suitable choice for the removal of these compounds. Heterogeneous TiO_2-photocatalysis belongs to the category of AOP due to the formation of surface reactive oxygen species such as the radicals HO•, $O_2^{\bullet -}$ or $HO_2^{\bullet}$, highly reactive, that induce the mineralization of the organic contaminants. However, these processes are prone to produce multiple transformation products (TPs), also of interest from an environmental point of view. A comprehensive evaluation of wastewater treatment processes requires the identification and monitoring of these TPs during the treatments.

In this work, a liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-ESI-QTOF-MS) system has been used in the characterization of the TPs generated during the treatment of a water solution of the b-blocker propanolol by TiO_2-photocatalysis. A number of reaction intermediates were identified based on accurate mass measurements recorded by the instrument operating in positive mode (ESI+). MS/MS spectra were acquired at optimized collision energies to increase the fragmentation and thus improve structural information. These measurements allowed elemental compositions to be proposed for the protonated $[M+H]^+$ molecular ions and their characteristic product ions, so providing a high degree of confidence in structure assignation. In the same way as propanolol, the characteristic fragmentation of oxidation by-products provided enough information for the identification of over thirty transformation products. The appearance of characteristic fragments in the set of product ions spectra indicates the prevalence of a certain fraction of the molecule and suggests the place it should occupy in the transformation pathway. Despite the advantages of the technique, some drawbacks are also discussed. Finally, a degradation route of propanolol under TiO_2-photocatalysis is proposed

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OP05

WORKING TOWARDS THE EUROPEAN WATER FRAMEWORK DIRECTIVE FOR THE GCMS AMENABLE CONTAMINANTS.

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The EU water frame work directive (DIRECTIVE 2008/105/EC) poses stringent new detection limits for a list of priority substances in various water matrices. Many of those substances are GC amenable and can be analyzed with different injection and detection techniques.

The compounds have been divided into different classes, according to the standard analytical workflow in the environmental lab and the analytical method for each class is described, together with the current detection limits and showing results in various surface water samples.

The volatile compounds are analyzed using headspace GC, with the MS in SIM mode. All compounds are analyzed using three different SIM ions which are monitored continuously for their ratio. A brief discussion on other volatile pollutants, that are not included in the directive is included in this section.

For the semivolatile compounds a triple quadrupole MS is being used in MS/MS mode. All compounds are measured using two transitions of which the ratio is monitored continuously.

For the semivolatile analysis six compound classes are discussed: polycyclic aromatic compounds, pesticides, phenols, short chain chlorinated parafins, organotin and polybrominated diphenyl ethers. All compound classes pose their own chromatographic challenges and some solutions will be presented.

[1] Directive 2008/105/ec of the european parliament and of the council
of 16 December 2008
[2] Stockholm Convention on persistent organic pollutants (POPs)
STARTUP GUIDANCE for the 9 new POPs (general information, implications of listing,information sources and alternatives)December 2010
[3]. Retention-time database of 126 polybrominated diphenyl ether congeners and two Bromkal technical mixtures on seven capillary gas chromatographic columns Peter Korytar, Adrian Covacic, Jacob de Boer, Anke Gelbind,Udo A.Th. Brinkman
[4]M. Takeuchi, K. Mizuishi and T. Hobo, Analytical Sciences, 2000, 16, 349.
[5]R. Morabito and Ph. Quevauviller, Spectroscopy Europe, 2002, 4, 18
[6]Quantification of Organotin Compounds Using S/SL Injection and LVI-GC-MS by R.A. Correa C. and D. Claus
[7]Qua G.Centineo, P.Rodriguez-Gonzalez, E. Blanco Gonzalez, J.I. Garcia Alonso, and A. Sanz Medel, J, Mass Spectrum, 2004, 39, 485-494.
[8]Analysis of Organotins by LVI GCMS SIM, Thermo Application Note 10305, by Jessie Butler, Eric Phillips

OP06

ANTIMONIALS ACTIVITY AND RESISTANCE MECHANISM IN *LEISHMANIA SPP*., A MULTIPLATFORM METABOLOMICS APPROACH

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Metabolomics has become an invaluable tool to unveil the biology of parasitic diseases. One of them is leishmaniasis which causes significant morbidity and mortality worldwide: it has so far spread throughout four continents and is considered endemic in 88 countries, 72 of them developed, where economic globalization and increased travel have extended its reach to people.

This disease is produced by *Trypanosomatids*, which belongs to the *Kinetoplastida* order where species from the genera *Leishmania spp.* are included. It has exhibited several clinical manifestations that can be grouped as cutaneous, mucocutaneous and visceral forms, which is potentially fatal.

Organic pentavalent antimonials have been the first-line treatment against leishmaniasis for the last six decades and clinical resistance to these drugs has emerged as a primary obstacle to successful treatment and control. Alternative therapies are necessary and new treatments, such as miltefosnie, have been introduced: nevertheless, the mechanisms of action and resistance are still poorly understood.

It is currently accepted that there is no one single technique capable of obtaining the whole metabolic fingerprint of a biological system; the knowledge of the metabolic profiles of the cells is very important to understand its physiology. In this study, we have explored the capability of capillary electrophoresis, liquid chromatography and gas chromatography coupled to mass spectrometry (CE-MS, LC-MS, GC-MS) to unveil metabolic changes associated with pentavalent antimonials treatment in *Leishmania infantum* promastigotes (resistant and control strains) through a non-targeted approach. The combined information provided by these multiplatform analysis has shown arginine pathway to be key in the process. The biochemical mechanisms of action of antimony is supposed to kill parasites via oxidative stress. Arginine route is responsible for providing spermidine to the glutathione pathway, where it is used with glutathione to produce glutathionyl-spermidine and trypanothione, the main antioxidant defence of the parasites against the host cell. According to them, this behaviour is related to the response to miltefosine but, as far as we know, no description of this relationship exists although it can explain the cross-resistance presented by several patients.

OP07

CE/LC-MS MULTIPLATFORM FOR BROAD METABOLOMIC ANALYSIS OF DIETARY POLYPHENOLS EFFECT ON COLON CANCER CELLS PROLIFERATION

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In this study, an analytical multiplatform is presented to carry out a broad metabolomic study on the anti-proliferative effect of dietary polyphenols on human colon cancer cells.

CE, RP/UPLC, and HILIC/UPLC all coupled to TOFMS were combined to achieve a global metabolomic examination of the effect of dietary polyphenols on HT29 colon cancer cells.

By the use of a non-targeted metabolomic approach, metabolites showing significant different expression after the polyphenols treatment were identified in colon cancer cells. It was demonstrated that this multianalytical platform provided extensive metabolic information and coverage due to its complementary nature. Differences observed in metabolic profiles from CE-TOFMS, RP/UPLC-TOFMS, and HILIC/UPLC-TOFMS can be mainly assigned to their different separation mechanisms without discarding the influence of the different tools used for data processing. Changes in glutathione metabolism with an enhanced reduced glutathione/oxidized glutathione (GSH/GSSG) ratio were detected in polyphenols-treated cells. Moreover, significant alterations in polyamines content with important implications in cancer proliferation were observed after the treatment with polyphenols. These results from metabolomics can explain the chemopreventive effect of the tested dietary polyphenols on colon cancer and may be of importance for future prevention and/or treatment of this disease.

OP08

DETERMINATION OF NEW "LEGAL HIGHS", THE SYNTHETIC CANNABINOIDS, BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Introduction: Synthetic cannabinoids with THC-like psychotropic effects have emerged into the market in 2004. They are artificially sprayed into herbal mixtures sold on the Internet or smart shops, advertised as incense or air fresheners, and labeled "not for human consumption". However, they are actually acquired for a recreational use [1]. Since the end of 2008, different European countries and US states established regulatory policies to control some of these drugs; in spite of this, minor structural modifications on these compounds are constantly performed to avoid its legal control [2]. No legislation on these new drugs has been enacted in Spain.

Aim: To develop and validate a LC-MS/MS method for the determination of THC and the synthetic cannabinoids JWH-200, JWH-250, JWH-7, JWH-18, HU-211, CP-47,497 and CP-47,497-C8 in oral fluid. To apply this method to real specimens.

Methodology: 0.5 mL of oral fluid were fortified with 50 µL of internal standard (IS) mixture (JWH-73-d11, JWH-18-d9 and THC-d3 at 0.125 µg/mL, and THCCOOH-d3 at 0.05 µg/mL) and conditioned with 1 mL acetic acid 0.1 M before performing solid phase extraction with reversed-phase Strata X cartridges (Phenomenex®, Torrance, USA). Chromatographic separation was achieved using a Sunfire™ IS column (20 x 2.1 mm, 3.5 µm, Waters, Mildford, USA), with formic acid 0.1% (A) and acetonitrile (B) as mobile phase. A different chromatographic gradient was applied for the separation of the analytes, depending on the detection mode employed and, therefore, two injections were required. Detection was performed in a Quattro Micro™ API ESCI triple quadrupole (Waters, Mildford, USA), using electrospray in positive mode (ESI+) for JWH-200, JWH-250, JWH-73, JWH-18 and THC, and ESI- for HU-211, CP-47,497 and CP-47,497-C8. Two MRM transitions were selected for each analyte, except for HU-211, for which a second transition was not available. Validation of the method included the study of linearity, limits of detection (LOD) and quantification (LOQ), intra- (n=5) and inter-assay (n=20) imprecision and accuracy (n=20), endogenous (n=10) and exogenous (n=43) interferences, matrix effect (n=10) and extraction efficiency (n=5). The method was applied to 33 real specimens remitted to our laboratory in 2008-2009, which had previously tested positive for THC.

Results: Total run time for the two chromatographic gradients was 14 minutes. Linearity was verified from 0.1-2.5 to 200 ng/mL, applying a 1/x or 1/x2 weighting factor, with coefficients of determination >0.99. LODs and LOQs were 0.025 and 0.1 ng/mL for JWH-200, JWH-250, JWH-73 and JWH-18, 0.5 and 1 ng/mL for THC, and 1 ng/mL and 2.5 ng/mL for HU-211, CP-47,497 and CP-47,497-C8, respectively. Intra- and inter-assay imprecision were <15%, and accuracy range from 85-115%. Selectivity was verified as no quantifiable interferences were observed in blank oral fluid or those fortified with other drugs and medicines. Matrix effect experiments showed signal enhancement for JWH-250 and JWH-73 (<40%), similar to that of the deuterated IS employed for these analytes, and suppression for THC, HU-211, CP-47,497 and CP-47,497-C8 (-26.5 to -51.4%). Matrix effect was reproducible (%CV<18%, n=10), except for THC and THC-d3 at the high QC concentration (%CV<34%). Extraction efficiency was 65-109%. Only THC was identified in the analyzed real specimens.

Conclusion: A LC-MS/MS method was developed and successfully validated for the determination of JWH-200, JWH-250, JWH-73, JWH-18, THC, HU-211, CP-47,497 and CP-47,497-C8. None of the synthetic cannabinoids were detected in the analyzed real specimens, probably because they were collected in the early years in which these drugs were detected in Europe.

[1] CD Rosenbaum, SP Carreiro, KM Babu. J Med Toxicol. 8 (2012) 15-32.
[2] KA Seely, J Lapoint, JH Moran, L. Fattore. Prog Neuropsychopharmacol Biol Psychiatry (2012). doi:10.1016/j.pnpbp.2012.04.017

OP09

CE-LIF METHOD FOR SERINE ENANTIOMERS IN PLASMA WITH APPLICATION TO BIPOLAR DEPRESSION

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INTRODUCTION. Amino acids play a major role in energy metabolism, neurotransmission, and lipid transport and their quantitative analysis is increasingly important in disease diagnostics, and in elucidating metabolic influences on physiology[1]. Depression is a chronic, severe mental illness and a leading cause of disability worldwide. Recent clinical studies have demonstrated that a single intravenous administration of ketamine exert rapid antidepressant effects relief in about 60–70% of patients with treatment-resistant bipolar depression (BD)[2]. Notably, reductions in the NR1 subunit – which is a target of D-serine – have been implicated in mood disorders[3]. These bases raise the possibility that D-serine could be implicated in the effect of response to ketamine in bipolar depression patients. The accurate quantification of L- and D-serine amino acids in plasma of bipolar depression patients after ketamine infusion is highly important. Capillary electrophoresis is a well suited technique for biofluid analysis. It requires small sample volumes, and has high efficiency to separate considerable number of components and high sensitivity with LIF detection. Our group developed and validated a previous method based on CE-LIF for determination of amino acids in urine and hippocampus tissue. The aim of this study was to optimize the previous analytical methodology[1] to measure both enantiomers in plasma.

MATERIALS AND METHODS. The CE-LIF method was performed on a P/ACE MDQ system (Beckman-Coulter, Fullerton, CA, USA) equipped with a LIF detector with an Argon source operating at λexc: 488 nm and λem: 522 nm; a bare fused silica column (Beckman Coulter, Madrid, Spain) 60 cm in total length, 75 µm (ID). The running buffer consisted of: 175 mM borate buffer at pH 10.25 and 12.5mM native β-cyclodextrin. The voltage applied was 21 kV and the current observed was 140 µA. Samples were kept refrigerated at 7 ± 2ºC in a sample garage in the CE apparatus. Plasma from sixteen patients with BD (men and women aged 24 to 62 years) was analyzed. Patients received lithium as mood stabilizer. Plasma samples were filtered through a Centrifree® system to separate proteins and derivatized with 40 mM NBD-F as labeling agent. Reaction only lasts 15 min.

RESULTS. Several analytical conditions were optimized including BGE concentrations, pH and sample injection. The new method achieves the maximum resolution for L- and D-serine isomers and also L-phenylalanine, L-proline, L-leucine, L-isoleucine, L-lysine, L-ornithine, L-alanine, L-glutamic acid, L-threonine, taurine, glycine, L-aspartic, aminoadipic acid, acid and L-glutamine were identified. The quantification of L- and D- serine was performed in plasma samples from patients with BD that were infused with ketamine, and statistical differences were found for those patients who responded to the treatment from remitters in treatment-resistant BD.

CONCLUSIONS. New analytical methodology was developed for serine racemic quantitation in plasma by CE-LIF, giving the opportunity to find new statistically differences in amino acids on plasma related to BD disease.

[1] M.P. Lorenzo, A. Navarrete, C. Balderas, A. Garcia, J: Pharm Biomed Anal. (2012) DOI information: 10.1016/j.jpba.2012.03.017.
[2] N. Diazgranados, L. Ibrahim, NE. Brutsche, A. Newberg, K, Kronstein, S. Khalife, WA. Kammerer, Z. Quezado, DA. Luckenbaugh, G. Salvadore, R. Machado-Vieira, HK. Manji, CA. Zarate Jr. J: Arch. Gen. Psychiatry. 67 (2010) 08.
[3] O. Malkesman, AB. Hamidi, DR. Austin, Z. Cui, T. Tragon, CA. Zarate, HK. Manji, G. Wang, WS. Young, G. Chen, J: Int. J. Neuropsychopharmacol. 12 (2011) 01.

OP10

HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY USING FOLDED FLIGHT PATH TECHNOLOGY FOR PROFILING OF STEROIDS AND STEROID METABOLITES IN URINE

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Introduction:
Analysis of endogenous steroid levels has many important applications. These assays are usually performed on plasma samples in a targeted manner. The determination of steroid metabolites in other biological fluids is of variable clinical utility. High-resolution time-of-flight mass spectrometry (TOFMS) allows sensitive measurement of targeted steroid metabolites while simultaneously capturing a global profile of other analytes present in the matrix. This technology allows qualitatively and quantitatively useful data to be collected not just on the same instrument, but at the same time. The different qualitative and quantitative functions are accomplished post hoc with data analysis software, not by performing serial experiments or by compromising duty cycle in a single run.

Method:
The urine samples were hydrolyzed followed by SPE extraction according to conventional practice. The methanolic extracts were analyzed by an UHPLC system interfaced to an high-resolution time-of-flight mass spectrometer using positive mode electrospray ionization. LECO's CitiusTM LC-HRT high resolution time-of-flight mass spectrometer uses patented Folded Flight Path(FFPTM) technology to allow flight paths as long as 40 m. Flow injection peaks were about 3 seconds wide; time-of-flight mass spectrometry maintains resolution approaching 100,000 even at high spectral acquisition rates. Pulsed in-source collision induced dissociation (isCID) was used to acquire alternating parent and product ion spectra that were recorded on separate data channels. Beside flow injection other direct analysis techniques including DESI and LESA were tested.

Preliminary Data:
Endogenous steroid metabolites in urine samples were analyzed by liquid-chromatography / high-resolution time-of-flight mass spectrometry (LC-HRT). After normalization based on other analytes present in urine, time-course profiles of endogenous steroid metabolites correlated with known and induced biological variation. For example, variation in conjugated testosterone levels followed previously reported patterns in response to physical training. Collision-induced dissociation (CID) spectra of known analytes generated by LC-HRT were consistent with spectra previously reported from triple quadrupole and other MS-MS instruments and provided favorable database search results. These analytes included both endogenous steroids and some abused as dopants. CID spectra generated by direct analysis techniques including DESI and LESA also matched the spectra generated by LC-MS. The accurate mass measurement and accurate isotope ratio measurements included in the precursor and product ion spectra from the LC-HRT instrument provided a level of confidence in analyte assignment superior to triple quadrupole instrument data. All this information was obtained from a single chromatographic analysis, demonstrating several advantages of high resolution time-of-flight mass spectrometry.

Novel Aspect:
Fast screening method using direct analysis techniques in combination with a high-resolution time-of-flight mass spectrometer

OP11

HPLC-MS/MS TRACE DETERMINATION OF MULTICLASS UV FILTERS IN MILK

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Nowadays, it is known that some environmental factors play an important role in human health being this effect decisive in the early stages of life, including the perinatal period, because of the major development taking place. Some authors, for instance, correlate a limited foetal growing and some other anthropometrics values with an exposition to organochloride compounds during the gestation process [1].
Sunscreens (also known as UV filters, UV Fs) have risen especial concern, having been regarded as emerging environmental contaminants. These compounds are being used more frequently and in greater extent as consequence of the major knowledge on the harmful effects of the sun-light

A few studies on the behaviour of some UV Fs in wastewater treatment plants (WWTPs) show that their elimination is incomplete (<50%) [2]. The large majority of these UV Fs are lipophilic, tending to accumulate in sediments [3] and sludge from WWTPs [4], as well as to bioaccumulate in biota [5]. Furthermore, it is known that these compounds display biological activity. Both “in vitro” and “in vivo” assays show that most of them produce endocrine disruption [6], reproductive disruption and thyroid malfunction [7].

Despite the strong concern about the consequences on human’s health of exposures to biological active chemicals, especially endocrine disrupting substances, in the scientific community, very little information is available on the presence of UV Fs in food. In order to start filling this knowledge gap, a highly sensitive method was successfully developed and validated for the simultaneous detection and quantification of nineteen largely used UV filters and their transformation products with a wide range of physicochemical properties, in milk. Samples were extracted by pressurized liquid extraction (PLE), and the extracts were analyzed by liquid chromathography-tandem mass spectrometry using a hybrid quadrupole-linear ion trap-mass spectrometer (HPLC-(ESI)-QqLIT-MS/MS). The performance and applicability of the developed method are presented.

[1] M.J. López-Espinosa, M. Murcia, C. Iñiguez, E. Vizcaino, S. Llop, J. Vioque, J. Grimalt, M. Rebagliato, F. Ballester Pediatrics 128 (2011) e127.
[2] W. Li, Y. Ma, C. Guo, W. Hu, K. Liu, Y. Wang, T. Zhu, Water Res. 41 (2007) 3506.
[3] P. Gago-Ferrero, M.S. Díaz-Cruz., D. Barceló, Anal. Bioanal. Chem. 400 (2011a) 2195.
[4] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, Chemosphere 84 (2011b) 1158.
[5] K. Fent, A. Zenker, M. Rapp Environ. Pollut. 158 (2010) 1817.
[6] P.Y. Kunz, H. Galicia, K. Fent, Toxicol. Sci. 90 (2006) 349.
[7] C. Schmutzler, A. Bacinsk, I. Gotthardt, K. Huhne, P. Ambrugger, H. Klammer, C. Schlecht, C. Hoang-Vu, A. Grüters, W. Wuttke, H. Jarry, J. Köhrle, J. Endocrinol. 148 (2007) 2835.

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OP12

EVALUATION OF GC-MS AND LC-MS^n FOR DETERMINATION OF IMINOSUGARS IN NATURAL SOURCES

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Among the different life-style related diseases, diabetes is currently recognized as a major global health problem. The use of dietary supplements obtained from natural sources for preventing the onset of this illness has attracted considerable attention. Different studies have demonstrated that iminosugars are intestinal glycosidase inhibitors which decrease the post-prandial blood glucose concentration, reducing the risk of developing insulin resistance [1, 2]. These bioactive carbohydrates are present at very low concentrations in vegetable sources and their characterization is not trivial. Therefore, the development of sensitive and high resolution methodologies is a required task for their detection and quantitation. Although different attempts have been proposed by both GC and HPLC [3, 4], none of the existing methods allow a comprehensive characterization of both iminosugars and other coextracted interfering compounds such as low molecular weight carbohydrates (LMWC), whose content should be controlled for the therapeutic use of iminosugars.

In this work, different methods based on GC-MS and HILIC-MS^2 have been optimised and applied to the analysis of iminosugars and other LMWC in extracts from *Hyacinthus* sp.*, Fagopyrum esculentum, Morus* sp. and *Aglaonema* sp.

Different conditions (freeze-drying, vacuum evaporation, humidity of the sample, etc) and reagents for iminosugar derivatization were assayed previous to their GC-MS analysis. The best results were achieved using oximation followed by trimethylsilylation with hexamethyldisilazane as silylation agent. Chromatographic conditions (injection temperature, oven ramp, etc) were also optimised to obtain well resolved and symmetric peaks.

Three stationary phases for operation in HILIC mode have been assayed and compared for the analysis of iminosugars and LMWC by HPLC. The best results in terms of separation were achieved using the ethylene bridge hybrid with trifunctionally-bonded amide column. MS^2 fragmentation in positive mode using a Q-TOF provided useful information for the identification of the different iminosugars present in the samples under study.

GC-MS, which provided a better separation between iminosugars, appears to be the most appropriate technique to simultaneously quantify these carbohydrates. However, lower detection limits were found for iminosugars by using HILIC-MS^2. The developed methods have been shown to be complementary tools for the analysis of iminosugars.

[1] T. Oku, Brit J Nutr. 95 (2006) 933.
[2] H. Yokoyama, Tetradedrom Asymmetry. 18 (2007) 852.
[3] A.F. Magalhães et al., Phytochem. Anal. 13, (2002) 215
[4] S. Amézqueta et al., Anal. Bioanal. Chem. 402 (2011) 1953.

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OP13

UNAMBIGUOUS CONFIRMATION OF CYCLIC IMINES AS EMERGING TOXINS IN SHELLFISH HARVESTING AREAS OF CATALONIA (NW MEDITERRANEAN SEA)

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Cyclic imines are a group of lipophilic marine toxins that can be bioaccumulated in seafood. They comprise three main types of toxins (spirolides, gymnodimines and pinnatoxins) that have several spiro-linked functional groups and an imino group in their structure [1]. Although their fast-acting neurotoxicity in mice, there are no regulatory limits for cyclic imines in shellfish because these toxins have not been directly linked to human intoxication. Therefore, the European Food Safety Authority (EFSA) performed a risk assessment to human health related to the consumption of shellfish contaminated with cyclic imines, but it was not conclusive due to the lack of data about the occurrence of these toxins in seafood [2].

This work presents the first detection of spirolides and pinnatoxins in shellfish sampled in Catalonia (Spain, NW Mediterranean Sea). Spirolides were first detected in the Atlantic coast of Spain (Galicia region) in 2006 [3], but to the best our knowledge, the presence of pinnatoxins in Spanish shellfish has never been reported. These two toxins have been also detected in sea water using solid-phase adsorption toxin tracking devices (SPATTs) [4]. The detection by LC-MS/MS of spirolides and pinnatoxins was performed under alkaline chromatographic conditions using a QTrap 3200 hybrid triple quadrupole (AB/Sciex). The further identification of the compounds was performed in an 6340 Ion Trap (Agilent) and in an Orbitrap Discovery (Thermo Scientific). The complementation of these mass spectrometric techniques provided the optimum sensitivity, resolution and mass accuracy to quantify, characterize and unequivocally identify these new emerging marine toxins in Catalonian samples. The results of this work support the requests of EFSA to include cyclic imines in the shellfish safety monitoring programs to collect exposure data of cyclic imines to consumers, required to perform accurate assessments of the risk posed by cyclic imines in seafood.

[1] A. Otero, M.-J. Chapela, M. Atanassova, J.M. Vieites, A.G. Cabado, Chemical Research in Toxicology 24(11) (2011) 1817.
[2] EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal 8 (6) (2010) 1628.
[3] A. Villar Gonzalez, M. L. Rodriguez-Velasco, B. Ben-Gigirey , L. M. Botana, Toxicon 48(8) (2006)1068.
[4] L. A. MacKenzie, Current Opinion in Biotechnology 21(3) (2010) 326.

OP14

TRANSIENT ISOTACHOPHORESIS OF PROTEINS ON GLASS MICROCHIP WITH LASER INDUCED FLUORESCENCE DETECTION

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Capillary electrophoresis (CE) is a powerful separation technique for protein analysis that has been successfully used in targeted proteomics and biomarker discovery [1]. Its implementation on microchip format (MCE) has grown in the last two decades because its promising features; integration of several analytical steps, portability, high speed, high efficiency, reduced reagent consumption, low waste generation and high throughput (multiple/parallel separations) [2]. However, due to the small injection plug and the short optical path, detection sensitivity is a drawback in MCE. For that reason, laser induced fluorescence (LIF) is the most widely used detection method for MCE, due to its superior selectivity and sensitivity [2, 3].

On the other hand, proteins of interest in biological samples, i.e., as molecular makers for illnesses, are often present in trace amounts so the development of preconcentration strategies, both outside and inside the microchips is necessary. In this sense, isotachophoresis (ITP), that is a separation technique, has also been successfully applied in conjunction with CE for sample preconcentration. ITP can be carried out in CE in several modes. In one of the ITP modes, called transient isotachophoresis (tITP) [3, 4], the same capillary is employed for both the ITP pre-concentration and the electrophoretic separation, thus enabling an in-line approach which is easily adaptable to microchip format, By on-chip tITP, 20000-fold concentration of BSA was reported by Baba's group under SDS-denaturing conditions [5]. It is worth mentioning that in the majority of works that use on-chip tITP for protein concentration, the separation is carried out by gel electrophoresis mode in SDS-denaturing conditions [4]. At this condition, proteins are highly charged and possess high electrophoretic mobility so it is very easy to find a terminating ion. However, the excellent selectivity achieved in CZE is usually lost when gel electrophoresis is used as a separation mode.

In this communication, we present preliminary results about the preconcentration and separation of three proteins (α-lactalbumin, β-lactoglobulin and carbonic anhydrase) by on-chip tITP using zone electrophoresis as separation technique. This methodology was carried out in a glass microchip with laser induced fluorescence detection. A commercial polyacrilamide derivative, EOTrol® (Target Discovery, Palo Alto, CA), was used as dynamic coating to avoid protein adsorption on the channel surface and to reduce the electroosmotic flow (EOF). This latter point is crucial in the analytical strategy developed because EOF disturbs ITP process brodening the stacked protein bands. Proteins were labeled off-chip with the fluorogenic reagent Chromeo™ P-503 (Active Motif, Carlsbad, CA). We chose chloride ion and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) as leading and terminating anions, respectively. Several parameters such as the composition of leading electrolyte (LE) and terminating electrolyte (TE), injection time and separation distance were optimized to get the maximum concentration index (CI) along with acceptable peak resolution. Using the optimized methodology, CI close to two orders of magnitude and limit of detections below nM range were obtained for the studied proteins.

[1] X. Fang, B.M. Balgley, C.S. Lee, Electrophoresis 30 (2009) 3998.
[2] F. Shang, E. Guihen, J.D. Glennon, Electrophoresis 33 (2012) 105.
[3] N.T. Tran, I. Ayed, A. Palandre, M. Taverna, Electrophoresis 31 (2010) 147.
[4] T. Stroink, E. Paarlberg, J.C.M. Waterval, A. Buly, W.J.M. Underberg, Electrophoresis 22 (2001) 2375.
[5] J. Wang, Y. Zhang, M.R. Mohamadi, N. Kaji, M. Tokeshi, Y. Baba, Electrophoresis 30 (2009) 3250.

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OP15

IN VIVO SPME MONITORING OF PLANT UPTAKE OF ORGANIC MICROCONTAMINANTS

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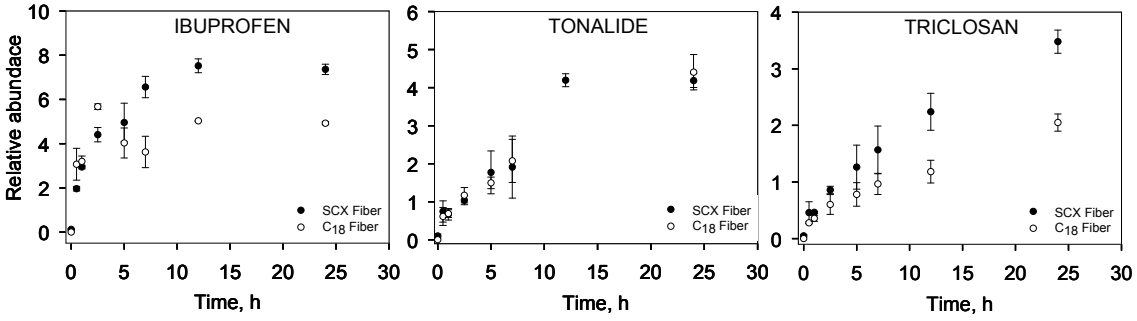
The use of reclaimed water for agricultural irrigation has become widespread in several countries (i.e., Spain, EE.UU., Israel, etc.) in order to cope with the increasing water demand resulting from a rise in population, climate change and limited amount of hydric resources. The use of reclaimed water in agricultural irrigation has direct benefits such mitigating water scarcity and providing the crops with nutrients (*1*). However, it is well known that reclaimed water is one of the main sources of organic micropollutants (OMs) in the aquatic compartment. Compounds recalcitrant enough can be uptaken by plants and possibly enter the food chain (*2;3*). In this regard, the uptake of OMs into crops irrigated with reclaimed water under field conditions has been already documented (*4*). Such uptake can be evaluated by means of SPME in vivo which allows the monitoring of environmental pollutants in plants and could assist in a spatio-temporal resolution of chemical processes in this living system (*5*).

The main goal of this study were firstly to assess the behaviour of the available SPME in vivo fibers with respect to analyte hydrophobicity and then apply the most suitable fiber in the monitoring of organic microcontaminants uptake in a fully functional plant.
There are two stationary phases for in vivo SPME, a commercially available C-18 coated silica and a experimental one SCX mixed mode (propylsulfonic acid on mixed mode with C-18 fiber) that contains an anionic exchange stationary phase. Both stationary phases were evaluated for the extraction of a variety of OMs from a celery extract from 0.5 to 24 h. Time extraction profiles were consistent with the analyte hydrophobicity. In this regard, the C-18 fibers were successful in the extraction of hydrophobic compounds like triclosan (log K_{ow} = 4.8) and tonalide (log K_{ow} = 5.9). Similar experiments performed with the SCX mixed mode fibers showed a broader range of applications from highly hydrophilic compounds like naproxen (log D_{ow} = 0.5 at pH 7) to highly hydrophobic compounds such as tonalide. Equilibrium conditions were reached in 10 h (*6*).



An experiment to assess uptake of target analytes on fully functional plants is underway using the SCX mixed mode fiber, taking into account two controls (a: soil+target analytes; b: soil+plant) and plants grown in soil spiked with the target analytes (PPCPs, antibiotics, stimulants, etc)..

[1] Graber, E. R.; Gerstl, Z., Israel J. Plant Sci. 59 (2011) 105.
[2] Boxall, A. B. A.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S., J. Agric. Food Chem. 54 (2006) 2288.
[3] Huang, A. T.; Batterman, S. Environ. Int., 36, 754.
Calderón-Preciado, D.; Jiménez-Cartagena, C.; Matamoros, V.; Bayona, J. M., Water Res. 45 (2011) 221.
[4] Vuckovic, D.; Risticevic, S.; Pawliszyn, J., Angew. Chem. Int. Ed. 50, 5618.
[5] Matamoros, V.; Calderón-Preciado, D.; Domínguez, C.; Bayona, J. M., Anal. Chim. Acta 722 (2012) 8.

OP16

IMPROVEMENT OF THE SELECTIVITY OF NICKEL SPE SORBENTS BY ION-IMPRINTING

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Metal ion extraction and quantification in the aqueous environment is a major issue. Quantification of a specific metal pollutant without using expensive laboratory equipments such as AAS or ICP-AES can be considered by selective extraction of that metal before measurement. Ion-imprinting technology is perfectly suited for that purpose.

Our objective was the elaboration of IIPs in a bead format for selective extraction of nickel(II) from an aqueous matrix by suspension polymerization. To avoid the transfer of the metal ion in the aqueous phase, inverse suspension polymerization was performed using mineral oil as the continuous phase. We thus reported the first attempt to prepare IIPs by a nonaqueous suspension polymerization[1]. We now compare two different ways of introducing the metal template during the IIP synthesis.

Vinylbenzyliminodiacetic acid (VbIDA) was polymerized with EDMA in the presence of nickel(II). IIP1 was prepared without prior complexation of Ni by VbIDA, whereas for IIP2 the $(VbIDA)_2Ni$ complex was previously isolated. The efficiency of the IIPs for nickel retention in presence of interfering species (Co, Pb and Zn) was measured by batch experiments with metal quantification by ICP-AES, and compared with non-imprinted polymers and Amberlite IRC-748 (a commercial resin with IDA moiety). Both resins IIP1 and IIP2 present high sorption capacities for nickel (10.8 and 10.7 mg/g). As shown in Fig.1, in presence of interfering ions, the sorption capacities remain very high with a significantly improved selectivity for IIP2. The comparison with Amberlite IRC-748 emphasizes the selectivity of both IIPs towards nickel.

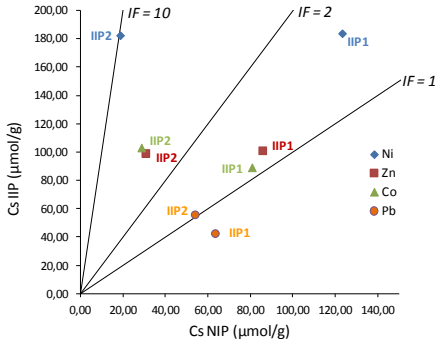
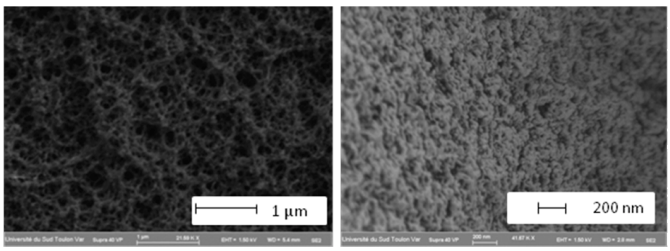


Fig.1: Sorption capacities of imprinted polymers vs non-imprinted polymers (IF = imprinting factor)



(a) (b)

Fig 2: SEM pictures of IIP1 (a) and IIP2 (b)

The characterization of the polymers porous structure by SEM (Fig.2) and nitrogen adsorption/desorption revealed that IIP1 present a micro-, meso- and macroporosity whereas IIP2 was only macroporous. The BET surface area calculations lead to 275 and 27$m^2.g^{-1}$ for IIP1 and IIP2 respectively. These results prove the importance of preforming the complex between the functional monomer and the metal template since IIP2 has a comparable sorption capacity with IIP1 and is much more selective with a less porous structure.

[1] W.Meouche, C.Branger, I.Beurroies, R.Denoyel, A.Margaillan, Macromol. Rapid Commun. 33(2012)928−932.

OP17

PCDDs, PCDFs AND PCBs IN WOMEN WITH ENDOMETRIOSIS: A CASE STUDY IN SPAIN

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There is evidence that polychlorinated dibenzo-p-dioxins (PCDDs) polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) may adversely affect the health of wildlife and humans[1]. Humans are daily exposed to complex mixtures of PCDD/Fs and PCBs mainly via trace amounts present in food[2].

Endometriosis is a gynecological disorder characterized by the presence of functional endometrial glands and stroma outside the uterus[3], which causes internal bleeding, inflammation and scarring, and often leads to pelvic pain and infertility[4]. Endometriosis has been estimated to affect as many as 10% of women of reproductive age[3]. It has been proposed that environmental compounds with endocrine-disrupting or estrogen-like activity, such as PCDD/Fs and DL-PCBs, may be involved in the pathogenesis of endometriosis[5]. The association of dioxins and PCBs to endometriosis has been supported by several experimental animal studies[6]. These studies prompted a series of hospital based endometriosis studies to investigate the association between dioxins and PCBs and endometriosis, which resulted in conflicting results[7]. Important differences in study design, patient selection, analytes assessed and assay methods affect the comparability of the results[8].

In a previous work, we reported a case-control design over 8 women with endometriosis and controls in Spain as a preliminary study[9]. This work is a continuation of the preliminary study started in 2008 and focused in women with endometriosis. The study consisted in determine whether an association between dioxins, DL-PCBs and marker PCBs and deep-infiltrating endometriosis (DIE) exists by analyzing blood serum and adipose tissue samples from women (n=20) who have been surgically confirmed to have DIE and a group of control women (n=20) in which endometriosis has been surgery discarded as a diagnosis. This study is remarkable as both adipose tissue and serum samples have been analyzed, whereas most other human studies have analyzed only blood samples. Results obtained in this study show that PCDD/F and PCB levels in serum from DIE subjects have slightly higher levels than the control subjects. However, this fact does not suggest a possible association of PCDD/Fs content with the occurrence of endometriosis. Since endometriosis is an understood disease, further studies are necessary to determine the factors that play a role in its etiology.

[1] World Health Organization, 1998. Executive summary— Assessment of the health risk of dioxins: Re-evaluation of the tolerable daily intake (TDI). WHO Consultation (Geneva).
[2] Fries G.F. *J. Anim. Sci*. 1995; 73:1639.
[3] G. Lebel, S. Dodin, P. Ayotte, S. Marcoux, L. Ferron, É. Dewailly. Fertil Steril 69 (1998) 221.
[4] J-F. Heilier, J. Donnez, D. Lison. Chemosphere 71 (2008) 203.
[5] J-F. Heilier, A. Ha, D. Lison, J. Donnez, R. Tonglet, F. Nackers. Fertil Steril 81 (2004) 456.
[6] E. De Felip, M Porpora, A. di Domenico, A. Ingelido, M. Cardelli, E. Cosmi, J. Donnez. Toxicol Lett 150 (2004) 203.
[7] B. Eskenazi, P. Mocarelli, M. Warner, S. Samuels, P. Vercellini, D. Olive, L. Needham, D. Patterson, P. Brambilla, N. Gavoni, S. Casalini, S. Panazza, W. Turner, P. Gerthoux. Environ Health Perspect 110 (2002) 629.
[8] A. Pauwels, P. Schepens, T. Hooghe, L. Delbeke, M. Dhont, A. Brouwer, J. Weyler. Hum Reprod 16 (2001) 2050.
[9] J. Parera, M. Ábalos, M.G. Martrat, E. Abad, F. Carmona, M.A. Martinez-Zamora, M.T. Galceran, J. Balasch, J. Rivera. Organohal. Compd. 70 (2008) 1554.

OP18

DEVELOPMENT OF A GC-LRMS METHOD FOR DETERMINATION OF NOVEL PERSISTENT FLAME RETARDANTS IN HUMAN SERUM

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Brominated flame retardants (BFR) are used to prevent or reduce the spread of fire in many consumer goods and household products. However, the use of several BFRs has been banned as they are toxic, highly persistent, lipophilic, and accumulate in food chains. Therefore, industry develops and introduces alternative flame retardants. In order to protect the environment and human health, it is important to survey the environmental occurrence and human exposure to such emerging and unregulated flame retardants [1,2]. Thus, analytical methods must be developed to determine the occurrence of novel halogenated flame retardants (HFR) in different matrices such as dust, air, animal tissues and human blood [3].

A fast, sensitive and effective method has been developed for the determination of the following HFRs in human serum; 1,2-bis[2,4,6-tribromophenoxy]ethane (BTBPE), decabromodiphenyl-ethane (DBDPE), hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO), hexabromo-benzene (HBB) decabromodiphenylether (BDE-209) and dechloranes (DPs syn and anti isomers).

We explored extraction of HFR from human serum using two different techniques: liquid-liquid extraction (LLE) and solid phase extraction (SPE). Heptane, toluene and methyl *tert*-butyl ether (MTBE) (and combinations of them) were tested for LLE and three different sorbents (Oasis HLB, Isolute ENV+ and Isolute C_{18}) were tested for SPE. Clean-up was performed on sulphuric acid–silica columns to remove lipids from the extracts. Highest recoveries with LLE were achieved with a mixture of heptane / toluene 1:1 (38 to 78 %, RSD < 22 %, n=5) and with Oasis HLB (20 to 82 %, RSD < 28 %, n=5).

It turned out that recoveries using SPE were comparable to LLE for the most hydrophobic analytes: DPs, BDE-209 and DBDPE, hence SPE was the technique chosen. Surprisingly, despite of their hydrophobicity, these compounds were not completely retained in the SPE column and they were detected in the aqueous waste. The percentages ranged from 8 to 25 % of the recoveries obtained in the SPE cartridge, whereas HBB, HCDBCO and BTBPE were fully retained on the sorbent.

HFR were separated on a DB-5MS column (15m x 0.25mm x 0.1µm) and quantified using gas chromatography coupled to mass spectrometry operating in electron capture negative ionisation mode.

[1] C.A. de Wit, Chemosphere 40 (2010) 583-624
[2] M. Alaee, P. Arias, A. Sjödin, A. Bergman, Environt. Int. 29 (2003) 683-689
[3] A. Covaci, S. Harrad, M.A.E. Abdallah, N. Ali, R.J. Law, D. Herzke, C.A. de Wit, Environ. Int. 37 (2011) 532-556

OP19

FULLY AUTOMATED ANALYTICAL METHOD FOR THE DETERMINATION OF PERFLUOROALKY SUBSTANCES ACCUMULATION IN DIFFERENT HUMAN MATRICES

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Perfluoralky subltances (PFAs) are ubiquitous contaminants in humans and animals worldwide [1]. Their properties of stability made them appropriates for their used in a lot of industrial and domestically applications. For their hydrophobic and lyophilise structure they shown affinity for proteins not for fat as classical POPs. PFAS are present in a number of environmental matrices [2-5] like water, food, soil, etc.

Furthermore, concern has increased about the toxicity of these compounds. In addition, different studies have shown that PFCs affects the lipid metabolism, disturbs the immunity system, can cause liver cancer and can be a cause of human infertility [6]. At the present time, few works exist reporting levels of PFAS in the human [7].

In the present work a high-throughput method for measuring trace levels of 21 PFCs in human in different human tissues have been developed. The method consists of an alkaline digestion with NaOH 10 mM in MetOH, followed by an on-line clean-up step using the TurboFlow™ technology coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method is sensitive, with LOD between 0.02 and 14 ng/g of tissue, involves minimal sample preparation. This approach requires the use of two extraction columns in tandem: C_{18} and Cyclone. The injection volume was 20 µl. The influent solvent consisted in water pH 3.4 at turbulent flow of 1.5 ml/min, 20 s. An extra clean-up step with water at 0.5 ml/min, 10 s, was included. The loop elution (250 µl) was performed with (water pH 3.4 : methanol (20:80)) and followed by (water at pH 3.4 : methanol (70:30)) at flow of 0.2 ml/min, 2 min. Separation was carried out in a LC-column Hypersil GOLD PFP (50 x 3) (Thermo Scientific). Chromatographic mobile phases were (A) aqueous ammonium acetate 20 mM, and (B) MeOH, and the total run time was16 min. Thermo Scientific TSQVantage mass spectrometer, coupled to TLX-2, was used for analytical purposes, equipped with a Turbo Ion Spray source operated in the negative mode and working in SRM. The analytical method was validated using animal matrices. The method showed high recoveries rates in the range from 50 to130%, and good reproducibility and repeatability were also shown. The applicability of the method was tested with 100 real samples of brain, rib, lung, kidney and liver.

[1] J.P. Giesy and K. Kannan, Environ. Sci. Technol. 35 (2001) 1339.
[2] Y. Pico, M. Farré, M. Llorca and D. Barceló, Critical Reviews in Food Science and Nutrition, (2010).
[3] M. Farré, S. Pérez, L. Kantiani and D. Barceló, TrAC - Trends Anal. Chem. 27 (2008) 991.
[4] Y. Pan, Y. Shi, J. Wang, X. Jin and Y. Cai, Bull. Environ. Contamin. Toxicol. 87 (2011) 152.
[5] J. Thompson, G. Eaglesham and J. Mueller, Chemosphere 83 (2011) 1320.
[6] C. Fei, J.K. McLaughlin, L. Lipworth and J. Olsen, Hum. Reprod. 24 (2009) 1200.
[7] A. Kärrman, J.L. Domingo, X. Llebaria, M. Nadal, E. Bigas, B. van Bavel and G. Lindström, Environ. Sci. Pollut. Res. 17 (2010) 750.

OP20

HIGH SUBMICELLAR LIQUID CHROMATOGRAPHY

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In 1980, the addition of a surfactant above the critical micellar concentration (CMC) in reversed-phase liquid chromatography (RPLC) was proposed as a way to modify the selectivity and analysis time. This gave rise to a new chromatographic mode, which was called micellar liquid chromatography (MLC). The total production of scientific reports in this technique up-to-date amounts to several hundreds. Many authors have demonstrated that the technique has several advantages regarding its large versatility produced by the interaction of solutes with different surfactants and organic solvents, the direct injection of physiological fluids which avoids the tedious sample pre treatment required in conventional RPLC, the suppression of peak tailing for basic drugs, and the analysis of samples containing compounds in a wide range of polarities using isocratic elution, among others

However, with conventional columns, solutions containing only surfactant were too weak and yielded poor peak shape. This was remediated by the addition of a small amount of organic solvent to the pure micellar mobile phase. Since then, in order to preserve the existence of micelles, analysts working in MLC avoid usually high amounts of organic solvent in the mobile phase. Nevertheless, there is no reason to neglect the potentiality of mobile phases containing a surfactant above its CMC in water and a high concentration of organic solvent, where micelles cannot be formed (submicellar conditions).

The new chromatographic mode has been called high submicellar liquid chromatography (HSLC), and can be considered as a bridge between MLC and conventional RPLC. There is no sudden breakdown of micelles with addition of organic solvent, and accordingly, the transition between MLC and HSLC is easily not noticeably. For this reason, in the literature, some authors have claimed to be working in MLC conditions, without being aware that no micelles were formed. The combination of stronger elution strength, larger selectivity and improved peak shape, with respect to MLC and conventional RPLC, makes HSCL a promising chromatographic mode, which achieves in practical times separations of compounds unresolved, or highly retained with other RPLC modes. The consumption of organic solvent in HSLC is higher with respect to MLC, which can be considered as a drawback. However, in the presence of surfactant, the risk of evaporation decreases due to the solubilisation of the organic solvent molecules by the surfactant. This facilitates mobile phase recycling.

This work offers some insights on the interactions that occur inside the chromatographic column, the modification of the stationary and mobile phases, modelling of retention, peak shape implications, and separation performance in HSLC, in comparison to MLC and conventional RPLC.

[1] A. Berthod, M.C. García-Álvarez-Coque, Micellar Liquid Chromatography, Marcel Dekker, New York, USA, 2000.
[2] X. Li, J.S. Fritz, J. Chromatogr. A 728 (1996) 235.
[3] J. Fischer, P. Jandera, J. Chromatogr. B 681 (1996) 3.
[4] M.J. Ruiz-Ángel, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, S. Carda-Broch, Anal. Chem. 80 (2008) 9705.
[5] M.J. Ruiz-Ángel, S. Carda-Broch, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, J. Chromatogr. A 1216 (2009) 1798.
[6] M.J. Ruiz-Ángel, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, S. Carda-Broch, J. Chromatogr. A 1216 (2009) 3199.
[7] M.J. Ruiz-Ángel, J.R. Torres-Lapasió, S. Carda-Broch, M.C. García-Álvarez-Coque, J. Chromatogr. A 1217 (2010) 7090.

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OP21

RETENTION BEHAVIOR OF PHTHALATE METABOLITES ON DIFFERENT LIQUID CHROMATOGRAPHY STATIONARY PHASES

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Phthalates, or phthalic acid esters (PAEs), are chemicals that have been used for more than 80 years in large quantities due to their wide range of applications, mainly in the plastic industry. Research conducted in recent years has evidenced that several phthalates and some of their metabolites (MPAEs) have activity as endocrine disruptors and, therefore, they are now considered as emerging contaminants and included in the priority list of dangerous substances in the legislation of many countries. Nowadays, it is widely accepted that an unambiguous assessment of the human exposure to phthalates can only be achieved by biological monitoring studies measuring the amount of PAEs and their metabolites.

The analysis of MPAEs has been carried out by a number of authors, using different analytical techniques. During the last years, and due to its robustness, sensitivity and selectivity, most procedures have been based on liquid chromatography coupled to mass spectrometry. In order to reach reliable results, a good chromatographic separation is needed before the ionization process and the subsequent mass detection. The most used stationary phases (SPs) to analyze MPAEs belong to octadecylsilane (ODS) and phenyl types, which separate these compounds using dispersive and π-π interactions, respectively. It is somehow surprising the lack of published separation procedures for MPAEs that employ SPs with alternative selectivity, such as the well-known polar-embedded type, capable of taking advantage of the ionizable nature of these compounds. Among these SPs, those with an amide group embedded into an alkyl chain show an especially high affinity toward substances capable of giving hydrogen bonds.

Therefore, the aim of this work was to study the chromatographic behavior of MPAEs, characterizing the mechanism that regulates their retention on octadecylsilane, phenyl and amide-embedded stationary phases. In order to carry out this study, a mixture of MPAEs was analyzed under isocratic conditions, using percentages of acetonitrile in the mobile phase ranging between 20 and 98%, with a final content of 0.1% of formic acid.

A markedly different behavior of MPAEs in each stationary phase was found. In the phenyl phase, the separation was driven by π-π interactions in the whole range of mobile phase compositions, but with low intensity at high contents of acetonitrile. On the other hand, the separation in the ODS phase was guided by dispersion forces, being the steric effects relevant up to acetonitrile contents as high as 80%. Finally, the plots of log k *vs.* acetonitrile percentage in the mobile phase for the amide phase revealed a combined retention process, due to the contribution of both dispersive and hydrogen-bond interactions, which were very intense, even at high contents of organic modifier in the mobile phase.

According to the results obtained, the use of an amide-embedded phase for separating MPAEs seems to be very promising, and could allow developing more versatile and faster liquid chromatography methods.

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OP22

USEFULNESS OF PARAFAC DECOMPOSITION AND D-OPTIMAL DESIGNS FOR THE DETERMINATION OF TRIAZINES IN ORANGES BY PTV-GC/MS WHEN DISPERSIVE-SOLID PHASE EXTRACTION IS USED FOR REGULATED ANALYSIS

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The setting of low harmonized minimum residue limits (MRLs) for pesticides in food [1] and the need of controlling their residues in a large number of food samples have highlighted the problem of working with complex matrices which require pretreatment stages to eliminate interferent compounds. Different techniques have been developed to address this problem, among them an approach known as the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multiresidue method [2], which is a rapid, straightforward and cost-effective procedure with which a large number of samples can be processed simultaneously.

The QuEChERS approach typically involves an extraction with acetonitrile followed by a clean-up step which consists of a dispersive solid-phase extraction (dSPE). But even after dSPE clean-up, QuEChERS extracts are usually relatively dirty because of the risk of removing pesticides along with other matrix compounds if refined clean-up steps are used, so the extracts can still contain co-extracted compounds. The presence of non-target compounds can cause false negatives during pesticides identification [3], since the maximum permitted tolerances for relative ion abundances established in the regulations will not be fulfilled if some fragments of the non-target compounds contribute to the abundance of the m/z ratios of the pesticides. The problem of overlapping peaks can be approached using parallel factor analysis (PARAFAC), a three-way technique which is capable of resolving signals if the data are trilinear [4]. The PARAFAC decomposition provides the same number of factors as there are compounds that coelute at the same m/z ratio, as well as the mass spectrum and chromatographic profile of each compound. And if some deviations in chromatographic profiles have to be modelled, then the PARAFAC2 model must be used.

In this work, we describe the determination of seven triazines (atrazine, prometryn, simazine, terbuthylazine, ametryn, simetryn and terbutryn) in orange, for which MRLs are established. Eight experimental variables that influence the PTV injection are optimized; the total number of experiments (384) is reduced to 16 using a D-optimal design for seven factors at two levels and one at three levels. Next, six variables of the QuEChER procedure are also optimized using another D-optimal design for five factors at two levels and one at three, reducing experiments from 96 to 10, which means an important saving in the optimization cost.

To calculate the permitted tolerance intervals for identification, three diagnostic ions are chosen for each triazine. The maximum permitted tolerances for the qualifier ions are exceeded when the analysis is carried out in the usual way, but this problem is obviated when PARAFAC and PARAFAC2 are used, leading to the correct conclusion that samples contain all the triazines. The use of these muti-way techniques makes it possible to unequivocally identify the triazines using the spectral and chromatographic profiles, as SANCO/12495/2011 states.

[1] Commission Regulation (EC) no. 149/2008 of 29 January 2008 amending regulation (EC) no. 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV. Off. J. L58 (2008)1.
[2] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412A.
[3] L. Rubio, L.A. Sarabia, A. Herrero, M.C. Ortiz, Anal. Bional. Chem. 403/4 (2012) 1618.
[4] M.C. Ortiz, L.A. Sarabia, J. Chromatogr. A 1158 (2007) 94.

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OP23

OPTIMIZATION OF A BREATHING TRAPPING METHODOLOGY FOR THE OFF-LINE GC-MS ANALYSIS OF RETRONASAL AROMA COMPOUNDS DURING DRINKING

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Retronasal aroma release during food consumption influences aroma perception and consumer preferences. Therefore, looking for analytical tools for *in vivo* monitoring aroma release during eating and drinking is becoming of great interest in flavour chemistry [1].

In this work, the optimization of a trapping aroma methodology for the GC-MS analysis of breath aroma during drinking has been performed. To do so, the efficacy of three aroma trapping polymers (Tenax, Lichrolut, and a PDMS/Carboxen/DVD SPME fibre) has been essayed in dynamic Purge&Trap experiments. The dynamic flow rate, amount of adsorbent, concentration of aroma compounds in the solution and the design of the trap (dimension and shape) were among the variables tested. Six aroma compounds with different physico-chemical properties (boiling points, polarities, hydrophobicities) were used to aromatise a synthetic alcoholic drink. In addition, a breathing trapping device (BTD) connected to a vacuum pump was design to fit the selected trapping material and to be used for trapping the breath aroma of different subjects during drinking. The aroma trapped in the adsorbent was eluted with pentane/diethyl ether or dicloromethane (for Tenax and Lichrolut samples respectively). An internal standard mixture was added to the aroma extract previous its concentration under gentle N_2 stream. The extract was injected into a GC-MS provided of a programmed temperature injector with a CIS and a Combi-PAL autosampler. The SPME fibre was thermally desorbed in the GC-injector.

Results from the Purge &Trap experiments showed that the best sensitivities were achieved when using the SPME fibre compared to Tenax and Lichrolut, although it exhibited the worst repeatability between fibers. Tenax and Lichrolut gave similar results; nonetheless, the repeatability within the same trap and between traps was better when using Tenax. In addition, this polymer could be easily thermically and chemically conditioned. Therefore, Tenax was used to set up the optimal trapping conditions in the BTD during drinking of a synthetic alcoholic drink. Qualitative analytical data (repeatability, linearity, LOD, LQD) were calculated showing that the BTD can be used for the trapping of retronasal aroma compounds during beverage consumption.

[1] C. Muñoz-González, J.J. Rodríguez-Bencomo, M.V. Moreno-Arribas, M.A. Pozo-Bayón, Anal. Bioanal. Chem. 401 (2011) 1501.

OP24

NEW GREEN ALTERNATIVES FOR THE EXTRACTION AND ANALYSIS OF 5-NITROIMIDAZOLES IN WATER AND MILK SAMPLES USING CAPILLARY ELECTROPHORESIS

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5-Nitroimidazoles (5-NDZs) are a group of antibiotics with wide spectra of action, being very effective against anaerobic protozoans and bacteria. Although some 5-NDZs as metronidazole, tinidazole and ornidazole are still used as human antibiotics, they are forbidden for treatment of animal diseases. 5-NDZs are believed to possess genotoxic, carcinogenic and mutagenic properties and also their use as veterinary antimicrobials can contribute to resistant bacteria appearance. Because of that, no residue of these substances can appear on any animal product destined to human consumption as it is established on Commission Regulation (EU) Nº37/2010 [1]. Besides, due to their high polarity and low biodegradability, what involves their bioaccumulation, they are environmental contaminants which are focus of health risk too. In order to monitor the illegal use of these substances, EU's Community Reference Laboratories (CRLs) recommend that analytical methods focused in 5-NDZ determination must detect at least 3 µg/L of these compounds in all matrices. Liquid Chromatography (LC) and Gas Chromatography (GC) are the most popular analytical techniques employed for 5-NDZs determination [2]. However, Capillary Electrophoresis (CE) has not been investigated so deeply for this purpose. For that reason, a new method based on the application of a CE mode so-called Micellar Electrokinetic Chromatography (MEKC) coupled with Ultraviolet (UV) detection has been developed for 5-NDZs determination in different matrices. Due to the lack of sensitivity offered by UV detection that makes hard to achieve the established minimum required limits of detection, preconcentration procedures are always needed. For this reason, in this work we propose the use of an on-line preconcentration procedure such as micelle stacking (sweeping) together with new green sample-treatments that include off-line preconcentration steps for the analysis of 5-NDZs in different water and milk samples, reducing the environmental impact and analysis time. Dispersive Liquid-Liquid Extraction (DLLE) has become one of the most attractive sample treatment procedures [3] being mostly limited to apolar extraction from aqueous samples. We present here, for the first time, the application of DLLE to the extraction of 5-NDZs from water samples with different origins, obtaining recoveries between 65-85% for at least seven 5-NDZs, including some metabolites. A preconcentration factor of 25 times has been obtained. A total of six extraction solvents, ten dispersive solvents and different combinations of them were tested. Finally, a mixture of 1600 µL of dibromethane as extraction solvent and 2000 µL of 2-propanol as dispersive solvent has shown to be the most effective. Extraction has been supporting by a salting out effect which involves the addition of 33% w/v sodium chloride to the sample. Moreover, this sample treatment was checked for the analysis of 5-NDZs in milk samples but not satisfactory results were obtained depending on the analyte due to the complexity of the matrix. As alternative, another simple and quick procedure based on Solid Phase Extraction (SPE) has been developed. This sample treatment simplifies other SPE process previously proposed for the extraction of antibiotics from milk and subsequent analysis by CE-UV. Fat removing, protein precipitation, clean up and pre-concentration of analytes have been carried out in only two steps. A volume of 3.5 mL of milk samples were treated by using Oasis MCX cartridges, achieving a preconcentration factor of 17.5 and detection limits lower than 1.80 µg/L. This methodology has been applied successfully to raw sheep's milk obtaining recoveries between 60-100% for nine 5-NDZs. Both sample treatments are useful alternatives for the analysis of 5-NDZs in real samples.

[1] Commission Regulation (EU) Nº37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
[2] C. Mahugo-Santana, Z. Sosa-Ferrera, Mª E. Torres-Padrón, J. J. Santana-Rodríguez, Anal Chim Acta 665 (2010) 113.
[3] A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, M.A. Rodríguez-Delgado Trac-Trend Anal Chem 29 (2010) 728.

OP25

DEVELOPMENT OF A COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY METHOD FOR THE CHARACTERIZATION OF GRAPE SEED PROCYANIDINS BASED ON THE COUPLING OF HYDROPHILIC INTERACTION AND REVERSED PHASE SEPARATIONS (HILICxRP).

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Procyanidins are a group of proantocyanidins, also called condensed tannins, widely distributed in the vegetable kingdom. These compounds consist of polymers of flavan-3-ols, in particular, catechin, its isomer epicatechin and epicatechin-3-O-gallate linked by C4-C8 or C4-C6 bonds (B type procyanidins) or by two inter-flavonoids bonds, a C-C bond and an ether C4-O-C7´ (A type procyanidins). Grapes (*Vitis vinifera*) are one of the richest sources of procyanidins, which are mainly found in the seeds.

These compounds have shown some interesting functional and bioactive properties such as antioxidant, antibacterial, antiinflammatory or anticancer activities. For this reason, there is a growing interest on the extraction and characterization of procyanidins. Nevertheless, these compounds constitute a rather complex and huge group of natural compounds, formed by different isomers with diverse degree of polymerization (DP, up to 37 units) and degree of galoilation. Consequently, conventional analytical techniques are not capable to provide the needed separation and identification power. In this regard, multidimensional chromatography offers enhanced separation abilities thanks to the coupling of different independent separations. Comprehensive two-dimensional liquid chromatography (LCxLC) provides much greater resolving power compared to mono-dimensional LC. In a comprehensive LCxLC system all fractions from the first column are continuously sampled and transferred by means of a switching valve to the second dimension column for further separation.

In this work, a novel LCxLC method has been developed for the characterization of grape seeds procyanidins. This new method was based on the challenging coupling between a first dimension separation based on hydrophilic interaction chromatography (HILIC) and a second dimension separation performed under reversed phase conditions (RPLC). A diol stationary phase microbore column was used in the first dimension whereas two different options (C18 solid core and C18 monolithic columns) were studied in the second dimension. The system was coupled to a diode array detector and to a mass spectrometer in series. The use of both detectors under optimum conditions allowed the separation and characterization of more than 50 different procyanidins (up to octamers) on grape seeds without the need of performing any sample pretreatment.

Consequently, this work contributes not only to the development of a new analytical procedure to analyze procyanidins, but also to increase the knowledge on the chemical composition of this interesting complex sample.

OP26

PESTICIDE ANALYSIS IN HERBAL TEAS BY LC AND GC TANDEM MASS SPECTROMETRY. SURVEY IN REAL SAMPLES

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Herbs are considered as any small plant with thin and tender stems which does not develop woody tissue and only live to bloom. Some of them are valued for its medicinal properties, flavour, or scent. Tea is undoubtedly one of the most consumed herbs all over the world. Increased interest in those beverages has resulted from perceived health benefits that may be associated with their consumption.

Taking into account the EFSA annual report of 2008 [1] it is very important to check extraction methods to analyze tea and herbs in general, because 5.8% and 4.5%, respectively, of the samples analyzed on the mentioned report contained more than 5 pesticide residues. Also, these matrices had the highest percentage of MRL exceedance rates (16% herbs and 9% tea). The aim of this work was to compare and choose the best method for extraction pesticides residues from green tea. Samples for studies were prepared from blank green tea. Tea was spiked with methanol solution of 86 pesticides (insecticides, fungicides and herbicides). Three popular extraction methods were selected for comparison. Those methods were: QuEChERS (to limit amount of coextractives $MgSO_4$ in clean-up step was replaced with $CaCl_2$), ethyl acetate extraction method and mini-Luke. Due to matrix complexity samples were diluted and 1 ml of final extract represented 0.2 g of tea sample. The analysis were carried out with a LC-QQQ-MS/MS and a GC-QQQ-MS/MS. Methods were compared in terms of recoveries, precision and matrix effects.

Once established the benefits and drawbacks of the studies extraction methods the modified QuEChERS method with $CaCl_2$ [2] was chosen. For the validation we used four different matrices (green tea, red tea, black tea and chamomile) and we determined linearity and matrix effects. Also we evaluated recoveries, precision (RSD %), repeatability and reproducibility (n=5) for the selected matrices at two different concentration levels, 25 and 100 µg/L in the sample, because for the majority of the compounds the maximum residue limit (MRL) in tea were 50 or 100 µg/L [3]. The validation procedure was made in a LC-QQQ-MS/MS and in a GC-QQQ-MS/MS following DG-SANCO guidelines [4].

Finally, we applied the validated extraction method to 75 real samples of different kinds of herbs purchased in 10 different countries (from European Union and third countries). Only six samples did not contain pesticides above the detection limit. Forty-eight compounds were found in 65 positive samples and 16 of them had exceeded MRL established by European Union [3].

[1] EFSA Journal 2010; 8 (6):1646; available at http://www.efsa.europa.eu/en/efsajournal/pub/1646.html
[2] http://quechers.cvua-stuttgart.de/pdf/q-tea.pdf
[3] EU MRLs, available at http://ec.europa.eu/sanco_pesticides/public/index.cfm
[4] European Commission DG-SANCO (2012) Method validation and quality control procedures for pesticide residues analysis in food and feed. No. SANCO/12495/2011

OP27

DETERMINATION OF MULTI-PESTICIDE RESIDUES IN DRIED TEA SAMPLES USING AN OPTIMIZED EXTRACTION / CLEAN-UP REGIME AND THE AGILENT 7000 SERIES TANDEM QUADRUPOLE GC/MS/MS SYSTEM

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A multi-residue pesticides analysis method by GC/MS/MS was evaluated for trace analysis of 135 representative pesticides in dried tea samples extracted by QuEChERS method.

This study showed different modifications of extraction procedure and validation of final method selected.

All analyses were done on an Agilent 7890 GC equipped whit an Agilent 7963B autosampler and an Agilent 7000 series GC/MS triple quadrupole system. An Agilent Ultra Inert GC column, HP5MS UI, was used to provide analyte separation and a highly inert flow path into the detector. We based to build up the MRM conditions in Agilent MRM database for the target analysis. Backflushing techniques were used to shorten analysis time for samples that contain high-boiling matrix residues.

For each pesticide, two MRM transitions were selected for quantitation and qualification. However, different transitions might be used for qualification according whit the high matrix effect in the Tea. Therefore was critical to review the data in matrix before setting up a quantitation method for this matrix.

After the validation of method, finally we can conclude:

- Simplified, rapid and optimized sample preparation for tea samples based on a modified QuEChERS regime whit Liq/Liq extraction.
- Sensitive detection – LCLs for most of analytes ≤ 0.005mg/Kg
- Excellent recoveries (70-120%) down to 0.01 mg/Kg
- Excelent repeatability ≤20%
- RTL for ease of method set-up/maintenance
-Capillary flow technology: Backflush – GC method robustness
- MS/MS Selectivity – low matrix interference
- Method suitable for detection and confirmation of pesticide residues well below the European Regulations MLRs (EC No.396/2005)

OP28

Comparative study of traditional SPE-LCMS approach and Direct Sampling Analysis (DSA) in the detection of target and non-target compounds.

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The analysis of trace compounds in complex matrix is often accomplished through a SPE purification and enrichment steps followed by LCMS analysis; this approach provides quality data and enables the detection of contaminants well below the legal limits, but the bottleneck of sample preparation is limiting the number of samples that a laboratory can analyze.

Direct Sampling Analysis (DSA) is an emerging technique enabling the acquisition of mass spectra directly from the sample without any purification or treatment for fast, accurate measurements; coupling DSA with target analysis software allows high throughput screening of samples and real time results.

In this study we'll compare the results provided by a last generation LC-TOF system with data acquired installing DSA probe on the same analyzer and we'll see how these techniques are complementing each other in the analysis of real life samples:

- Analysis of Pharmaceutical and personal care products in river water downstream of sewage treatment plant
- Common food contaminants (Pesticides, mycotoxins)
- Steroids and aminoacids in blood

The study will also report Direct Sampling Analysis of solid, liquid, pastes and gas samples for quality control and forensic use with unambiguous identification of analytes using the high mass accuracy and isotopic abundance data provided by Time of Flight mass spectrometer.

OP29

A NOVEL ELECTROSPUN MAGNETIC-BASED POLYURETHANE NANOCOMPOSITE FOR MICROEXTRACTION OF FLUOXETINE

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The electrospinning process is a simple and convenient method for producing nanocomposites with adjustable diameters, polarities and porosities. These nanofibers have malleability to conform to a wide variety of sizes and shapes and it is possible to control their composition to achieve the desired properties and functionality [1,2].

Following our research interests in electrospun nanofibers and their use in different microextraction systems [3-6], for the first time, a novel electrospun magnetic nanocomposite was fabricated and used as a medium for selective isolation and preconcentration of fluoxetine form aquatic and biological samples. In this work, a magnetic-based nanocomposite was fabricated by dispersing the magnetic nanoparticles in polyurethane solution and subsequent electrospinning of the doped polymer solution (Fig. 1). Taking advantage of the magnetic property of nanocomposites, these nano-scaled particles could be separated from sample solution easily and conveniently by a permanent magnet. After performing the extraction process using a piece of magnetic nanocomposite sheet, it was immersed in a tinny amount of acetonitrile for complete desorption in an interval of 5 min and its fluorescence spectrum was recorded.

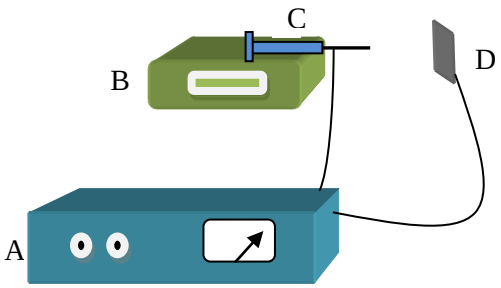

Fig. 1. Scheme of the electrospinning set up; A: power supply, B: Syringe pump, C: syringe containing polymer solution, D: Al foil, collector.

Various parameters affecting the extraction and desorption processes such as eluting solvent, amount sorbent, extraction time ,component ratio, pH and salinity of aqueous samples were optimized. The detection limit of the method under optimized conditions was 100 ng L^{-1}. The relative standard deviations (n = 5) at a concentration level of 400 ngL^{-1} was 3 %.

The method was linear for at least three orders of magnitude with correlation coefficient of 0.9997. The whole procedure showed to be conveniently rapid, efficient, selective and economical for extraction of fluoxetine from environmental and biological samples.

[1] X. J. Kang, C. Pan, Q. Xu, Y.F. Yao, Y. Wang, D.J. Qi, Z.Z. Gu, Anal. Chim. Acta 587 (2007) 75.
[2] N. Bhardwaj, S,C. Kundu, Biotechnol. Adv. 28 (2010) 325.
[3] H. Bagheri, A. Aghakhani, Anal. Methods 3 (2011) 1284.
[4] H. Bagheri, A. Aghakhani, M. Akbari, Z. Ayazi, Anal. Bioanal. Chem. 400 (2011) 3607.
[5] H. Bagheri, A. Aghakhani, Anal. Chim. Acta 713 (2012) 63.
[6] H. Bagheri, A. Aghakhani, M. Baghernejad, A. Akbarinejad, Anal, Chim. Acta, 716 (2012) 34.

OP30

IMPROVEMENT OF GC-MS CAPABILITIES BY USING ATMOSPHERIC PRESSURE CHEMICAL IONIZATION IN PESTICIDE RESIDUE ANALYSIS

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Although the use of gas chromatography (GC) coupled to mass spectrometry (MS) under electron ionization (EI) has been widely used for the determination of pesticides in food commodities, the interest in developing methodologies that include a larger number of compounds has increased in recent years. This would require not only selective and sensitive techniques, as tandem mass spectrometry (MS/MS) but also the use of more adequate ionization techniques. During the ionization process that takes place in EI source, the electron transfer at high energy usually produces high fragmentation of the molecules, leading to the loss of the molecular ion ($M^{+\cdot}$). Thus, transitions when working in MS/MS selected reaction monitoring (SRM) methods can loss in sensitivity (due to the numerous fragments in the MS spectra) and in selectivity (due to the common use of a precursor ion with a low m/z value).

In recent years, an atmospheric pressure chemical ionization (APCI) source has been developed, offering a robust approach for GC/APCI/MS technique with attractive analytical capabilities towards GC analysis. In this work, a multiresidue method for the determination of 150 pesticides in fruit samples has been developed using an APCI source coupled to GC-MS/MS with a triple quadrupole analyzer. The advantageous ionization of the APCI source has allowed to carry out method optimization selecting, in most cases, the $M^{+\cdot}$(or the protonated molecule $[M+H]^+$) as precursor ion in SRM transitions, which was possible for most compounds due to the soft ionization occurred in the APCI source.

As a previous step, the behavior of the different types of pesticides during the APCI ionization has been deeply studied in order to establish the capabilities of this new ionization technique, considering three groups: the first one for those compounds for which the absence of the $M^{+\bullet}$ in electron ionization spectra forces to select a lower m/z value as precursor ion in MS/MS, leading to a possible loss in specificity. The second group includes compounds that are highly fragmented, resulting in EI spectra rich in fragment ions. Thus, the complete spectrum shows many ions but all of them with poor intensity, leading to MS7MS transitions that are not sensitive enough to perform trace analysis. The third group includes compounds whose EI spectra show similarities to other compounds, making difficult to find selective and specific MS/MS transitions. It usually occurs to pesticides belonging to the same chemical family.

The method has been validated using orange, tomato and carrot matrices in terms of linearity, accuracy, precision, limit of quantification and limit of detection. Recovery experiments have been carried out at two fortification levels (0.01 and 0.1 mg/kg), obtaining satisfactory results for most analytes. Sample treatment has been based on QuEChERS procedure (AOAC Official Method 2007.01). In order to test the applicability of the method, real samples of orange, tomato and carrot have been analyzed.

As a conclusion, the GC-(APCI) MS/MS system has demonstrated a notable potential for quantification purposes by taking profit of the enhancement of the sensitivity and selectivity due to the good characteristics of APCI spectra with respect to EI ionization.

OP31

EXTRACTION TACTICS FOR A SUSTAINABLE WINEMAKING PROCESS: ANALYTICAL AND MULTIDISCIPLINARY APPROACH

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Solid and semi-solid byproducts from wine industry are a very rich source of bioactive polyphenols with a diverse range of bio-physicochemical properties implicated in different functional roles. Additionally, environmental and economic reasons support the interest to recover and exploit wastes from the food industry; being winery byproducts of particular interest, since grape (*Vitis vinifera*) is amongst the largest crop worldwide. On the other hand, one of the objectives of any industrial process is to become as sustainable as possible, and ideally self-sufficient.

In this context, our research group has been exploring the possibilities of green extraction techniques to get plant phenols from grape marc, obtained in the winemaking process of high quality white wines from Galicia (NW Spain). Matrix Solid-Phase Dispersion (MSPD) [1] and Pressurized Solvent Extraction (PSE) [2] have both been evaluated. Different factors that influence the extraction processes have been investigated by means of experimental design tools (mainly Screening and Response Surface Methodology). Advantages and drawbacks of the two different extraction approaches are discussed in comparative terms. Finally, the optimized extraction methods were applied to a wide range of bagasse samples from different *Vitis vinifera* white varieties.

These extracts have several potential ways of exploitation, as a source of bioactive phytochemicals with demonstrated antibacterial and antifungal activities; and as a raw extract with a high level of antioxidant activity. In this way, we could demonstrate that the white grape marc extracts showed specific Gram + antibacterial activity, the intensity of which is discussed in terms of the grape variety of origin. On the other hand, all the polyphenols extracts showed relevant antioxidant activity; the correspondent data will also be shown and comparatively discussed with the antiradical power of extracts from other origins.

Keeping in mind that the objective is proposing a sustainable exploitation of the winemaking process, the “technology transfer potential” to real wineries of both polyphenol extraction methodologies is also explored.

[1] M. Lores, M. Iglesias-Estévez, M. Álvarez-Casas, M. Llompart and C. García-Jares, Australian J. Grape & Wine Res. (2012) Submitted
[2] M. Álvarez-Casas, C. García-Jares M. Llompart and M. Lores, Journal of Chromatography A (2012) Submitted

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OP32

EFFECTS OF SFC CONDITIONS ON ENANTIOSELECTIVITY AND PERFORMANCE OF POLYPROLINE DERIVED CSPs

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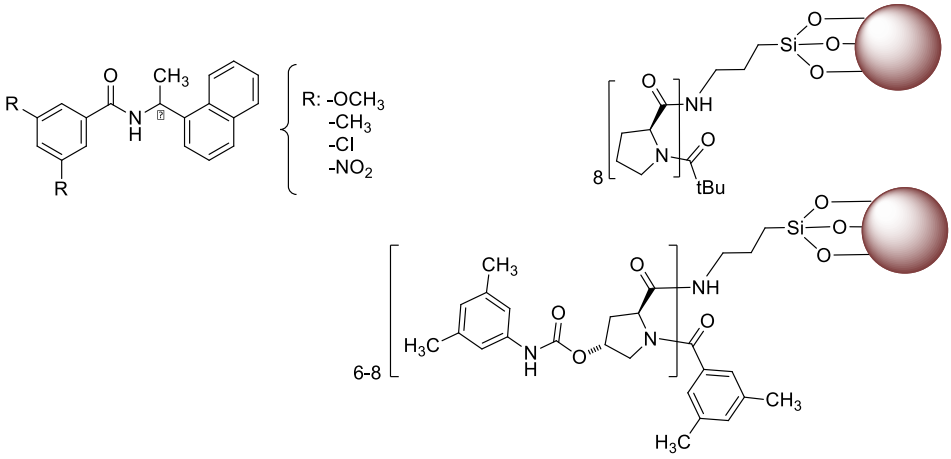
Chirality has a significant impact on drug discovery and development processes in the pharmaceutical industry. As the number of enantiopure drugs launched onto the market increases, the need for fast and high performance enantioseparation methods with minimal costs is becoming more overpowering. In this context, sub- and supercritical fluid chromatography (SFC), being applicable at an analytical, as well as at a preparative scale, is gaining more interest. In this context, the new ACQUITY UPC^2 (UltraPerformance Convergent Chromatography^{TM}) instrument used in this study is specially focused in lowering the dispersion of the system and providing reproducible and reliable data. Consequently, higher efficiency is obtained for the same column when compared with normal phase HPLC analytical conditions. These properties are advantageously applicable to enantioselective separations in which resolution constitutes a key point in the development of an appropriate analytical method.

In order to perform enantioselective chromatography, a chiral stationary phase (CSP) is required. In our group, polyproline helical structures acting as chiral selectors (CS), constitute one of the centers of interest [1,2]. On the basis of the chromatographic behavior, this is a new kind of CS [3,4], whose enantiorecognition mechanism has not been elucidated at present. Also, CSPs containing CSs of this kind are not commercially available yet.

Different solvents in normal phase conditions and isocratic mode were tested on our columns, obtaining the complete separation for a number of racemic analytes when hydrocarbon/alcohol mobile phases were used. The results obtained seem to confirm an enantiorecognition mechanism different from the commonly accepted for CSPs containing low-molecular-weight CSs (Pirkle type CPSs).

In this work, two distinct polyproline CSPs were tested using a series of compounds bearing aromatic rings of different electronic density in order to contribute to the study of the enantiorecognition mechanism for this kind of CS. Also, the comparative use of normal and SFC conditions confirms the hypothesis and demonstrates the advantages of the later respect resolution and analysis time.



[1] R. Sancho, A. M. Pérez, C. Minguillón, J. Sep. Sci. 29 (2006) 905-914.
[2] R. Sancho, C. Minguillón, J. Chromatogr. B, 875 (2008) 93-101.
[3] J. Huang, P. Zhang, H. Chen, T. Li, Anal. Chem. 77 (2005) 3301.
[4] M. Lämmerhofer, J. Chromatogr. A, 1217 (2010) 814-856.

OP33

CAPILLARY IONIC CHROMATOGRAPHY: A FURTHER STEP IN BROMATE ANALYSIS

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Ozone is one of the most powerful and used drinking water disinfectants. However, ozonation of drinking water containing bromide can result into the formation of the disinfection by-product bromate, a potential human carcinogen even at low µg/L concentrations. Nowadays, the U.S. Environmental Protection Agency (EPA) and the European Commission have established a regulatory maximum contaminant level (MCL) of 10 µg/L bromate in drinking waters. More recently, the European Commission set a lower MCL of 3 µg/L bromate for natural mineral waters and spring waters treated by ozonation.

One of the first published EPA methods for determining low concentrations of bromate in drinking waters used the direct injection ionic chromatography (IC) and was focused primarily on using columns designed specifically for carbonate eluents combined with suppressed conductivity detection (EPA 300.1). Despite the introduction of new Dionex hydroxide selective columns that improved the minimum detection limits (MDL), this option was not sensitive enough in those cases that the sample matrix contained the usual Chloride concentration (around 200 mg/L). A new step was done with the introduction of post-column reaction methods (EPA 317 and 326), followed by UV/Vis detection, to achieve lower limits. In those cases, even that the MDL were reached easily, the complexity and the lack of robustness were the main disadvantages.

To solve these drawbacks and to broad the range of bromate analysis methods, the U.S. EPA has recently approved a new method (EPA 302) where the two dimension (2D) ionic chromatography has been used. The reliability of the method has motivated our application laboratory to go one step further: include the use of capillary columns in the 2D ionic chromatography. With the aim to improve MDL and to contribute with the advantages of Capillary IC, this new configuration has been used for the bromate analysis in drinking water.

OP34

HPLC-MS/MS DETERMINATION OF FULLERENES IN SOLID SAMPLES

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Fullerenes are carbon-based nanomaterials which are known to be present in geologic registers (Premian-Triassic and Cretacious-Tertiary boundary layers) and rocks (shungites and fulgurites) all over the world due to several natural events (including volcanic activity [1], meteoritic impacts [2], lightnings and wildfires). In addition to this natural background, anthropogenic combustion and industrials processes have been directly linked to the recent detection of C_{60} and C_{70} fullerenes in atmospheric particulate [3] and wastewater samples [4]. Finally, the increasing relevance of nanotechnology industry opens new paths for the emission of functionalized fullerenes to the environment. Because all of this, a general consensus exists in considering fullerenes as a new family of emerging pollutants [5]. The analysis of fullerenes in soil samples is a challenging issue that has received relatively low attention while some traditional approaches, such as for instance Soxhlet, have exhibited poor performance in [6]

In the present work, ultrasound assisted extraction (USE) and accelerated solvent extraction (ASE) techniques have been explored. Several clean-up and acidic digestion procedures have been evaluated in order to solve the matrix effect issues and improve the recovery yields respectively. USE and ASE methods have been validated and several real samples have been analyzed. The presence of fullerenes in real soil and sediment matrices has been assessed.

58 real soils from Saudi Arabia (fuel-burning/industrial/urban activity) have been analyzed. Due to interference issues, the chromatographic performance has been especially optimized for the analysis of these samples. Moreover, sediment samples from the Llobregat River have been analyzed, and influence from the wastewater treatment plants discharges has been observed.

Preliminary results on the analysis of carbonaceous materials and about the performance of DART ionization source will also be explore

[1] Jehlička *et al*., Fullerene Sci. Technol. 8 (2000) 449.
[2] Becker *et al*., Nature 400 (1999) 227.
[3] J. Sanchís *et al*., Environ. Sci. Technol. 46 (2012), pp.1335-43.
[4] M. Farré *et al*., J. Hydrol. 383 (2010) 52.
[5] Richardson *et al*., Anal. Chem. 82, (2010) 4742.
[6] Jehlička *et al*.,, Carbon 43 (2005) 1909.

OP35

ANALYSIS OF POLYBROMINATED DYPHENYL ETHERS (PBDES), METHOXILATED PBDES (MEO-PBDES) AND HALOGENATED NORBORNENES IN DOLPHINS FROM SOUTHERN MEDITERRANEAN SEA (SPAIN) BY GC-MS-MS

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants for many years. Concentration levels of these compounds have been severally reported in different environmental matrices such as sediment, air and household dust, and also in biota samples such as human milk, fishes, marine mammals and different species of birds. Along with other harmful effects, PBDEs are endocrine and thyroid disruptors. Due to their toxicological effects, the production and use of commercial PBDE mixtures are banned in Europe. However, and in response to increasing international regulations on BFR formulations, alternative BFRs for achieving commercial product fire safety standards are being developed and used. Some of these non-BDE BFRs are pentabromoethylbenzene (PBEB), hexabromobenzene (hexaBBz) and decabromodiphenylethane (deBDethane) [1].

On the other hand, Methoxilated PBDEs (MeO-PBDEs) come from natural sources like sponges or blue mussels. Due to that, they are only found in the marine environment. They are considered thyroid disruptors and cytotoxic effects have been reported, but the information of its toxicity and metabolism is still limited. Levels of MeO-PBDEs have been reported in different top predators of aquatic food chains such as dolphins, polar bears or seals. Thus, it seems that they can bioacummulate and biomagnify among the food web [2].

Dechlorane plus (DP), Dechlorane 602, 603 and 604 (Dec 602, Dec 603 and Dec 604) are halogenated norbornenes used as flame retardants as substitutes for Mirex, which was banned as flame retardant in 1976. Although there were not supposed to have bioaccumulation capacity, these compounds have been found in biological matrices such as fish or bird eggs, and also in environmental matrices such as sediments, air or sewage sludge. Due to the increase in the restrictions for other classical flame retardants like PBDEs, the use of these compounds will probably increase. Therefore, more information about its current levels in the environment is needed [3].

Both PBDEs, non-BDE FRs and MeO-PBDEs where analysed by GC-EI-MS-MS. The EI-MS-MS methodology for PBDEs has been previously reported; on the other hand, the methodology for MeO-PBDEs and non-BDE FRs was previously developed. This methodology allows the determination of these compounds with an adequate sensitivity and selectivity and represents an alternative for the wide used GC-NCI-MS methodology. Halogenated norbornenes were analysed by a previously developed methodology by GC-NCI-MS-MS.

Twenty eight samples of two different species (*Tursiops truncatus* and *Delphinus delphis)* were collected in two different points, Gulf of Cadiz and Strait of Gibraltar, in February 2012. The strait of Gibraltar and the gulf of Cadiz are known worldwide as an important spot for study the cetaceans. Moreover, the fact the Atlantic ocean and the Mediterranean Sea converge gives makes this site really interesting. There are actually no published data about MeO-PBDEs nor Dechloranes in biota of this area.

In this work, we present the quality parameters for the GC-EI-MS-MS methodology for the analysis of MeO-PBDEs and non-BDE FRs, and also the levels found of the different compounds studied. Moreover, the isomer ratio of both isomers of DP (*syn*- and *anti*-) was studied and compared with the commercial mixture available. In addition, natural levels (MeO-PBDEs) were compared to the anthropogenic levels (Dechloranes, PBDEs and non-BDE FRs).

[1] A. Covaci, S. Harrad, M.A.E. Abdallah, N. Ali, R.J. Law, D. Herzke, C.A. de Wit, Environ. Int. 37 (2011) 532.

[2] L. Weijs, S. Losada, K. Das, L. Roosens, P.J.H. Reijnders, J.F. Santos, H. Neels, R. Blust, A. Covaci, Environ. Int. 35 (2009) 893.

[3] E. Sverko, G.T. Tomy, E.J. Reiner, Y.F. Li, B.E. McCarry, J.A. Arnot, R.J. Law, R.A. Hites, Environ. Sci. Technol. 45 (2011) 5088.

OP36

CHARACTERIZATION OF METHACRYLATE MONOLITHIC COLUMNS GRAFTED WITH EPINEPHRINE AND DERIVATIVES FOR CEC AND NANO-LC

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Organic-polymer based monoliths constitute a major category of monolithic materials. Advantages are easy preparation, no need of retaining frits, high permeability and easy tuning of pore size, large surface area and wide column functionalities. Control of the surface chemistry of the monoliths has been generally achieved using either direct copolymerization of functional monomers, chemical modification of reactive groups of the monolith, or grafting of functional polymer chains on the pore surfaces. Within this concern, the use of glycidyl methacrylate monomers has been strongly recommended for multi-step *in situ* functionalization of monoliths. In this work, epinephrine and its derivatives were used to functionalize glycidyl-based methacrylate monoliths. Owing to the presence of alcohol groups in its molecular structure, the epinephrine grafted columns allowed additional grafting, which constitutes a promising way to further manipulate the surface chemistry of the monoliths. In this work, the preparation of one- and two-step functionalized columns has been considered, where several experimental aspects (grafting reaction conditions, mobile phase composition and pH) were optimized. To study the separation mechanisms of the resulting columns in the CEC mode, neutral and ionizable compounds were used as probes. Subsequently, a comparison in terms of chromatographic performance between CEC and nano-LC was considered. Finally, the grafted monolithic columns were also employed as stationary phases for the separation of basic pharmaceutical compounds.

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OP37

PLASTICIZERS IN URINE AS MARKERS OF BLOOD TRANSFUSION IN SPORT

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One of the most common used plasticizers in polyvinyl chloride (PVC) products is di-(2-ethylhexyl)phthalate (DEHP). DEHP is present in PVC bags used for blood storage. Due to the presence of DEHP in bags used for transfusions there is a high exposure to this compound during a blood transfusion procedure. In this regards, the DEHP metabolites may be used as markers of the misuse of blood transfusion in athletes.

In this study, a method to quantify the main five DEHP metabolites, mono-(2-ethylhexyl)phthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate, mono-(2-ethyl-5-oxohexyl)phthalate, mono-(2-ethyl-5-carboxypentyl)phthalate, and mono-(2-carboxymethylhexyl)phthalate in urine has been developed and validated. The method involves a first step of hydrolysis, a liquid liquid extraction and a final analysis by ultraperformance liquid chromatography tandem mass spectrometry. The validation assays indicated an adequate reproducibility and reliability in the measurement of the metabolites.

Regarding the possible application of the analysis of the DEHP metabolites as a screening test for suspicion of blood transfusion in sportsmen, the concentrations of these metabolites were evaluated in different population groups.

In a first study, the urinary concentrations of the metabolites were determined in patients subjected to blood transfusions, in hospitalized patients subjected to medical treatments different to blood transfusions, and in a control group. The concentrations were significantly higher in patients receiving blood transfusion.

In order to verify the results obtained in the first study, the DEHP metabolites concentrations were evaluated in samples from healthy volunteers subjected to an experiment of autologous blood transfusion. The results confirmed that high concentrations of DEHP metabolites are excreted in urine after a blood transfusion process.

Additionally, the basal concentrations of the DEHP metabolites have been evaluated in samples of general population (control group) and in sportsmen samples. The results were in the low range and they were clearly different from those obtained in the previous transfused subjects; thus indicating a normal DEHP exposure of the general population. Moreover, threshold concentrations were proposed to determine whether a high result may be related to a transfusion process.

In conclusion, the DEHP metabolites are good markers of the use of blood transfusions and they could be used in the antidoping control laboratories to screen for the detection of these prohibited practices.

OP38

EVALUATION OF DIFFERENT POLAR COATINGS FOR STIR BAR SORPTIVE EXTRACTION OF EMERGING POLLUTANTS FROM ENVIRONMENTAL WATER SAMPLES

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Stir bar sorptive extraction (SBSE) is a sorptive technique that overcomes the limited capacity of solid-phase microextraction (SPME) fibers. Till very recently, the only commercially available phase for SBSE has been polydimethylsiloxane (PDMS), which, due to its non-polar nature, is ideally designed to extract non-polar compounds [1]. In recent years, efforts have been made to develop new materials for the SBSE of polar compounds. Currently, due to the increasing demand for suitable materials for extracting polar compounds, Gerstel has commercialised new stir bars with more polar phases, such as polyethylene glycol (PEG) Silicone, commercialised as EG Silicone Twister, and another that compromises polyacrylate with a proportion of PEG, currently at pilot stage as Acrylate Twister. With respect to the in-house prepared stir bar different approaches have been employed, that include sol-gel technology and monolithic materials, that improve the degree of polarity in the coating and so, enhance their retentions towards polar compounds [2,3].

New monolithic materials in stir bar form have been synthesised using different hydrophilic precursor monomers such as 2-hydroxyethyl methacrylate and pentaerythritol triacrylate using thermal-initiated free radical polymerisation. These new polar coatings were then applied to SBSE followed by liquid chromatography coupled to a triple quadrupole mass spectrometry (LC-MS-MS) for the determination of a group of emerging pollutants that covers different polarities from environmental water matrices. The main parameters affecting the efficiency during both the extraction (sample pH, ionic strength, matrix characteristics, agitation speed and extraction time) and the desorption (type of solvent, desorption mode and time) of the presented methodology were optimised.

The performance of these in-house coatings was also compared to the commercial available coatings: PDMS Twister, EG Silicone Twister and Acrylate Twister. As expected, under the conditions tested, the more polar the coating the higher affinity presented to polar analytes.

Moreover, the SBSE developed method was applied for the determination of these target analytes in different complex environmental samples, including river, effluent and influent waste water from treatment plant samples and showed good results of most of the analytes studied.

[1] R. Lourdes, J. Chromatogr. A 1221 (2012) 84.
[2] F.M. Lancas, M.E. Costa, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813.
[3] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernandez, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642.

POSTERS

CHE

Chemometrics

CHE01

COMPARATION OF TWO APPROACHES FOR COLUMN COUPLING

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Although there are a great deal of stationary phases having different selectivity properties, these very rarely act as a parameter to be optimized during the method development. The chromatographist selects first the stationary phase, in a trial-and-error fashion, and then optimizes the mobile phase composition, temperature and flow-rate. Recently, the advantage of a novel approach was demonstrated: the optimization of the best combination of serially connected columns of different length and nature, which has been called "stationary-phase optimized selectivity liquid chromatography" (SOS-LC). Some practical aspects of this approach are here described, using the same mobile phase and constant operating conditions. Usually, the overall selectivity of the combination of columns is better than that obtained with a single column varying the mobile phase composition. By use of different combinations the selectivity can be finely adjusted and even different elution order might be achieved.

This idea is highly attractive, but there are only few reports in the literature using this approach. In 2006, a system to couple columns was commercialized with this purpose, with the trade name POP-LC. This system suffers of a number of mechanical and technical problems:

(i) the high pressure of the instrument makes the pieces to fit tightly, which makes unscrewing difficult;
(ii) the holders that join the columns may not be sufficiently robust and suffer damage after some days of use;
(iii) the void volume between columns may be not negligible;
(iv) stabilizing the system at the beginning of the analysis may be time consuming (there may be problems of leakage and pressure stabilization);
(v) column packing may be not sufficiently homogeneous, making the results change when the order of the columns is altered or a new combination is made.

All these features result in poor reproducibility (i.e. the retention times are not really additive). These problems have been solved in our laboratory by coupling columns of different length and nature, using couplers with PEEK ferrules, recently developed by several manufacturers. This mechanism provides highly reliable results and the work is really comfortable. This will facilitate the future development of "stationary-phase optimized selectivity liquid chromatography", which is especially useful to resolve complex mixtures.

[1] K. Outinen, H. Haario, P. Vuorela, M. Nyman, E. Ukkonen, H. Vuorela, Europ. J. Pharm. Sci. 6 (1998) 197.
[2] P.H. Lukulay, V.L. McGuffin, J. Chromatogr. A 691 (1995) 171.
[3] Sz. Nyredy, Z. Szüs, L. Szepesy,Chromatographia 63 (2006) S3.
[4] M. Kuehnle, J. Rehbein, K. Holtin, B. Dietrich, M. Gradl, H. Yeman, K. AlbertJ. Sep. Sci. 31 (2008) 1655
[5] M. De Beer, F. Lynen, M. Hanna-Brown, P. Sandra, Chromatographia 69 (2009) 609.

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CHEO2

QUALITY ASSURANCE IN THE DETERMINATION OF TOTAL PETROLEUM HYDROCARBONS (TPH) IN SOIL BY GC – FID

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Mineral oil hydrocarbons are among the pollutants most often found on contaminated sites. These compounds can cause risk to health human and in the environmental receptors. Its quantification is reasonably done as a summation parameter that covers a defined boiling range and is often referred to as total petroleum hydrocarbons (TPH).

The objective of this work is the validation of the analytical procedure and the uncertainty estimation of the measurement to assure the obtention of consistent, reliable and accurate results of TPH determination in soils. The final purpose of the validated analytical method is the monitoring of the occurrence of TPH in soils from a northern Spanish region (Cantabria).

In this work TPH determination in soil was carried out by following the international standard ISO 16703:2004 [1] that describes the extraction procedure, clean-up of the extract and the boiling range to be quantified. TPH content was quantified using a GC2010 *Shimadzu* gas chromatograph equipped with a flame ionization detector (GC-FID) and a HP-1 capillarity column (15 m x 0.53 mm x 0.15 µm).

The validation of an analytical method consists in the demonstration of the fact that the studied procedure is adequate to obtain the expected result within the defined concentration range. The uncertainty is the parameter associated with the result of a measurement which characterizes the range within which the true value of the quantity is found [2]. Figure 1 illustrates the set of tools for quality assurance used in this work to evaluate the quality of the analytical method.

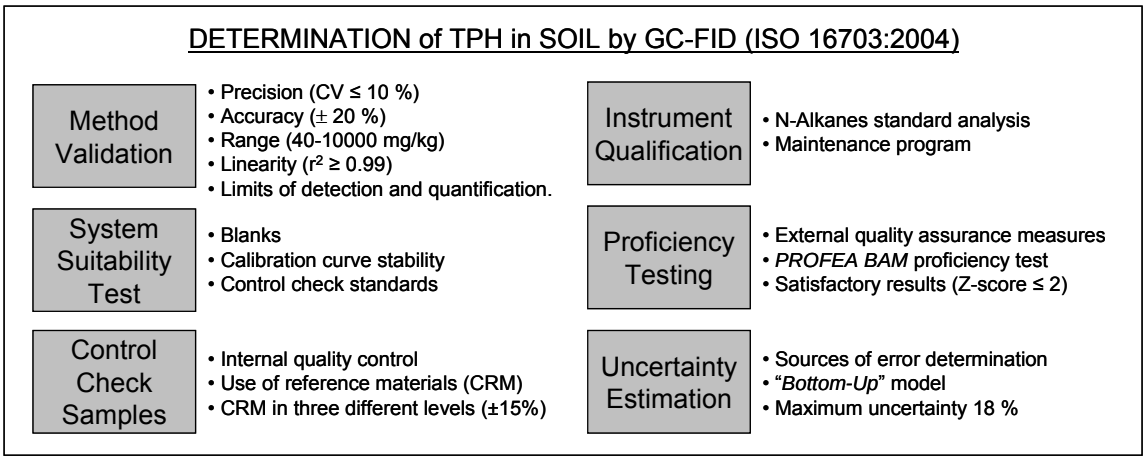

Figure 1. Quality assurance of the analytical method.

These results were used to assess positively the laboratory analytical activities and to obtain accreditation status according to the criteria in ISO 17025:2005 [3] by the National Accreditation Authority (*ENAC*). This international standard specifies general requirements to guarantee the technical and analytical competence and it is widely used as a quality system in environmental testing laboratories.

[1] International Standard ISO 16703:2004 "*Soil quality – Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography*" 1st edition 2004-11-01.
[2] EUROCHEM / CITAC Guide "*Quantifying uncertainty in analytical measurement*" (2000).
[3] International Standard ISO 17025:2005 "*General requirements for the competence of testing and calibration laboratories*".

CHEO3

RESPONSE SURFACE METHODOLOGY APPLIED TO SIMULTANEOUS OPTIMIZATION OF RESOLUTION, SENSITIVITY AND ANALYSIS TIME OF PHTHALATES METABOLITES ON A POLAR-EMBEDDED LIQUID CHROMATOGRAPHY STATIONARY PHASE

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Phthalates (PAEs) are well-known chemical compounds widely used as commercial plasticizers and in various applications and products, including textiles, medical equipment, electronics, and personal care products. In 2000, the European Union estimated a production of phthalates around 1 million tons per year in Western Europe (worldwide approximately 7 million tons), being diethylhexyl phthalate the 60% of the production. Humans are exposed to PAEs in numerous ways, e.g., by migration of phthalates into foodstuffs, by dermal absorption of cosmetics or simply by inhalation. Once in the organism, PAEs are rapidly cleaved to their respective monoesters and a portion is further metabolized to different oxidation products.

Several phthalates and some of their metabolites (MPAEs) have shown teratogenic, reproductive and developmental effects. Today, it is widely accepted that an unambiguous assessment of the human exposure to phthalates can be achieved by biological monitoring studies measuring not only the amount of PAEs but also their metabolites. As a result, a large quantity of research is now being conducted in order to know its behavior in the environment and in humans, their main pollution sources, their levels, and the way of decreasing them.

MPAEs are usually quantified by liquid chromatography coupled to mass spectrometry, most of the times using an octadecylsilane or a phenyl chromatographic column. The separation process in these columns is driven by dispersive and $\pi-\pi$ interactions, respectively. Bearing in mind that MPAEs share a very similar structure, relatively time consuming chromatographic runs are necessary to obtain an acceptable separation. The use of columns with stationary phases showing a greater selectivity to this type of compounds would be an alternative to reduce the analysis time. It has been reported that an amide group embedded into an alkyl chain presents an especially high affinity toward substances capable to give hydrogen bonds, which is the case of MPAEs.

Consequently, the objective of this work was to develop and optimize a liquid chromatography method to separate MPAEs in a stationary phase with an amide group embedded.

A Response Surface Methodology was used for method optimization, applying a Box-Wilson Central Composite design and choosing the initial content of acetonitrile in the mobile phase, the gradient time, and the pH value of the modifier (formic acid) as relevant parameters. A global optimum was obtained by using the Derringer's function value and selecting elution time, sensitivity and resolution as responses to optimize.

The resultant optimized model for the amide-type stationary phase, allowed a 50% of reduction in the analysis time, with good sensitivity and acceptable resolution.

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CHEO4

CHEMOMETRIC OPTIMIZATION OF ANTISOLVENT FRACTIONATION OF ROSEMARY HYDROALCOHOLIC ANTIOXIDANT EXTRACTS

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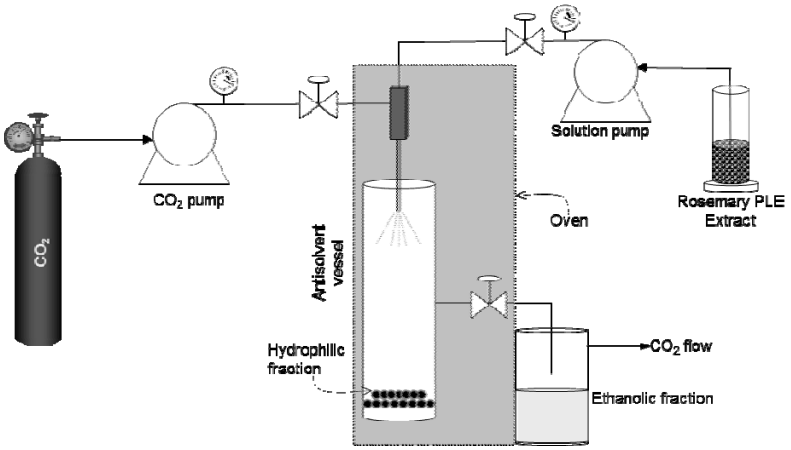
Rosemary (*Rosmarinus officinalis*, L.) is an aromatic plant widely known by its antioxidant properties. Several authors have correlated this antioxidant activity with the presence of two chemical families of compounds: phenolic terpenes (such as carnosic acid, carnosol…) and flavonoids (such as rosmarinic acid).

Pressurized Liquid Extraction (PLE) using ethanol has proved to be one of the main techniques for the isolation of those compounds, while other green extraction techniques such as SFE (Supercritical Fluid Extraction) and PHWE (Pressurized Hot Water Extraction) provide extracts mainly enriched in one family of compounds [1].

The objective of the present work was to develop a continuous method for the fractionation of PLE extracts in different families of bioactive compounds. To do this a self-designed equipment was set up (Figure) based on the one described by Catchpole et al [2]. In this system the fractionation of phenolic terpenes and flavonoids took place mediated by the antisolvent effect of supercritical CO_2 in the ethanol:water mixture.

The optimization of the process was carried out using a response surface methodology (RSM) based in two factors: Pressure and ratio (CO_2:PLE extract) flow rate at 3 levels. The selected responses for the optimization were the relative amount of each compound (rosmarinic and carnosic acid) in the vessels. Those compounds were quantified using HPLC-UV-ESI-MS.

The RSM allowed the optimization of the process by providing a mathematical model with a very high correlation with experimental data, which granted the prediction of the composition of further fractionation experiments. Moreover the system developed can be used as green preparative technique for further fractionations, without the need of using tedious preparative LC steps, because it can be run in continuous mode.



[1] M. Herrero, M. Plaza, A. Cifuentes, E. Ibáñez, J. Chromatogr. A 1217 (2011) 2512.
[2] OJ. Catchpole, JB. Grey, KA. Mitchell, JS. Lan, J. Supercrit. Fluids 29 (2004) 97.

CHEO5

SIMULTANEOUS DETERMINATION OF MALACHITE GREEN AND LEUCOMALACHITE RESIDUES IN FISH TISSUES AND FISH FARMING WATER BY MEANS OF A FIA SYSTEM WITH ELECTROCHEMICAL DETECTION

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Malachite green (MG), a triphenylmethane dye, has been used since 1933 as a veterinary medicine in aquaculture, fisheries and related food industries. The reason for its popularity derives from its broad antimicrobial spectrum and its effectiveness in preventing and treating fish diseases compared with other chemicals, especially against infections caused by fungus *Saprolegnia* and the parasite *Ichthyophthirius multifiliis*.

In fish MG is easily absorbed into tissues during waterborne exposure and extensively metabolized to the reduced colourless compound, leucomalachite (LMG), which is more accumulated in fish fat tissues for long periods of time than the chromatic form. Further, the control of the use of MG in aquaculture fish is also by LMG as marker residue.

Although malachite green is a suspected mutagen and teratogen [1, 2], recent studies suggest that leucomalachite green shows greater mutagenicity and tumorigenicity [3]. Therefore, malachite green is not authorised for use in food producing animals in the EU, despite its widespread use. However, consumers are exposed to MG residues from illegal use which makes it very significant to monitor these residues in aquatic products.

In routine analysis flow injection systems are interesting because of their fast response and low-cost instrumentation. The combination of this technique with electrochemical detection significantly increases the advantages offered by these systems which are at the same time highly sensitive. In this work, a procedure is proposed for the simultaneous determination o MG and LMG using a flow injection system with amperometric detection and a previous step of preconcentration and clean-up based on solid-phase extraction (SPE). The simultaneous determination of MG and LMG is described by means of a calibration model function based on a PLS model.

In the optimization of analytical procedures the response surface methodology [4] provides efficient experimental designs to model a response. In this work, the optimized experimental factors were: i) analytical cell potential; ii) flow rate and iii) percentage of acetonitrile of the carrier solution. However, in many occasions, these procedures are multi-response either because several analytes are simultaneously determined and/or because it is desired to have great signals with small variability, that is, more sensitivity and better repeatability are looked for. Here, the size and variability of the signal of MG and LMG are optimised using a desirability function, and under the optimal conditions the simultaneous determination of MG and LMG is carried out in fish tissues and fish farming water.

[1] S. Srivastava, R. Sinha and D. Roy, Toxicology, 66 (2004) 319.
[2] K. Mitrowska and A. Posyniak, Medycyna Weterynaryjna, 61 (2005) 742.
[3] R.A. Mittelstaedt et al., Mutation Reasearch, 561 (2004) 127.
[4] L.A. Sarabia and M.C. Ortiz, *Cap. 1-12: Response Surface Methodology*, in *Comprehensive Chemometrics. Chemical and Biochemical Data Analysis*, S. D. Brown, R. Tauler y B. Walczak, Editors. Elsevier, Oxford, 2009.

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POSTERS

CPA

Clinical and Pharmaceutical Analysis

CPA01

TOTAL SOLUTION FOR THE ANALYSIS OF 25-HYDROXY VITAMIN D2 AND D3 IN HUMAN SERUM USING AUTOMATED SAMPLE PREPARATION AND UHPLC-TOF

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Vitamin D (where D refers to D2 and D3) is a fat soluble vitamin. The major physiological function of vitamin D metabolites is to maintain calcium and phosphate homeostasis. Vitamin D insufficiency can result in rickets in children and osteoporosis in adults. Vitamin D status has been associated with a variety of diseases including cancer, diabetes, cardiovascular disease, osteoporosis and multiple sclerosis. 25-Hydroxy vitamin D is the metabolite measured in blood serum to determine the vitamin D status of patients.

The goal of this work was to develop a complete and robust Vitamin D analysis using a kit based solution with fast automated sample prep and UHPLC/ToF with ESI source for routine measurement of 25- hydroxyvitamin D22 and D3 in serum with a LOQ ≤ 2 ng/ml

This job has been accomplished using a PerkinElmer Janus robotic system equipped for liquid handling and SPE extraction. After cleanup the purified serum fraction has been analyzed using UHPLC-MS system based on PerkinElmer Flexar UHPLC and AxION2 Time of Flight mass spectrometer.

The high resolution, accurate mass data of AxION2 TOF allowed analysis of Vitamin D eliminating all the possible isobaric interferences in a linearity range of 1.4 to 150 ng/ml for hydroxyvitamin D in human serum.

Retention time reproducibility is less than 0.5% and LOQ for 25-hydroxyvitamin D2 and D3 in serum was<2ng/ml.

CPAO2

PROSTATE-SPECIFIC ANTIGEN (PSA) ISOFORMS STUDIED BY OFFGEL, 2-DE, AND CE-UV

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The concentration of prostate-specific antigen (PSA) in serum has been employed as prostate cancer biomarker for the last 20 years. However, in spite of being approved by the U.S. FDA for this aim, it is far from being an ideal molecular marker since an excessive number of false positives from the test originate an elevated number of unnecessary biopsies. In the last few months there has been a big controversy about the advantages and harms of PSA-based screening for prostate cancer in the general population, after the US Preventive Force Task recommended against it [1].

Other biochemical characteristics of PSA could improve its value as prostate cancer biomarker. In particular, it should be taken into account that PSA is a glycoprotein that presents heterogeneities due to variability in glycosylation and peptidic chain length. In fact, different spots (isoforms) of PSA can be resolved by two-dimensional gel electrophoresis (2-DE). These PSA isoforms have shown to be in different proportions in serum from patients with non-malignant prostatic diseases, such as benign prostate hyperplasia, compared to patients with prostate cancer [2].

Capillary electrophoresis (CE) allows separating peaks (isoforms) of PSA and it has advantages over conventional gel electrophoresis in terms of speed of analysis, quantification, and ease of automation [3]. However, 2-DE using Western blot for detection provides higher sensitivity than CE with UV monitorization and allows analyzing several samples simultaneously. Thus, both techniques could be complementary to study the role of the proportions of PSA isoforms as prostate cancer biomarker. In this regard, it would be of interest to correlate the PSA isoform profiles obtained by 2-DE with those obtained by CE-UV.

Because of CE-UV is not a preparative technique and the PSA fractions isolated from the spots of 2-DE are denatured, it is not possible neither to analyze by 2-DE the individual isoforms separated by CE-UV nor viceversa. To try to correlate the PSA patterns obtained by both analytical techniques, in this work PSA has been fractionated by OFFGEL, a separation preparative technique based on isoelectric focusing.

The aim of this study is to test the feasibility of analyzing by 2-DE and by CE-UV the PSA isoforms in the fractions obtained by the OFFGEL technique.

Since OFFGEL fractions are contaminated with chemicals used in this technique, different ways to eliminate these substances that interfere in the CE-UV analysis of PSA isoforms have been addressed.

The results obtained by the electrophoretical techniques indicate that each of the PSA isoforms does not focus on a single well of the OFFGEL system. However, enrichment of the different isoforms in different wells is observed.

[1] V.A. Moyer, U.S. Preventive Services Task Force, Ann Intern Med. 157 (2012) 1.
[2] A. Sarrats, J. Comet, G. Tabarés, M. Ramirez, R.N. Aleixandre, R. de Llorens, R. Peracaula, Prostate 70 (2010) 1.
[3] R. Garrido-Medina, J.C. Diez-Masa, M. de Frutos, Electrophoresis *32 (*2011) 2036.

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CPAO3

EVALUATION OF THE INCORPORATION OF SINGLE-WALL CARBON NANOTUBES IN POLYMERIC MONOLITHIC COLUMNS BY CAPILLARY ELECTROCHROMATOGRAPHY

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The considerable interest of polymeric columns in CEC results from the ease and flexibility of their *in situ* preparation, good permeability due to their large porosity, and easy tunning of column selectivity. On the other hand, the employ of carbon nanotubes (CNTs) has attracted much attention because of their special chemical and physical properties. Thus, CNTs have been used as adsorbents in solid-phase extraction and pseudostationary phases in electroseparation techniques. Then, the combination of monolithic column architecture and the specific features of CNTs could be an attractive way to obtain novel stationay phases for CEC.

The main objective of this study was to investigate the influence of single-wall carbon nanotubes (SWNTs) on the morphological and chromatographic properties of photo-polymerized butyl methacrylate (BMA)-based monoliths. The morphology of the resulting columns was characterized by scanning electron microscopies, whereas the CEC performance of different monoliths was evaluated by the separation of mixtures of anti-inflammatory drugs as probe solutes.

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CPA04

IN-LINE SOLID PHASE EXTRACTION-CAPILLARY ZONE ELECTROPHORESIS FOR THE DETERMINATION OF BARBITURATE DRUGS IN HUMAN URINE

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Barbiturate drugs are typical sedative-hypnotic drugs and, depending on the substituting groups, exhibit a wide variety of responses in the body. Barbiturates, in high doses, depress the respiratory system, which accounts for their toxicity [1]. These kind of drugs as other drugs of abuse, are mainly excreted in the urine in its original form, so this kind of sample matrix is very suitable for their determination in medical and forensic science [2]. The abuse of barbiturates is now widespread and the development of methods for their efficient separation and precise identification and quantification is needed [3].

The increasing interest in capillary electrophoresis as analytical technique is certainly based on its high efficiency, high resolution power, low reagent consumption, automation and in that it is a low-cost alternative compared with other chromatographic techniques. However, CE suffers from inherent low concentration sensitivity and the limits of detection (LODs) achieved when using this technique in comparison with liquid chromatography and gas chromatography are generally higher. In order to overcome that issue, it has been an increasingly interest in the development of several on-line preconcentration techniques in CE [4-9]. Among them, solid phase extraction (SPE) in-line coupled to CE is a very attractive combination because low LODs can be achieved due to the high sample volume that can be injected [6-11]. The most common setup as in-line SPE-CE design is the use of a small packed bed in which the sorbent is placed near of the inlet tip of the capillary.

The principal aim of this work was the determination of three barbiturate compounds in urine samples by in-line SPE-CE. Secobarbital, phenobarbital and barbital were preconcentrated and determined by in-line SPE-CE in urine samples. The separation buffer (BGE) used was 20 mM sodium tetraborate anhydrous (pH 9.2). The analyte concentrator consisted of a small segment of capillary filled with Oasis HLB sorbent and inserted into the inlet section of the electrophoretic capillary. Different parameters affecting preconcentration were evaluated, such as sample pH, the volume of the elution plug and sample injection time. The obtained results show that this strategy enhanced detection sensitivity in the range between 170 and 1840-folds compared with normal hydrodynamic injection. The developed method provides LODs for standard samples in the range between 0.5 and 5 ng/mL with good repeatability (values of relative standard deviation, %RSD < 7). Reproducibility values (expressed in terms of relative standard deviation) were below 9% for standard samples. The applicability of the optimized method was demonstrated by the validation with human urine samples spiked with the studied compounds. Repeatability and reproducibility values were under 8.6 and 10.4 respectively. The LODs obtained for urine samples were in the range between 5 and 60 ng/mL.

[1] A. Ashnagar, N.G. Naseri, B. Sheeri, Chin. J. Chem. 25 (2007) 382.
[2] L.-Y. Fan, T. He, Y.-Y. Tang, W. Zhang, C.-J. Song, X. Zhao, X.-Y. Zhao, C.-X. Cao, J. Forensic Sci. 57 (2012).
[3] G.A. Shabir, T.K. Bradshaw, S.A. Arain, G.Q. Shar, J. Liq. Chromatogr. & Rel. Techn. 33 (2010) 61.
[4] M.C. Breadmore, M. Dawod, J.P. Quirino, Electrophoresis 32 (2011) 127.
[5] L.A. Kartsova, E.A. Bessonova, J. Anal. Chem. 64 (2009) 326.
[6] P. Puig, F. Borrull, M. Calull, C. Aguilar, Anal. Chim. Acta 616 (2008) 1.
[7] P. Puig, F. Borrull, M. Calull, C. Aguilar, Trends Anal. Chem. 26 (2007) 664.
[8] R. Ramautar, G.W. Somsen, G.J. Jong, Electrophoresis 31 (2010) 44.
[9] R. Ramautar, G.J. de Jong, G.W. Somsen, Electrophoresis 33 (2012) 243.
[10] I. Botello, F. Borrull, C. Aguilar, M. Calull, Electrophoresis 33 (2012) 528.
[11] I. Maijo, F. Borrull, M. Calull, C. Aguilar, Electrophoresis 32 (2011) 2114.

CPA05

URINARY METABOLITES OF METHYLPREDNISOLONE AFTER ORAL AND TOPICAL ADMINISTRATIONS

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Glucocorticosteroids are included in the list of banned substances in sports of the World Antidoping Agency (WADA). All glucocorticosteroids are prohibited when administered by oral, intravenous, intramuscular or rectal routes. However, inhalation, intra-articular administration and topical preparations are allowed. Since some glucocorticosteroids are marketed in different administration forms, the distinction between different routes of administration through the analysis of urine samples is needed. In an attempt to distinguish between allowed and forbidden administration ways, WADA established a reporting level of 30 ng/mL for all corticosteroids. The aim of this study was to elaborate analytical strategies to be used for the differentiation between authorized and forbidden administration routes for methylprednisolone (MP) in sport.

MP was administrated to two healthy male volunteers by oral route (40 mg), and to two healthy volunteers by topical (10 mg/day for 5 consecutive days) and oral administrations (4 mg). The samples were analysed using an LC/MS/MS method specifically developed to detect MP and 15 metabolites.[1] In order to investigate the best marker to distinguish between oral and topical administration, concentrations and ratios of the different metabolites were calculated. All metabolites were detected in urines collected after oral intake up to at least 36h. Only, MP and 5 metabolites were detected in urine samples obtained after topical treatment. As expected, concentrations of MP after topical administration were well below the current threshold (30 ng/mL). However, after the low oral dose (4 mg), 3 out of 4 samples in 8-24h range were also below that threshold. Considering all metabolites detected after both administration routes, 11β,17α,20α,21-tetrahydroxy-6α-methylpregna-1,4-diene-3-one and 16β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,11,20-trione appear to be best markers to differentiate between topical and oral administrations. In all cases, their concentrations after a topical administration were lower than those obtained during the first 36 h after the intake of both oral doses assayed.

[1] OJ. Pozo, J. Marcos, X. Matabosch, R. Ventura, J. Segura. Using complementary mass spectrometric approaches for the determination of methylprednisolone metabolites in human urine. Rapid Commun Mass Spectrom. 2012, 26, 541-53.

CPA06

CAPILLARY ELECTROPHORESIS OF GLYCOSYLATED AND DEGLYCOSYLATED ALPHA-1-ACID GLYCOPROTEIN ON SU-8 MICROCHIPS

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In the last few years, the biotech industry has been one of the fastest growing segments in the pharmaceutical area, due to the development of new products, named biologics, which are usually obtained from DNA recombinant techniques. Biologics marketing requires the use of novel bioanalytical tools to address new regulatory aspects [1-2]. On the other hand, their fabrication and purification processes are highly complex; this fact prompted the development of new analytical methodologies which entail comprehensive characterization by sensitive and highly efficient analytical techniques. These methods allow the control of the active principle during the fabrication process and the quality of the finished product in a fast and simple way. Some of these biopharmaceutical drugs have glycans attached to the polypeptidic chain, which are essentials for their activity. For this reason, the control of the glycosylation and/or -deglycosylation along the down-streaming process is one of the key point of the production of some of these biologics [3].

Some of the most employed analytical methodologies in quality control of biologics are SDS-PAGE and capillary gel electrophoresis (CGE) of denatured proteins. Capillary electrophoresis has allowed the analysis of these products in less time and with higher automatization than with SDS-PAGE. However, it is known that for analysis of some proteins even better results can be obtained employing capillary electrophoresis in microchips format (MCE). That is due to several advantages of MCE such as high speed analysis, high efficiency, reduced reagent consumption, high throughput analysis (multiple/parallel separations) and integration of several analytical operations in the same chip [4].

In this communication we have evaluated the potential of SU-8 microchips in analysis of glycosylated and deglycosylated proteins. We have selected the alpha-1-acid glycoprotein (AGP) as a model protein of biologics, because this protein has a high content of glycans (more than 40% in weight) and it is easier and cheaper to obtain and purify than most of the commercial biologics. First, AGP was deglycosylated with PNGase F according to the previous experience in the group [5]. Then AGP samples both glycosylated and deglycosylated were labeled off-chip with the fluorogenic reagent Chromeo™ P-540. Finally, we have developed a simple method to separate glycosylated AGP from deglycosylated forms of this protein by CGE in a SU-8 microchip with laser induced fluorescence detection. Dextran was employed as sieving matrix and EOTrol® was employed as a dynamic coating to suppress the EOF. The electrophoretic results were compared with the results obtained by MALDI-TOF of these samples with good correlation between them. The methodology developed by CGE allows separating these proteins in a few minutes, with good resolution, demonstrating the potential of the SU-8 microchips for quality control of glycosylated biologicals.

[1] A. Guttman, A.S. Rathore,I.S. Krull, LC GC North America, 2012. http://www.chromatographyonline.com/lcgc/Column%3A+Biotechnology+Today/Bioanalytical-Tools-for-the-Characterization-of-Bi/ArticleStandard/Article/detail/773765.
[2] T. Logtenberg , Trends in Biotec., 25, 9 (2007) 5.
[3] M.J. Little, D.M. Paquette, P.K. Roos, Electrophoresis, 12 (2006) 2477.
[4] B. Antes , P. Oberkleiner, A. Nechansky, O. Szoar, J. Pharm. Biomed. Anal. 51 (2010) 7.
[5] S. Ongay, C. Neussuss, Anal. Bioanal. Chem. 398 (2010) 11.

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CPAO7

ANALYSIS OF FREE AMINO ACIDS BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY–ELECTROSPRAY–MASS SPECTROMETRY EMPLOYING A VOLATILE SURFACTANT

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The determination of amino acids (AAs) in biological samples is important for metabolic profiling and the screening of deficiencies in amino acid metabolism. Capillary electrophoresis–mass spectrometry (CE-MS) has shown strong potential for AAs analysis, discriminating between analytes and matrix compounds. Micellar electrokinetic chromatography (MEKC) is a CE mode in which compounds are separated by differential partitioning between micelles (pseudo-stationary phase) and the surrounding aqueous buffer solution. MEKC offers potential resolution of compounds with similar structure. However, commonly used surfactants (as SDS) are non-volatile and can cause analyte signal suppression and contamination of the mass spectrometer. In this study direct coupling of MEKC to MS is pursued using ammonium perfluorooctanoate (APFO) as a volatile surfactant. The aim of the study was to enhance the electrophoretic separation of AAs while maintaining good MS compatibility. Sheath-liquid electrospray ionization was used for MEKC-MS interfacing.

The influence of APFO on the MS signal of AAs was evaluated by infusion experiments showing that APFO hardy affects signal intensities and presents significantly less ion suppression than equal concentrations of ammonium acetate. In order to obtain efficient MEKC separation of AAs, the pH and APFO concentration of the BGE was optimized. Overall best resolution, including baseline separation of Leucine and Isoleucine, were obtained using 150 mM at pH 9.0 representing a considerable improvement over capillary zone electrophoresis at pH 9.0. Further optimization of sheath liquid composition and flow, and interface and MS settings led to limits of detection ranging from 0.01 to 0.10 mg l^{-1} for the 20 tested AAs. Good linearity ($r^2 > 0.99$) and satisfactory reproducibility were obtained for all AAs with RSD (%) values for peak area between 3.0 and 6.7 %. The applicability of the new method is demonstrated by the quantitative determination of AAs in urine employing minimum sample pretreatment (i.e. 1:1 mixing with BGE).

CPA08

ANALYSIS OF FREE HYDROXYTYROSOL IN HUMAN PLASMA FOLLOWING THE ADMINISTRATION OF OLIVE OIL

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Hydroxytyrosol (HOTYR), the main polyphenol of olive oil is purported to be one of the key components of olive oil that contributes to its health benefits in humans in particular in the prevention of cardiovascular diseases [1]. HOTYR is well absorbed in the gastrointestinal tract but its bioavailability is poor because an important first pass metabolism both in gut and liver, leading to the formation of biologically inactive sulfate and glucuronide conjugates, to the extent that concentrations in body fluids in its free form (free-HOTYR) are deemed undetectable [2,3]. HOTYR chemical instability in biological matrices and low concentrations are factors contributing to this observation. It is therefore necessary the development of an analytical methodology that takes into account these factors to determine free-HOTYR in plasma for a better understanding of its biological activity.

An analytical method based on the selective derivatization of catechols with benzylamine in the presence of an oxidant to form fluorescent benzoxazoles has been developed [4]. The reaction of HOTYR with benzylamine (molar ratio 1:2) forms a compound with a higher molecular weight and highly ionizable, susceptible to be detected by LC/MS-MS. The sensitivity achieved is 0.3 ng/mL. The method of analysis was as follows: after the addition of tetra deuterated hydroxytyrosol as internal standard, plasma samples were deproteinized with methanol and the supernatant was incubated with a reaction mixture of benzylamine and potassium ferrocyanate. The reaction products were purified by HLB solid phase extraction and analyzed by LC/MS-MS in the positive electrospray ionization mode. The chromatography was done in an ACQUITY C18-CSH column (Waters) with a gradient of 0.01% formic acid in water and acetonitrile. The instrument was an Agilent triple quadrupole (6410). The fragmentor was set at 135V and the monitored transitions in MRM were m/z 345→223 (CE 35V) and 345→254 (CE 15V) for HOTYR and 349→225 (CE 35V) for its deuterated analogue. The calibration curves were prepared in plasma spiked with known amounts of HOTYR at the working range of 1 to 7.5 ng/mL. The determination coefficient was greater than 0.99 and the typical recovery was above 60%.

Plasma samples were collected at 15, 30, 45, 60, 90, 120 and 180 min. following the administration of 50 mL of extra virgin olive oil to a human volunteer. The Cmax and Tmax found were 3 ng/mL and 30 min respectively. The same samples were analyzed to determine the concentration of total HOTYR after the hydrolysis of the glucuronides by GC/MS. Free HOTYR was estimated to be 1.2% of total HOTYR after comparison of the AUC of both determinations. To our knowledge this is the first time that free-HOTYR pharmacokinetics in plasma after the ingestion of a dietary dose of olive oil in humans is reported.

[1] O. Castañer et al., Am. J. Clin. Nutr. 95 (2012) 1238.
[2] R. de la Torre, Inflammopharmacology. 16 (2008) 245.
[3] O. Khymenets et al., Drug Metab. Dispos. 38 (2010) 1417.
[4] J.P. Pennington et al., Chromatographia 66 (2007) 649.

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CPA09

APPLICABILITY OF A SURFACTANT-ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION – FIELD AMPLIFIED SAMPLE INJECTION – CAPILLARY ZONE ELECTROPHORESIS – LASER INDUCED FLUORESCENCE DETECTION (SA-DLLME/FASI-CZE-LIF) METHODOLOGY FOR THE DETERMINATION OF LSD, iso-LSD, nor-LSD AND nor-iso-LSD IN BODY FLUIDS

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Lysergic acid diethylamide (LSD) is considered a potent hallucinogenic drug. Small doses of LSD result in a number of psychotropic effects. LSD is extensively metabolized in the liver and less than 1% of the drug is eliminated unchanged in urine [1]. Due to the extremely low doses and its extensive metabolism [2], the determination of LSD and its metabolites in body fluids is considered a challenging analytical problem for forensic laboratories. Iso-LSD (an inactive isomer at C-8) is often found in urine of consumers and it can be also employed as marker of consume, but it is a contaminant of LSD itself.

We present, in this abstract, a sensitive method for the analysis of LSD, iso-LSD, nor-LSD and nor-iso-LSD, based in the combination of on-line sample stacking in capillary electrophoresis with laser induced fluorescence detection. The separation of these analytes in presence of methylergonovine (used as internal standard, I.S.) was performed in a fused silica capillary. The best results, in terms of resolution and time of analysis were obtained at pH values between 6.0 and 6.5. Then, the influence of the nature of the separation buffer was studied and it was found that the fluorescence quantum yield for the four analytes of interest was higher in organic buffers (e.g. citrate and acetate) than in phosphate. Citrate was finally selected as separation buffer, since it provided the highest performance and good sensitivity. Its concentration was optimized to 25 mM. The analysis was carried out in less than 6.5 min (55-cm effective length capillary) under the optimized separation conditions.

Field amplified sample injection (FASI) was the selected stacking technique. FASI basically consists in the injection of the sample diluted in a solvent of lower conductivity than that of the separation buffer. When applying voltage, an enhancement of the electric field strength occurs at the tip of the capillary, in the low conductivity zone, with the subsequent increase in the amount of analyte introduced into the capillary, improving sensitivity. Several parameters need to be optimized for the proper implementation of FASI in order to obtain good results in terms of sensitivity and performance. The optimization of injection time and voltage was chemometrically carried out by means of experimental design and the response surface methodology. Optima values of 15 seconds and 7 kV, respectively, were obtained. The influence of the sample solvent was also studied and ultrapure water with 100 µM H_3PO_4 resulted to be the optimum one. FASI involves electrokinetic injection, with its inherent irreproducibility, however this problem was solved by the use of methylergonovine as I.S.

Differences in samples conductivity is the main problem to perform FASI on real samples, however this problem was successfully solved by using surfactant-assisted dispersive liquid-liquid microextraction (SA-DLLME) as clean-up procedure for human whole blood, plasma and urine samples. DLLME is a novel microextraction technique [3], based on a ternary solvent system. It consists in the rapid injection of an appropriate mixture of extractive solvent plus a disperser agent, into the aqueous sample containing the analytes of interest, resulting in the formation of a cloudy solution. Dibromomethane was selected as extractive solvent and Tween-80 in a concentration below its critical micellar concentration (CMC), as disperser agent. The method has been successfully applied to the analysis of the LSD, iso-LSD, nor-LSD and nor-iso-LSD in human whole blood, plasma and urine. It has been validated in these matrices in terms of accuracy and precision and it can be concluded that the combination SA-DLLME/FASI-CZE-LIF makes it possible the determination of LSD and its metabolites at very low levels (low pg/mL) in important forensic samples, being one of the most sensitive methods for these compounds, very convenient for routine analysis in forensic laboratories.

[1] O.H. Drummer, (Ed.) The forensic pharmacology of drugs of abuse, Arnold, London, 2001, p.323, chapter 7
[2] K.H. Lim, d. Andrenyak, P. Francom, R.L. Foltz and R.T. Jones, Anal. Chem, 60 (1988) 1420.
[3] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116, 2006, 1.

CPA10

CHROMATOGRAPHIC EVALUATION OF THE LIPOPHILICITY OF DRUGS: THE ROLE OF HYDROGEN BOND ACIDITY

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The evaluation of the lipophilicity of drugs, expressed as the 1-octanol/water partition coefficient (log $P_{o/w}$), in a high-throughput way is nowadays of main interest for the pharmaceutical industry due to its relevance in the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of the drugs[1]. Thus, liquid chromatography is a convenient technique since it provides many advantages such as the separation power or the fast analysis, improved with the development of the UHPLC.

A previously reported method to determine the lipophilicity[2] is based on the combination of the chromatographic retention with structural information of the compound by means of a quantitative structure-property relationship (QSPR). The retention is expressed as the polarity of the solute ($p_{reference}$) and the structural information is contained in descriptors which express the hydrogen bond acidity, the polarizability and the polarity of the compounds, and the polar interactions between molecules[3].

In this study, the relevance of the hydrogen bond acidity is assessed by comparison of the 1-octanol/water partitioning and the chromatographic retention when both systems are described by means of the solvation parameter model proposed by Abraham[4]. As expected, the hydrogen bond acidity of the compounds has to be taken into account when the log $P_{o/w}$ is determined from the chromatographic retention.

Then, several hydrogen bond acidity descriptors are evaluated by means of the generation of suitable QSPR models. These descriptors comprise a 2D one, the calculated Abraham hydrogen bond acidity *A*, and three 3D descriptors computed using diverse commercial software. The use of 3D descriptors involves a geometrical optimization to generate the global minimum energy of the structure. Therefore, the stereoisomery and the conformation of the compounds have to be considered to calculate the appropriate descriptor values.

The results obtained showed that there is no significant difference between the several hydrogen bond descriptors assessed in this work. Thus, the use of the calculated Abraham hydrogen bond acidity is recommended for practical use since it is obtained with much less effort and the effect of stereoisomery and conformation of the 3D structures are not relevant in the descriptor values.

[1] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 23 (1997) 3.
[2] J.M. Pallicer et al, J. Chromatogr. A, 1217 (2010) 3026; J. Chromatogr. A, 1218 (2011) 6356 J. Chromatogr. A, 1240 (2012) 113.
[3] R. Bosque, J. Sales, E. Bosch, M. Rosés, M.C. García-Álvarez-Coque, J.R. Torres-Lapasió, J. Chem. Inf. Comput. Sci. 43 (2003) 1240.
[4] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.

MEASURING THE LIPOPHILICITY OF IONIZABLE COMPOUNDS: CHI vs log $P_{o/w}$ or log $D_{7.4}$

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An accurate measurement of the lipophilicity of a potential drug candidate is very convenient in order to describe its pharmacokinetic properties: namely absorption, distribution, metabolism, excretion, and toxicity (ADMET). The most widely used lipophilicity index for compounds with acid-base properties is the 1-octanol/water distribution ratio (log *D*), and expresses the quotient of the concentration of all ionized and unionized species on both immiscible phases at equilibrium. Clearly the lipophilicity of ionizable compounds is pH-dependent, and its value at pH 7.4 is interesting in order to mimic the blood conditions. The reference method for log *D* measurement is shake-flask, but it is time-consuming and requires a relatively high amount of high purity sample. With the aim of overcoming these limitations and only for uncharged compounds, Valkó and co-workers proposed the Chromatographic Hydrophobicity Index (CHI) as a new high-throughput lipophilicity index based on a on a fast-gradient reversed-phase method. These studies included octadecylsilane (CHI_{ODS}) [1] and immobilized artificial membranes (CHI_{IAM}) [2] stationary phases. For unionized compounds, good correlations were found between log $P_{o/w}$ and CHI_{ODS} by means of multilinear relations involving the Abraham's hydrogen-bond acidity descriptor [3] of the compounds.

In the present work the dependence of CHI on the particular column used, even of the same stationary phase (ODS or IAM), and the organic modifier is examined. Furthermore, the HPLC methodology developed by Valkó and co-workers is successfully transferred to the UHPLC system, obtaining excellent results in terms of resolution, repeatability and time-saving. In addition, the proposed gradient allows the direct injection of the analyte solved in DMSO at a concentration of 0.5 mg/mL, using only a few tenths of microgram of substance in each run.

In the case of ODS stationary phase and with the aim of obtaining good correlations between CHI_{ODS} and log $P_{o/w}$ for unionized compounds, several hydrogen-bond acidity descriptors calculated from different software have been tested. When dealing with ionized compounds, direct correlations between both lipophilicity indexes are very poor. Instead, the log $D_{7.4}$ value can be estimated from the pK_a and the CHI_{ODS} value obtained using mobile phases a pH that ensures the quantitative presence of the unionized species.

The IAM stationary phase allows good correlations between log $P_{o/w}$ and CHI_{IAM} values, and the calculation of molecular descriptors is not necessary. Similarly to ODS, there is no direct correlation between log $D_{7.4}$ and CHI_{IAM}, but with the drawback that IAM presents some limitations in the calculation of log $D_{7.4}$ from pK_a and CHI_{IAM} of neutral species due to its narrow pH stability range (from 2.5 to 7.5). This is a serious weakness for basic compounds, because the pH corresponding to unionized species is normally above this working range.

[1] K. Valkó, C. Bevan, D. Reynolds, Anal. Chem. 69 (1997) 2022.
[2] K. Valkó, C.M. Du, C.D. Bevan, D.P. Reynolds, M.H. Abraham, J. Pharm. Sci. 89 (2000) 1085.
[3] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.

POSTERS

ENV

Environment

ENV01

EVALUATION OF ANTIFOULING BOOSTER BIOCIDES IN SEA MULLET (Mugil cephalus) USING A MICROWAVE ASSISTED EXTRACTION COMBINED WITH LC-MS/MS METHOD

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Biofouling is a problem for any structure placed in the aquatic environment that it can be controlled through chemical biocides like antifouling paints. According to the Biocides Directive (98/8/EC) [1], biocides are active substances or preparations that are intended to destroy, deter, render harmless and exert control or prevent the action of any other harmful organism through chemical or biological means. The widely use of booster biocides in antifouling paints represent an important source of pollution to the marine environment and the transfer of these toxic pollutants to the higher throfic levels is a topic of major concern.

This work present a method for the extraction, preconcentration and determination of two booster biocides commonly employed, Irgarol 1051 and Diuron, in samples of muscle tissue of *Mugil cephalus* based on microwave assisted extraction followed by solid phase extraction as preconcentration and clean-up step (MAE-SPE) coupled with liquid chromatography-tanden mass espectrometry (LC-MS/MS). Optimum conditions of MAE were established in this work and SPE clean-up and LC-MS/MS detection were optimized previously [2].

In established conditions, limits of detection (LOD) obtained were in the range between 0,1 and 0,4 $ng \cdot g^{-1}$. Recoveries, calculated at three concentration levels, were greater than 74%. Precision in, %RSD, was for intra-day assays less than 7,5% and for inter-day less than 12,7% respectively.

The optimized method was employed for the monitorization of these compounds in muscle and liver tissues of *M. cephalus* in different harbors of Gran Canaria Island. Samples were collected bimonthly and processed following the optimized method.

Highest level of Irgarol (6,9±1,03 $ng \cdot g^{-1}$) were found in the liver whereas Diuron was not detected in this tissue. However, Diuron was found in muscle (1,41±0,45 $ng \cdot g^{-1}$).

The proposed sentinel organism could be use in tropical and subtropical regions for a continuous biomonitoring of booster biocides during long time of periods. This could be a useful tool to improve the ocean and coastal management

[1] European Parliament. Directive DIRECTIVE 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market (1998).
[2] A. Rodríguez-Sánchez, Z. Sosa-Ferrera, J.J. Santana-Rodríguez. International Journal of Environmental Analytical Chemistry. 91 (2009) 1166.

ENVO2

SOLID PHASE EXTRACTION COMBINATED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY PROCEDURE TO DETERMINATION OF PERFLUORINATED COMPOUNDS IN COASTAL WATERS

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The quality of the environment affects the quality of life in Earth. The problems associated with contaminated sites plays a key increasing in many countries, because it is a potential threat to human health. Water has suffered an alarming deterioration as a result of rapid economic and human development and the inappropriate use has been made of it as a means of disposal. The fundamental objective of the Water Framework Directive has been the establishment of a framework for the protection of the inland surface waters, transitional waters, coastal waters and groundwater to prevent any deterioration and further protects and enhances the status of aquatic ecosystems, among other objectives [1].

One of the most environmentally important groups of emergent pollutants are perfluorinated compounds (PFCs). Due to their chemical properties, it is useful for a wide range of industrial applications, like surfactants and dispersants, lubricants and fire fighting foams. Many of these compounds can be toxic, and they are regularly found in environmental matrices.

Concentrations of PFCs in aquatic systems are very low, typically in the pg/L up to the low ng/L range; therefore, it requires a system of preconcentration. SPE is the traditional method used for enrichment and isolation of trace levels of PFCs from water matrices [2]. For determination of specific perfluorinated compounds in water samples, HPLC combined with mass spectrometric is the chosen process [3].

In this study, we develop a SPE procedure combined with HPLC tandem mass spectrometry (SPE-HPLC-MS/MS) for some most environmentally important PFCs, such us, perfluorobutane sulfonate (PFBS), perfluorooctane sulfonate (PFOS), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA). The parameters involved in SPE process and LC-MS/MS are optimized. The developed method could be applied to determine perfluorinated compounds in coastal waters.

[1] M. Kuster, M.J. López de Alda, M.D. Hernando, M. Petrovic, J. Martín-Alonso, D. Barceló, J. Hydrol. 358 (2008) 112-123.
[2] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen, L.Vanhaecke, Trac-Trend. Anal. Chem. 35 (2012) doi:10.1016/j.trac.2011.12.003
[3] M. Trojanowiez, J. Musijowski, M. Koc, M.A. Donten, Anal. Methods 3 (2011) 1039-1045.

ENVO3

DETERMINATION OF STEROID HORMONES IN TREATED WATER FROM WASTEWATER TREATMENT PLANTS OF GRAN CANARIA (SPAIN) BY SPE-UHPLC-MS/MS

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The water quality is profoundly related to its characteristics and the quantity of compounds dissolved in it. The industrialization favors the increasing presence of organic compounds in the water, which, at present, have been detected at trace levels. A group of these compounds are the steroid hormones, of which exists a large quantity of natural hormones that regulate the vital bodily functions. Furthermore, in the last years, a great amount of synthetic hormones has been created to use in human medicine (e.g. contraceptives, menopause controlling medicaments) and veterinary medicine (e.g. cattle fattening). These hormones, once excreted, arrive to the wastewater treatment plants, where, if they aren't eliminated, can reach the natural waters, and this contaminated water can alter the hormonal system and harm humans, flora and fauna, and even more noticeable in the marine environment. [1]

For that reason, the hormones are named as endocrine disruptor compounds (EDCs). Nowadays, there are some researches that determine the presence of estrogenic compounds in environmental water and wastewater samples. [2, 3]

In this study, several synthetic and natural steroids have been analyzed, such as estrogens (17β-estradiol, estriol, estrone, 17α-ethinylestradiol and 17α-estradiol glucuronide), androgens (testosterone) and progestins (norgestrel, megestrol). Due to low concentrations of these compounds in the environment, it is necessary to carry out an extraction and preconcentration step. The chosen extraction and preconcentration method is solid phase extraction (SPE) and the detection method will be by ultra-high performance liquid chromatography coupled to mass spectrometry detection (UPLC-MS/MS). The variables that affect the process of extraction and detection were studied and optimized, and these methods were carried out for the determination of selected compounds in water samples of wastewater treatment plants in Gran Canaria, with different water treatment methods. One of the wastewater treatment plants (WWTP1) uses the traditional method of active mud technology, while the other one (WWTP2) has a membrane biorreactor (MBR).

[1] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli. Science. 313 (2006) 1072-1077
[2] T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodríguez. J. Hazard. Mater. 183 (2010) 701-711.
[3] C. Almeida, J.M.F. Nogueira. J. Pharmaceut. Biomed. 41 (2006) 1303-1311

ENVO4

ESTIMATION OF MEASUREMENT UNCERTAINTY OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY MULTIRESIDUE METHOD FOR THE DETERMINATION OF PHARMACEUTICALS IN WATERS.

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Nowadays, the estimation of measurement uncertainty associated with quantitative results is essential to assure the reliability of analytical methods. Many accreditation agencies are now requiring the measurement or estimation of uncertainty, for example, when a laboratory implements ISO standard 17025 [1]. The purpose of an analytical method validation is to prove that it is good enough to give results close to the real value of the analytes in the samples. In this sense, the estimation of the uncertainty of an analytical value is essential from the standpoint of analytical accuracy.

In this work, we have used the information obtained from method validation to estimate the expanded uncertainty and the uncertainties contribution of the different individual steps of the method [2, 3] used for the determination of pharmaceuticals at trace levels in waters. A multiresidue solid-phase extraction and liquid chromatography-tandem mass spectrometry method was used. The expanded relative uncertainty obtained ranged from 5% to 39% being the recovery in the SPE process the main contribution to uncertainty.

The developed methodology is proposed for the analysis of pharmaceuticals (analgesics, anti-inflammatories, antibiotics, lipid regulating agents, cholesterol lowering stating agents, gastric drugs, X-ray, and miscellaneous compounds such as: sildenafil, prednisone, triclosan, chlorhexidine and miconazole) in water and it has been validated according ISO standard 17025. Linearity (0.1-250 ng/l range), intra and inter-day precision (3 - 16 ng/l and 1 - 17 ng/l respectively), matrix effects (low matrix effects were observed for 50% of compounds), limits of quantification (0,2 – 40 ng/l in surface water and 0,2- 30 ng/l in drinking water) were calculated. Only three out of 53 pharmaceuticals presented sensitivity problems and were excluded from the proposed method. The recoveries at 100 ng/l were >80% for 72% and 79% of the target compounds in surface and drinking waters, respectively.

[1] *General Requirements for the Competence of Testing and Calibration Laboratories*; ISO 17025; International Standards Organization: Geneva, Switzerland, 2000.
[2] Maroto, A.; Boque, R.; Riu, J.; Rius, F. X. Measurement uncertainty in analytical methods in which trueness is assessed from recovery assays. Anal. Chim. Acta 2001, 440, 171-184.
[3] Eurolab. 2007 Measurement Uncertainty revised: Alternative approaches to uncertainty evaluation. Technical Report nº 1/2007 March 2007.

ENVO5

MULTIRESIDUE ANALYSIS OF NINE ANTICOAGULANT RODENTICIDES IN SOIL BY LC-DAD-FLD

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In Spain authorized or illegal rodent control campaigns using anticoagulant rodenticides have been carried out to reduce potential crop damages, particularly during vole plagues that usually appear in a cyclic way. The most concerning collateral problem is related to the persistence of these products in the countryside and particularly in the soils, which could condition their future uses. The last plague of *Microtus arvallis (Arvicola terrestris)* in Castilla and León region was so intense that it was finally necessary the employ of an extensive treatment with chlorophacinone and afterwards with bromadiolone. But nowadays, it is still unknown the fate of the rodenticides that were deposited in the soil surface or in burrows, or their persistence and degradation in the main soil types of this Spanish region. Moreover, it was observed, when fighting against the last vole plague of 2007, that some vole collectives were able to resist up to 25 times the lethal dose. It implies the existence of resistance phenomena, which makes amendable the searching for other compounds as alternative treatments. The most used anticoagulant rodenticides are the 4-hydroxycoumarin and indandiones. The different chemical structures present an interesting task for their liquid chromatography (LC) simultaneous determination because indandiones are subjected to molecular absorption detection (DAD) while coumarins present fluorescence (FLD). For this reason, we have also employed a LC system equipped with both detectors (DAD and FLD) to determine traces of the studied rodenticides (chlorophacinone, bromadiolone, pindone, coumafuryl, warfarin, coumatetralyl, brodifacoum, floucomafen and difenacoum) in soils.

Rodenticides in soil were extracted with methanol by mechanical shaking and a centrifugation step. After the LC optimization study, it was selected a Gemini 5 µ C_{18} (150 x 4.6 mm) column and a mobile phase constituted by a mixture of ammonium formate 30mM,30 mM di-n-butylamine (DBA) (pH 3.5), ammonium formate 30mM + DBA 20 mM (pH 4.4) and methanol in a gradient elution mode (varying pH, modifier and additive percentages) at a flow rate of 1 mL/min, with the aim of decreasing as much as possible the chromatographic run and obtain the best resolution between peaks. Finally, the LC-DAD-FLD method was fully validated and applied to analyze soils samples collected in different zones of Castilla y León region.

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ENV06

THERMAL DESORPTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY TO DETERMINE ADIPATE, PHTHALATES AND ORGANOPHOSPHATES ESTERS IN GAS PHASE FROM HARBOUR AIR SAMPLES

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The widespread use of phthalates esters and organophosphates in polymeric materials, such as cellulose esters and vinyl chloride copolymers (PVC) and the classification of buthyl benzyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) as human carcinogens for the US Environmental Protection Agency (EPA) has stimulated the study of these compounds worldwide in a variety of environmental samples, including aerosols [1], particulate air matter [2], indoor and outdoor air and dust [3] and environmental water [4]. Phthalates and organophosphates esters are a group of organic compounds that are widely used as plastic additives to alter the physical properties such as the mouldability and flame resistance of synthetic and building materials. These compounds are present in the majority of daily used objects such as plastic toys [5] or food wrappers.

The goal of this study is to optimise a suitable method to determine adipate, some phthalates esters and some organophosphates in gas phase from harbour air samples. The method to determine these compounds in gas phase is based on thermal desorption gas chromatography-mass spectrometry (TD-GC-MS), using Tenax TA as a sorbent tubes to collect these compounds present in air phase during a period of 15 minutes with a total sampling volume of 1.5 L.

The optimised method has allowed the identification of this group of compounds. Despite of semi-volatile characteristics TD-GC-MS was a suitable technique to determine them in air samples, except for DEP and DiBP, whose determination was difficult due to their presence in the blanks.

Different sampling areas (urban and harbour) were studied. The most abundant compounds were buthyl benzyl phthalate (BBP) at 0.15 and 0.03 µg m^{-3}, adipate at 0.21 and 0.20 µg m^{-3}, di(2-ethylhexyl) phthalate (DEHP) at 114.54 and 136.42 µg m^{-3} and di-n-octhyl phthalate (DnOP) at 0.40 and 0.35 µg m^{-3}, at maximum concentrations in urban and harbour samples, respectively.

[1] S.S.H. Ho, J.Z. Yu, J.C. Chow, B. Zielinska, J.G. Watson, E.H.L. Sit, J.J. Schauer, J. Chromatogr. A 1200 (2008) 217.
[2] M. Aragón, R.M. Marcé, F. Borrull, Talanta (2012) Submited.
[3] C. García-Jares, J. Regueiro, R. Barro, T. Dagnac, M. Llompart, J. Chromatogr. A 1216 (2009) 567.
[4] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 872 (2000) 191.
[5] T. Yuzawa, C. Watanabe, R.R. Freeman, S. Tsuge, Anal. Sci. 25 (2009) 1057.

ENVO7

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COMBINED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE SIMULTANEOUS DETERMINATION OF 25 SULFONAMIDE AND QUINOLONE ANTIBIOTICS IN WATER SAMPLES

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Antibiotics are one of the most important xenobiotic contaminants because of their potential development of antimicrobial resistance. They are also considered to be "pseudopersistent" pollutants due to their continual input into the environment and permanent presence. Quinolones (Qs), which are ADN gyrase inhibitors, and sulfonamides (SAs), which difficult the synthesis of bacterial folic acid, represent two of the most important families of antibiotics because of their widespread use worldwide to treat both human and animal diseases. As a result, they are finally released in the aquatic environment by means, mostly, of waste treatment plants which are considered the main discharge sources of pharmaceutical residues [1]. For these reasons, it is of importance to develop feasible analytical methods able to determine antibiotic residues at low concentrations in the aquatic systems.

In recent years, liquid-phase microextraction (LPME) techniques have attracted great attention as extraction method for contaminants in food and environmental samples. Particularly, the dispersive liquid-liquid microextraction (DLLME) methods, introduced in 2006 by Rezaee et al. [2], are performed by the rapid introduction of a suitable mixture of disperser and extractant solvents into an aqueous sample to form a cloudy solution. These techniques are extremely simple, quick, efficient, and with a very low consumption of solvents [3]. However, and despite its inherent advantages, DLLME have been scarcely applied to extract Qs and SAs.

The aim of this work was to develop a procedure combined with ultra-high performance liquid chromatography with diode-array detection to determine 25 antibiotics in mineral and run-off waters. Optimum DLLME conditions allowed the repeatable, accurate and selective determination of 11 sulfonamides (sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfadimidin, sulfamethoxypyridazine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfadimethoxine and sulfaquinoxaline) and 14 quinolones (pipemidic acid, marbofloxacin, fleroxacin, levofloxacin, pefloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, moxifloxacin, oxolinic acid and flumequine). The method was validated by means of the obtention of calibration curves of the whole method, with determination coefficients (R^2) higher than 0.993, as well as an accuracy and precision study. In this last case, the developed Student´s t test demonstrated that there were no significant differences between real and spiked concentrations. Finally, the LODs of the method were in the range between 0.41 µg/L for sulfadoxine and 9.87 µg/L for sulfacetamide in mineral water and between 0.35 µg/L for enrofloxacin and 10.5 µg/L for ciprofloxacin in run-off water.

[1] V. Andreu, C. Blasco, Y. Picó, TrAC-Trends Anal. Chem. 26 (2007) 534.
[2] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S.J. Berijani, J. Chromatogr. A 1116 (2006) 1.
[3] A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, M.A. Rodríguez-Delgado, TrAC-Trends Anal. Chem. 29 (2010) 728.

ENV08

DETERMINATION OF MACROCYCLIC MUSK FRAGRANCES IN WASTEWATER AND SEWAGE SLUDGE BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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A fully automated solid-phase microextraction (SPME) procedure has been developed for the determination of eight macrocyclic musk fragrances [1, 2] from wastewater and sewage sludge samples prior to analysis by gas chromatography ion trap mass spectrometry (GC-IT-MS). Five different fibers previously used to determine musk fragrances [3, 4] (PDMS 30 µm, PDMS 70 µm, PDMS 100 µm, PDMS/DVB 65 µm and PA 85 µm) were tested. For all the analytes, the highest enrichment factors were achieved by using a PDMS/DVB 65 µm fiber exposed in the headspace of 10 ml water samples stirred at 750 rpm, without NaCl addition, at 100ºC for 45 min for wastewater or when the same fiber was exposed directly in the headspace of 0.25 g sewage sludge sample, at 80ºC for 45 min, which contained 0.5 ml of ultrapure water to improve the extraction efficiency [5, 6] for sewage sludge.

Under optimized conditions, the method gave good levels of repeatability and reproducibility between days for both wastewater and sewage sludge samples with relative standard deviation (n=3, 1 µg l^{-1} or 5 ng g^{-1}) less than 8% and 15%, respectively. Method detection limits ranging between 0.75 ng l^{-1} and 5 ng l^{-1} and 25 pg g^{-1} and 50 pg g^{-1} in wastewater and sewage sludge respectively were found.

The applicability of the method was tested with influent and effluent wastewater and sewage sludge samples from different wastewater treatment plants (WWTPs). The analysis of influent samples revealed the presence of the majority of the target analytes studied at concentrations ranging between <MQL (Method Quantidication Limit) and 1.13 µg l^{-1} being the most abundant exaltone, habanolide and MC4.The analysis of effluent wastewater showed a decrease in macrocyclic musk concentrations and some of them (exaltolide and muscone) were detected at concentrations below the MQL or not detected. In sewage sludge samples most of the macrocylcic musk were found at concentrations between <MQL and 3.15 ng g^{-1}. According to the origin of the sample analysed the most abundant compounds were: exaltolide, muscone and habanolide or ambrettolide, MC4 and musk NN.

[1] K. Bester, Personal Care Compounds in the Environment, VCH-Wiley Weinheim, 2007.
[2] L. Vallecillos, E. Pocurull, F. Borrull, J. Chromatogr. A submitted (2012).
[3] M. Polo, C. García-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 388 (2007) 1789.
[4] S. Machado, C. Gonçalves, E. Cunha, A. Guimarães, M. F. Apendurada, Talanta 84 (2011) 1133.
[5] H. Liu, L. Liu, Y. Xiong, X. Yang, T. Luan, J. Chromatogr. A 1217 (2010) 6747.
[6] S.-F. Wu, W.-H. Ding, J. Chromatogr. A 1217 (2010) 2776.

ENVO9

EVALUATION OF EXPOSURE TO VOCS CONCENTRATIONS INDOORS

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Indoor air quality represents a major concern regarding human exposure to atmospheric pollutants. Among them, volatile organic compounds (VOCs) constitute one of the most important families, including toxicologically relevant substances such as benzene, ethylbenzene, xylenes or trichloroethylene. With regards to indoor air, sources of VOCs include outdoor air and many other inputs related to a large number of chemical products (building materials, furnishings, paints and solvents, cleaning agents, electronic devices, air fresheners and cosmetics). This work deals with indoor VOCs concentrations in a university building located in an urban area (the main classroom building of the University of the Basque Country in Donostia-San Sebastián).

Two points inside the building and one outside were selected for parallel sampling, which was conducted by means of sampling pumps (SKC) on activated charcoal tubes (Supelco) three times a week (on weekdays and weekends) during 24 h periods from 0 h to 0 h (UTC). After collection, tubes were desorbed with carbon disulphide and analysed by GC-FID and GC/MS, both equipped with a HP-1701 capillary column.

A total of 50 compounds were identified, from which 38 were detected in both outdoor and indoor environments. The composition profiles for these compounds (Figure 1) indicate that: a) VOCs concentrations inside the building were larger than those outdoor, with average indoor/outdoor (I/O) ratios between 1 and 15; b) the light hydrocarbons profile was similar in both ambients reflecting the incidence of outdoor concentrations on inside levels; c) several VOCs were related with both indoor and outdoor sources, mainly substituted aromatics, C10-C16 n-alkanes and terpenes. Regarding heavier substituted aromatics, no significant differences were found between weekdays and weekends, pointing to indoor sources being independent of occupancy.

In addition, some VOCs were detected in only one of the environments. Outdoor-only compounds were mainly oxygenated VOCs (linear aldehydes and cetones), whose concentrations increased as temperature increased from winter to summer and did not show significant differences between weekdays and weekends. This suggested non-anthropogenic sources. On the other hand, indoor-only VOCs (cyclosiloxanes D4 and D5) were related to personal care products and had strong weekday-weekend differences.

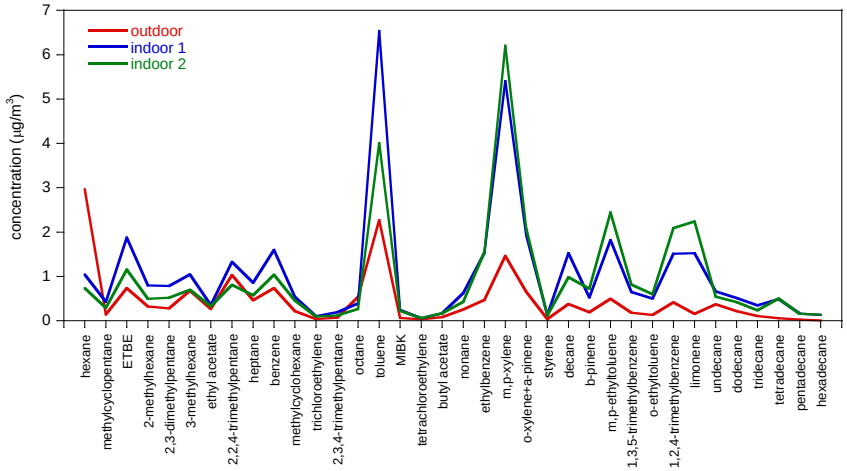

Figure 1. Average VOCs concentrations determined outdoors and indoors.

ENV10

STUDY OF VOLATILE ORGANIC COMPOUNDS (VOCs) CONCENTRATIONS IN AN URBAN AREA AROUND A PETROLEUM REFINERY

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In urban areas, the emissions originating from motor vehicular exhausts are usually the main source of Volatile Organic Compounds (VOCs). However, the location of industrial parks close to these zones can directly affect the composition of urban atmospheres. This work focuses on the study of VOC concentrations in two residential areas situated in the immediate surroundings of a petroleum refinery in the Basque Country and the evaluation of the influence of meteorological parameters (wind speed and direction) on those pollutants levels. Air samples were collected during 24 hours periods twice a week over one year (February 2011-March 2012), simultaneously at two sites (*Site 1*, about 400 m Southwest of the refinery; *Site 2*, less than 200 m to the Northwest).

VOCs were adsorbed on charcoal tubes attached to personal sampling pumps (SKC) operating at a flow rate of 1 L/min. Once collected, samples were desorbed with carbon disulphide (CS_2) and extracts analysed by gas chromatography (GC-FID and GC/MS) equipped with an HP-1701 capillary column.

An automatic station of the Basque Government's Air Quality Monitoring Network also measured the concentrations of some major pollutants (NO, NO_2, O_3, $PM_{2.5}$, SO_2) and meteorological variables (temperature, relative humidity, atmospheric pressure, solar radiation and wind speed and direction), which were used to interpret the results from the multivariate statistical analysis.

A total of 36 VOCs were identified and quantified, from methylcyclopentane (C6) to hexadecane (C16), which were classified into five groups: aliphatic and aromatic hydrocarbons, and biogenic, chlorinated and oxygenated compounds.

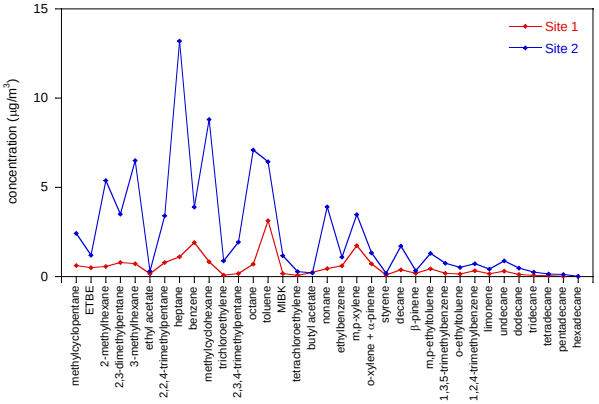

Figure 1. Example of VOCs concentrations profile.

The obtained results showed differences between studied areas:

- Pollutants levels were higher in *site 2* than in *site 1*, especially light aliphatic hydrocarbons (Figure 1). Annual averages of benzene (0.60 and 1.18 µg/m^3) were below the limit value (5 µg/m^3) set by the European Directive 2000/69/CE.
- The study of specific VOCs relationships (ej. heptane/toluene) contributed to the identification of different emission sources in these urban atmospheres.
- The correlation analysis carried out with the most characteristic compounds (Figure 2) showed a significantly high correlation between VOCs detected in each area and lack of correlation of each individual pollutant between sites.

In addition, the overall evaluation with meteorological parameters highlighted the influence of the wind direction on the VOCs concentrations obtained at each sampling point.

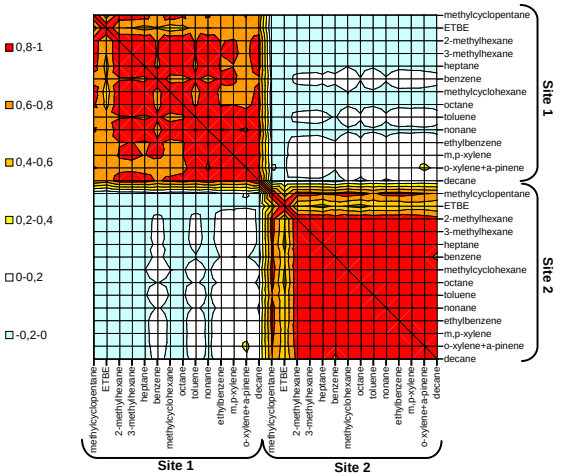

Figure 2. Correlation matrix between characteristic VOCs concentrations in the two sites.

ENV11

MULTI-WALLED CARBON NANOTUBES DISPERSIVE SOLID-PHASE EXTRACTION FOR THE ANALYSIS OF PESTICIDES RESIDUES IN ENVIRONMENTAL WATER SAMPLES

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Pesticides large production and wide application in agriculture has resulted in their high presence in aquatic systems, causing an environmental problem which has attracted great public attention. To monitor them in these samples at the extremely low levels required by current legislation, it is necessary to develop an effective sample preconcentration step with high enrichment factors prior to their chromatographic or electrophoretic determination. In this sense, solid-phase extraction (SPE) is one of the procedures most commonly used. However, it can often be time consuming, especially when high sample volumes are required. In contrast, the so-called dispersive-SPE (dSPE) method, though not frequently developed, offers an interesting and faster alternative, maintaining at the same time its high extraction efficiency. In this approach, the analytes are extracted in the bulk solution usually assisted by agitation and not on a stationary phase, without any kind of previous conditioning.

In this work, a dSPE method based on the use of MWCNTs has been proposed for the determination of 15 organophosphorus pesticides residues including some of their metabolites (disulfoton sulfoxide, ethoprophos, cadusafos, dimethoate, terbufos, disulfoton, chlorpyrifos-methyl, malaoxon, fenitrothion, pirimiphos-methyl, malathion, chlorpyrifos, terbufos sulfone, disulfoton sulfone and fensulfothion) from real environmental waters (runoff, mineral and tap water) by gas chromatography with nitrogen phosphorus detection. Based on the optimized conditions of a previous report [1], some factors that affect the enrichment efficiency were studied such as sample volume, MWCNTs amount and volume of eluent. The optimized method was validated in terms of matrix-matched calibration, recovery, precision and accuracy for the three analyzed samples. Limits of detection achieved were between 1.16 and 93.6 ng/L with absolute recovery values in the range 70-107% (relative standard deviation values were below 10%).

[1] M.A. González-Curbelo, M. Asensio-Ramos, .A.V. Herrera-Herrera, J. Hernández-Borges, Anal. Bioanal. Chem. DOI 10.1007/s00216-012-6103-7 (2012).

PRESSURIZED LIQUID EXTRACTION (PLE) AND DETERMINATION OF ANTIBIOTICS (TETRACYCLINES AND SULFONAMIDES) IN BIOSOLIDS BY LC-MS/MS.

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Tetracyclines (TCs) and sulfonamides (SAs) are widely used antibiotics in human and veterinary medicine practice and as a result of there are not completely metabolized, they were detected in wastewaters. Several studies have also shown that antibiotics are not eliminated during waste water treatment and sewage treatment plants (STPs) are referred to be the major source of their release into environment [1]. Due to their physical and chemical properties, TCs and SAs are distributed in STPs between sludge and water phases and they are accumulated in bio-solids. The determination of antibiotics in sewage sludge it is important both in the evaluation of the efficiency of the wastewater treatment and for determining the potential reuse of bio-solids as a manure.

Recently several analytical methods were developed for the determination of various antibiotics in environmental matrices by using liquid chromatography- mass spectrometry [2, 3]. In sewage sludges, pharmaceuticals are present in very low concentrations and coexist with a large number of potentially interfering compounds. For this reason, a clean-up step is sometimes required. SPE is preferred as clean up technique because it is fast, requires a low volume of organic solvent and has a low contamination risk. However, the use of SPE has resulted in low recoveries for tetracyclines and sulfonamides [3]. The main aim of this study is to develop an analytical methodology based in a PLE extraction procedure followed by SPE clean up and LC-MS/MS analysis for the quantification of tetracyclines and sulfonamides in sewage sludges.

Eight antibiotics from two major antibiotic classes, tetracyclines and sulfonamides, have been selected for this study. Veterinary antibiotic agents oxytetracycline (OTC), tetracycline.HCl (TC), dioxycycline.HCl (DC), chlorotetracycline (CTC), sulfathiazole (STZ), sulfapyridine (SPY), sulfamethazine (SMN) and sulfamethoxazole (SMX) were extracted from sewage sludge samples by pressurized liquid extraction (PLE) using citric acid (pH 4.7) and methanol as extracting buffer. The clean-up of the extract was carried out with Oasis HLB Cartridge solid-phase extraction cartridges. The samples were analyzed with a UHPLC-MS/MS (1290 Infinity LC System and 6430 Triple Quad, Agilent Technologies, USA) instrument operating in positive ion mode and multiple reaction monitoring (MRM) by recording two MRM per compound.

The parameters for pressurized solvent extraction were optimized as well as the SPE conditions resulting in recoveries of about 53-85% for the sulfonamides and quantitative for the tetracyclines. The validation of the method and determination of the analytical parameters were carried out by analyzing spiked blank samples (rabbit excrement) at different concentration levels. The method was applied to the analysis of sewage sludges collected from different STPs.

[1] Y. Ding, W. Zhang, C. Gu, I. Xagoraraki, H. Li, J: Chromatogr. A 1218 (2011) 10-16.
[2] L. Zhou, G. Ying, S. Liu, J. Zhao, F. Chen, R. Zhang, F. Peng, Q. Zhang J: Chromatogr. A 1244 (2012) 123-138.
[3] M. S· Dror Avisar, Water, Air, Soil, Pollut 223 (2012) 2571-2587.

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ENV14

DETERMINATION OF SYNTHETIC MUSKS IN SOIL

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Synthetic musks are often used as substitutes of natural odorous components and are widely used in many personal care products such as deodorants, perfumes, body lotions, soaps and laundry detergents. After application of the personal care product, musks are released into the environment, and parts were adsorbed by derma and inhalation. As a result, musks have been found in almost the entire environment, including air [1,2], water [3,4], sediment [4-6] and fish [7-9]. Moreover this compounds have been detected in human fat tissues [10,11], blood [12,13] and breast milk [10,14,15]. Furthermore, potential adverse effects of musks such as musk xylene, musk ketone have been shown by some scientist [16,17] and regulatory agencies in recent years. The major source of synthetic musk compounds in the environment through the wastewater discharge to aquatic environment. Therefore the study in the area focused on the analysis of these compounds in wastewater, sediment and aquatic animals such as fish, mussel etc. The evidences about the distributions of the musks in environmental media, aquatic food chain, as well as adipose tissue and mother's milk brought the attention on children and elders with weakened immune systems due to particular susceptibility to synthetic musks. Each and every system of the body may be adversely affected by synthetic musks (Wolff, 2005). According to the Environmental Protection Agency, like semi-volatile organic compounds (SVOCs), synthetic musk compounds affect the health [18].

In this work, a new rapid and simple method for the determination of synthetic musks compounds in soils has been developed. Soils samples are extracted by sonication with a acetone:hexane mixture. The extract was then evaporated and dissolved in 1ml of hexane.

Analysis of musks was carried out by GC/MS/MS in multirresidue (MRM) mode. The identification of compounds was based on retention time and on transition ions. This GC/MS/MS method presents a good linearity, RSD, repeatability and reproducibility. The obtained LOD and LOQ show that this method is robust and sensible for the determination of this compounds in soils.

[1] R. Kallenborn, R. Gatermann, S. Planting, G.G. Rimkus, M. Lund, M. Schlabach, I.C. Burkow, J. Chromatogr. A 846 (1999) 295.
[2] A.M. Peck, K.C. Hornbuckle, Atmos. Environ. 40 (2006) 6101.
[3] A.M. Peck, K.C. Hornbuckle, Environ. Sci. Technol. 38 (2004) 367.
[4] I.-S. Lee, S.-H. Lee, J.-E. Oh, Water Res. 44 (2010) 214.
[5] K. Bester, Chemosphere 57 (2004) 863.
[6] K. Bester, J. Klasmeier, ouml, rg, T. Kupper, Chemosphere 71 (2008) 2003.
[7] T. Yamagishi, T. Miyazaki, S. Horii, S. Kaneko, Bull. Environ. Contam. Toxicol. 26 (1981) 656.
[8] H. Nakata, Environ. Sci. Technol. 39 (2005) 3430.
[9] Y. Wan, Q. Wei, J. Hu, X. Jin, Z. Zhang, H. Zhen, J. Liu, Environ. Sci. Technol. 41 (2007) 424.
[10] G.G. Rimkus, M. Wolf, Chemosphere 33 (1996) 2033.
[11] K. Kannan, J.L. Reiner, S.H. Yun, E.E. Perrotta, L. Tao, B. Johnson-Restrepo, B.D. Rodan, Chemosphere 61 (2005) 693.
[12] H.P. Hutter, P. Wallner, H. Moshammer, W. Hartl, R. Sattelberger, G. Lorbeer, M. Kundi, Chemosphere 59 (2005) 487.
[13] H.P. Hutter, P. Wallner, H. Moshammer, W. Hartl, R. Sattelberger, G. Lorbeer, M. Kundi, Sci. Total Environ. 407 (2009) 4821.
[14] L. Duedahl-Olesen, T. Cederberg, K.H. Pedersen, A. Hojgard, Chemosphere 61 (2005) 422.
[15] J.L. Reiner, C.M. Wong, K.F. Arcaro, K. Kannan, Environ. Sci. Technol. 41 (2007) 3815.
[16] P. Steinberg, T. Fischer, M. Arand, E. Park, I. Elmadfa, G. Rimkus, H. Brunn, H.P. Dienes, Toxicol. Lett. 111 (1999) 151.
[17] G. Carlsson, L. Norrgren, Arch. Environ. Contam. Toxicol. 46 (2004) 102.
[18] Doctoral Thesis. Nihan KIYMET, İzmir Institute of Technology, Turkey (2009).

ENV15

ANALYSIS OF STEROID HORMONES IN SOILS AMENDED WITH BIOSOLIDS BY MATRIX SOLID-PHASE DISPERSION AND ISOTOPE DILUTION GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Steroid hormones, progestagens, androgens and estrogens, may act as potent endocrine disruptors and cause adverse effects when released into the environment. The emission from farm animals is potentially a major source of environmental pollution by these chemicals. Steroid hormones may be released to the soil environment when biosolids such as manure and sludge are used as soil amendments. These compounds have frequently been found in wastewater effluents, whereas few studies have been published on levels of steroid hormones in soil amended with biosolids. This paper presents the development of a sensitive yet robust analytical method for the simultaneous determination of steroid hormones in soil amended with biosolids. The chemicals studied in this work are the following: natural compounds such as estrone (E1), 17 β–estradiol (E2), estriol (E3), and progesterone (Pg); and synthetic estrogens, as, 17α-ethynylestradiol (EE2), diethylstilbestrol (DES) and mestranol (MeEE2). Thermally dried sewage sludge and chicken litter samples used as soil amendment. In this work, an isotope dilution method for the determination of steroid hormones in soil amended with biosolids by gas chromatography-tandem mass spectrometry was developed.

The compounds were extracted from soil by matrix solid-phase dispersion (MSPD) with a low volume (10 ml) of acetonitrile:methanol (90:10, v/v) as extraction solvent. After extraction, solvent was evaporated and analytes were derivatized. An aliquot (0.1 ml) of the standard or extract solution was transferred into a 2 ml reaction vial, followed by the addition of 25 µl of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 25 µl of pyridine. The vials were closed and the mixture left to react for 1 h at 70 ^{o}C. The compounds were determined by isotope dilution gas chromatography-tandem mass spectrometry, using $^{13}C_{12}$ labeled compounds as internal standards. With the aim to reduce detection limits different types of injection were studied. For recovery studies, samples were spiked to reach concentrations of 50 ng g^{-1}, 30 ng g^{-1} or 10 ng g^{-1} and the recoveries achieved were satisfactory for most of the compounds. A good linearity was obtained with correlation coefficients equal or higher than 0.990 for all the compounds studied. The validated method was used to investigate the levels of these steroid hormones in soil amended with biosolids collected from different locations in Spain.

ENV16

DETERMINATION OF METHYL JASMONATE AND OTHER VOLATILES OF INTEREST IN PLANT BEHAVIOR

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Plants respond to environmental stresses such as pathogen attack, mechanical or herbivorous insect wounding and the presence of pollutants by the synthesis of secondary signaling molecules that mediate interplant communication for defense responses.
Salicylic and jasmonic acid are considered among the major regulators of plant defense responses. The role of salicylic acid consists in influencing plant resistance to pathogens whereas jasmonic acid is believed to have an important function in plant responses to insect herbivores and abiotic stress. The volatile forms act as an airborne signal in plant cellular responses, plant herbivore interactions and plant-plant interactions. Methyl jasmonate serves as an indirect defense mechanism as it stimulates the production of jasmonic acid in surrounding plants even though they are not under stress.

Other compounds of interest released by plants are C_6-volatiles. These volatiles are emitted at low levels by healthy plants and are rapidly released in response to insect feeding and mechanical damage.

The last group of target compounds are those responsible for aroma, which have already been studied in this group in tomato matrix [1], and now are extrapolated to the plant itself.

In this work, a semi-quantitative method for volatile extraction from plant material based on solid phase microextraction and on purge-and-trap has been developed for the determination of up to 45 volatile compounds in tomato plants. In both cases analytical determination has been carried out by GC-MS (or GC-MS/MS) measurement using an ion-trap mass spectrometer in the electron ionization mode coupled to a gas chromatograph. After optimization of the different variables involved in GC-MS and GC-MS/MS around 50 volatiles could be correctly identified and quantified at low levels in a chromatographic run time of 40 minutes.

The extraction procedures were applied to four kind of treated plants: control, infected by Pseudomonas syringae, control with hexanoic acid treatment and infected with Pseudomonas syringae followed with hexanoic acid treatment. The experiences were carried out both with whole plants (in a higher flask) and with teased leaves in order to compare the difference between volatiles response.

[1] J. Beltran, E. Serrano, F.J. López, A. Peruga, M. Valvarcel, S. Rosello, Anal. Bioanal. Chem. 385 (2006) 1255.

ENV17

TWO-DIMENSIONAL HPLC CHARACTERIZATION OF FATTY ALCOHOL ETHOXYLATES AND OTHER CLASSES OF NON-IONIC AND ANIONIC SURFACTANTS

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Fatty alcohol ethoxylates (FAEs) are non-ionic surfactants widely used in cleaners and body care products. FAEs are industrially obtained as complex mixtures of oligomers, molecular consisting of a linear hydrocarbon chain (C10-C18) bound to an oxyethylene (EO) chain terminating in a hydroxyl group. The low volatility and the thermal instability of the EO chain at high temperatures, limits the use of GC to the oligomers with $m < 4$ [1, 2]. Also the lack of a chromophore hinders the use of HPLC with UV-vis detection. For these reasons, derivatization prior to oligomer separation by HPLC is often carried out [3-5]. However, the separation of FAE derivatives using HPLC in one dimension is limited by overlapping between consecutive hydrocarbon series. Enhanced separations of both hydrocarbon series and oligomers within the series can be achieved by heart-cut 2D-HPLC [4, 6-8]. In this work, two novelties respecting to our previous approach [4] were introduced. First, separation of the hydrocarbon series in the 1st dimension was achieved by using high temperatures, thus to reduce the polarity of the EO chains. To prevent excessive eluent strength of the eluate segments entering the 2nd dimension, stationary phases of low and high hydrophobicities (as C1-C4 and C18) were selected for the 1st and 2nd dimensions, respectively. Second, system configuration was improved. An auxiliary 6-port 2-position valve (V_1) was used first to select two alternative paths: one position of V_1 to perform 1st dimension separations, and the other position to by-pass the 1st column. Separation along the 2nd dimension was carried out with columns inserted within the channels of a column selection valve (V_2). One of the 6 channels of V_2 was shorted with a PEEK tube to derive the eluate of the 1st dimension towards the detector, and also to flush the system with new mobile phases. The other 5 channels of V_2 were used to eluate segments of the 1st dimension eluate along the 2nd dimension. This configuration allowed the use of up to 5 different columns along the 2nd dimension. Thus, flexibility was gained. The system was applied to the resolution of mixtures of alcohol ethoxylates, alkenylsulfates and other surfactants families with success.

[1] P. Rudewicz, Anal. Chem. 58 (1986) 674.
[2] C. Crescenzi et al., Anal. Chem. 67 (1995) 1797.
[3] A. Micó-Tormos et al., J. Chromatogr. A 1216 (2009) 3023.
[4] id., J. Sep. Sci. 33 (2010) 1398.
[5] M. Beneito-Cambra et al., J. Chromatogr. A 1218 (2011) 8511.
[6] R.E. Murphy et al., Anal. Chem. 70 (1998) 4353.
[7] B. Trathnigg et al., J. Chromatogr. A 952 (2002) 149.
[8] id., J. Chromatogr. A 953 (2002) 89.

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ENV18

HIGH-MOLECULAR MASS YESSOTOXINS REVEALED BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING OF MARINE DINOFLAGELATES

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Yessotoxins (YTXs) are a class of lipophilic marine toxins, structurally characterized by sulfate moieties and a ladder-shape backbone based on alternated ether rings. The YTX group is extremely complex with more than 90 different analogs tentatively identified by liquid chromatography-mass spectrometric methods [1]. YTXs are produced by the marine dinoflagellates *Protoceratium reticulatum*, *Lingulodinium polyedrum* and *Gonyaulax spinifera* [2], which are ubiquitous microalgae species present in seas around the world. Despite the low acute oral toxicity of yessotoxin in mice (LD_{50} > 10 mg/kg, [3]) the toxicity and mode of action has been confirmed *in vivo* and *in vitro* [4], and the study of their biological activity has resulted in increasing interest in YTXs for potential medical applications [5, 6].

In this work profiling of yessotoxins was performed with liquid chromatography-mass spectrometric methods (*i.e.* neutral loss scan, product ions spectra and accurate masses) in several YTX producing strains of *P. reticulatum* and *G. spinifera* from Spain, Italy, Canada, United Kingdom, and USA. Results showed different toxin profiles and different relative abundance among YTX analogs in every strain analyzed, which could be used to perform bio-regional studies. Moreover, some previously unreported analogs including several with high molecular weights and lower polarity compared to that of parent YTX were identified. This study increases the mass range in which YTXs may be found, and adds to the complexity of this toxin group. The biological activity and/or toxic potency of the new YTX analogs remain unknown.

[1] C. O. Miles, I. A. Samdal, J. A.G. Aasen, D. J. Jensen, M. A. Quilliam, D. Petersen, L. M. Briggs, A. L. Wilkins, F. Rise, J. M. Cooney, A. L. MacKenzie, Harmful Algae 4(6) (2005) 1075.
[2] B. Paz , A. H. Daranas, M. Norte, P. Riobó, J. M. Franco and J. J. Fernández, Marine Drugs 6 (2008) 73.
[3] K. Terao, E. Ito, M. Oarada, M. Murata, T. Yasumoto, Toxicon 28, 9 (1990) 1095.
[4] H. J. Dominguez, B. Paz, A. H. Daranas, M. Norte, J. M. Franco, J. J. Fernández, Toxicon 56, 2(15) (2010) 191.
[5] Therapeutic use of yessotoxin as human tumor cell growth inhibitor. European Patent EP 1875906 B1.
[6] Use of yessotoxin in the treatment of allergic and asthmatic processes. European Patent EP1875907 B1.

ENV19

THERMAL DESORPTION AS A PRECONCENTRATION TECHNIQUE FOR THE DETERMINATION OF ODOUR-CAUSING COMPOUNDS AND OTHER VOLATILES IN AIR

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The interest for the determination of volatile organic compounds (VOCs) in air has increased over the last decades since their emissions have been associated with environmental effects such as the depletion of stratospheric ozone and the greenhouse effect, and several adverse effects on human health [1,2]. Furthermore, some VOCs have been related to the perception of odours in environments such as the surrounding areas of wastewater treatment plants (WWTPs) [4]. Gaseous emissions from WWTPs are complex mixtures which include a huge amount of VOCs, being hydrogen sulphide, ammonia, carbon dioxide, and methane the compounds present at higher concentrations. In addition, other odorous compounds can be found at lower concentrations. For example, mercaptans, organic sulphides, nitrogen-containing compounds (e.g. amines, indole, and skatole) and oxygenated compounds (e.g. aldehydes, alcohols, organic acids, and ketones) might also be present [3].

The complexity of the air matrix and the low concentrations usually found in atmospheric air point out the requirement of very sensitive analytical techniques for the determination of VOCs. The present work describes the development of an analytical method based on active collection on multisorbent tubes, followed by thermal desorption and gas chromatography-mass spectrometry for the determination of 16 volatile organic compounds in air samples. The studied compounds include ozone precursors and odour-causing compounds representative of different chemical families (sulphur- and nitrogen-containing compounds, aldehydes and terpenes). Two types of sorbents were tested and desorption conditions (temperature, time, and sampling and desorption flows) were evaluated. Using a multisorbent bed of Tenax TA/Carbograph 1TD an external calibration was carried out and detection limits in the range 0.2 – 2.0 $\mu g \cdot m^{-3}$ were obtained for 1L samples. The method was applied for determining the target compounds in air samples from two different WWTPs in Girona and Tarragona. Most of them were detected, being toluene, limonene and nonanal the compounds found at higher concentrations.

[1] USEPA, Cancer Risk from Outdoor Exposure to Air Toxics, PA-450/1-90-004a. Research Triangle Park, NC, Office of Air Quality Planning and Standars, 1990.
[2] WHO, Air Quality Guidelines for Europe, WHO Regional Office for Europe, 2000.
[3] F. Dincer, A. Muessinoglu, J. Environ. Sci. Health, Part A 43 (2008) 1569.

Acknowledgements: this study has been financed by the MICINN (Spanish Ministry of Science and Innovation), project CTM2011-28765-CO2 and the Department of Innovation Universitiers and Enterprises (Projects 2009 SGR 223 and 2009 SGR 1425). A. Godayol gratefully acknowledges a grant from the Generalitat de Catalunya (2011FI_B 00020) and the financial support of the University of Girona for her research stay at the University Rovira i Virgili (DRAC program).

ENV20

ANALYSIS OF VOLATILE METHYLSILOXANES IN BIOTA BY HEADSPACE - SOLID PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Volatile polydimethylsiloxanes are high-production volume chemicals used during years in industrial products and consumer goods, such as personal care products, cleaning agents, electronics, cookware, and medical devices. As a result of their widespread use and high volatility, they have been detected in a wide variety of environmental samples, including water, sediments, soils, dust, air and biota.[1-3] Recent studies suggest that some cyclic methylsiloxanes, such as octamethylcyclotetrasiloxane (D4), decamethylcyclopenta siloxane (D5) and dodecamethylcyclohexasiloxane (D6), are potential carcinogens, have endocrine-disrupting properties and can produce impair fertility.[1] In addition, they tend to bioconcentrate and biomagnificate through the food web.[4] Although information about their toxicity is still limited, it is important to have an understanding of the occurrence and distribution of these compounds in the environment. Therefore, there is interest to dispose of rapid and sensitivity methods with enough selective for the determination of these compounds.

The analysis of methylsiloxanes is not easy due to their volatility and the potential sources of background contamination that affect their determination. At present, very few papers have been published regarding their presence in biota samples. Purge and trap[4,5] and solvent extraction,[1] both combined with gas chromatography-mass spectrometry, are applied for their determination. Nevertheless, purge and trap requires extraction times of 24-48 h per sample,[4,5] and the method proposed using solvent extraction is not sufficiently rugged because the extracts are directly analysed by GC-MS without any clean-up procedure.[1]. In this way, headspace solid-phase microextraction can be an excellent alternative to the published methods for the analysis of volatile compounds at low concentrations levels in biota samples.

In the present work, a method based on headspace–solid phase microextraction (HS-SPME) combined with gas chromatography – mass spectrometry (GC-MS) has been developed for the analysis of linear and cyclic methylsiloxanes in biota samples. The proposed method involves a solvent extraction of the target compounds from the biological matrix followed by the analysis by HS-SPME-GC-MS. Parameters affecting the efficiency of the extraction and desorption of methylsoloxames were optimized, and the effect of matrix components on the sensitivity of the HS-SPME method was studied. In addition, the instrumental and environmental contributions of siloxanes to procedural blanks were minimised in order to improve limits of quantification. The HS-SPME-GC-MS method provides good precision (RSD, <15%) and low limits of detections (linear methylsiloxanes: 0.001 – 0.012 ng g^{-1}, cyclic methylsiloxanes: 0.62 – 4.7 ng g^{-1}) and it was applied to the analysis of linear and cyclic methylsiloxanes in biota samples from different origin.

[1] L. Kaj, M. Schlabach, J. Andersson, A. P. Cousins, N. Scmidbauer, E. Brorström-Lundén, Siloxanes in the Nordic Environment, Nordic Council of Ministers,Copenhagen,2005, available at: http://nordicscreening.org/index.php?module=Pagesetter&type=file&func=get&tid=5&fid=reportfile&pid=4 (2010).
[2] E.Y. Companioni-Damas, F.J. Santos, M.T. Galceran, Talanta 89 (2012) 63.
[3] C. Sparham, R. V. Egmond, C. Hastie, S. O´Connor, D. Gore, N. Chowdhury, J. Chromatogr. A 1218 (2011) 817.
[4] K. Borga, E. Fjeld, A. Kierkegaard, M. S. McLachlan, Env. Sci. Technol. (2012) (doi: 10.1021/es300875d)
[5] A. Kierkegaard, M. Adolfsson-Erici, M. S. McLachlan, Anal. Chem. 82 (2010) 9573.

DETERMINATION OF PRIORITY PERSISTENT ORGANIC POLLUTANTS IN RIVER WATER BY STIR BAR SORPTIVE EXTRACTION FOLLOWED BY THERMAL DESORPTION AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Water is the mainstay of the environment and its degradation has serious consequences: protecting the water means protecting the ecosystems of which it forms an inseparable part. Waters are subject to great risk, primarily due to pollution and the growing need for quality water. The European Union is going to great lengths to improve water quality, one sign of which is the European Water Framework Directive (WFD) adopted in the year 2000 [1]. The WFD is a highly complex legal and technical document and the quantification limits required are extremely low. The WFD states that the methods used for the control of substances must comply with a LOQ equal to or less than 30% of the annual average environmental quality standard (AA-EQS), which are in the pg L^{-1} range of in some cases. The listed substances are classified into three main groups: metals (Cd, Pb, Hg and Ni), volatile organic compounds (benzene, carbon tetrachloride, 1,2-dichloroethane, dichloromethane, tetrachloroethene, trichloroethene naphthalene and trichlorobenzenes), and semi-volatile organic compounds.

For metal analysis, the most appropriate technique is ICP-MS and for volatile organic compounds purge and trap and subsequent analysis by GC-MS. However, semi-volatile compounds belong to very different families, and sometimes each must be analysed by an exclusive technique or method. To our knowledge, no methods are able to analyse all the compounds together. Some compounds are amenable to analysis by GC (PBDEs, organophosphorous pesticides), by liquid chromatography (PAH) or by both (tributyltin). Multi-residue methods that can analyse a larger number of substances with low detection limits are needed [2-4].

The main objective the work is to validate a muti-residue method for the analysis of semi-volatile organic pollutants in inland groundwater (river water) at ultra-trace levels in compliance with the WFD. Stir bar sorptive extraction and thermal desorption coupled with gas chromatography - mass spectrometry (SBSE-TD-GC-MS/MS) is used. The method includes various families of compounds included in the WFD and other compounds listed as persistent organic pollutants that are banned in the Stockholm Convention of Persistent Organic Pollutants, such as polychlorinated biphenyls, polycyclic aromatics hydrocarbons, and other pesticides not included in the WFD. Extraction conditions were optimised in order to analyse simultaneously analytes with very different polarities and octanol-water partition coefficients, which is an important parameter in the optimisation of a SBSE method. The quantification limits (LOQs) obtained ranged from 0.14 to 10 ng L^{-1}, lower that others presented in previous publications, and complies with the requirement for analytical methods to be used in the analysis of the compounds included in the WFD. Several quality parameters as linearity, trueness and precision were studied with good results, and also uncertainty was estimated. The WFD requires that the level of uncertainty must be lower than 50%, and this requirement was met for all compounds. Precision (in terms of RSD) was lower than 30%, recoveries ranged between 74 and 111%, and determination coefficients were higher than 0.990 for all analytes. Different factors that affect the SBSE procedure were optimised. GC-MS/MS parameters have also been revised. The accuracy of the method was tested participating in a proficiency testing scheme for each group of analytes.

[1] European Commission. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000. Off. J. Eur. Comm. C, L 327 22/12/2000.
[2] M. Coquery, A. Morin, A. Bécue, B. Lepot, Trends Anal. Chem. 24 (2005) 117.
[3] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.
[4] P. Lepom, B. Brown, G. Hanke, R. Loss, P. Quevauviller, J. Wollgast, J. Chromatogr. A 1216 (2009) 302.

ENV22

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY DETERMINATION OF BISPHENOL A AND ITS CHLORINATED DERIVATIVES IN SEWAGE SLUDGE SAMPLES. STUDY OF DIFFERENT EXTRACTION TECHNIQUES

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Endocrine Disrupting Chemicals (EDCs) are a group of natural and synthetic chemicals that may interfere with the normal function of the endocrine system in animals and humans [1]. In men, exposure to EDCs is associated with problems in reproductive capacity and testicular or prostate cancer [2]. In women, abnormal endocrine function may be associated with increased risk for endometriosis, reproductive and endocrine-related cancers [3]. Most EDCs are synthetic compounds, some of which are designed to act as estrogens (*e.g.*, oral contraceptives). However, many are designed for other purposes and accidentally have estrogenic activity (plasticizers and surfactants), or are components of pharmaceuticals and personal care products (parabens or UV-filters) [4].

BPA is probably the most widely used synthetic chemical in the world with an annual production of more than 3 million tonnes [5]. Studies conducted over the past 20 years show that BPA is not only a ubiquitous pollutant. Many studies have documented the adverse effects of BBA on human health including structural and neurochemical changes in the brain, associated with behavioural changes, fertility problems, recurrent miscarriage, and polycystic ovary syndrome in women [6].

Wastewater treatment plants (WWTPs) are the major point sources of BPA and other EDCs to the environment [7]. Sewage sludge, or the sludge that is reused for the production of compost, can be applied directly to agricultural lands as a means of soil amendment representing a way for the release of BPA into the environment, from where it may eventually enter the human food chain. The need for detection of trace level of BPA presents an important challenge, making it necessary the development of efficient pre-treatment procedures and highly sensitive and selective analytical methods.

This work presents a comparison of 3 extraction techniques—ultrasound-assisted extraction, microwave-assisted extraction and pressurized liquid extraction—in order to evaluate their efficiency in the determination of BPA and its chlorinated derivatives in sewage sludge samples. Extraction parameters for each technique were accurately optimized and the compounds were quantified using LC-APCI-MS/MS, operating in MRM mode. The analytes were separated in less than 6 min. BPA-d_{16} was used as internal standard. Three selective, sensitive, robust and accurate analytical methods were developed. The limits of detection of the methods ranged from 2 to 9 ng g^{-1} and the limits of quantification from 8 to 26 ng g^{-1}, while inter- and intra-day variability was under 6% in all cases. The methods were validated separately by using matrix-matched calibration and recovery assays with spiked samples. Recovery rates ranged from 97.7% to 103.1%. The sewage sludge samples used for experiments were collected from two different wastewater treatments plants located in the province of Granada (Spain). The comparison of the methods demonstrated no statistically significant differences between the extraction techniques.

[1] US EPA, Office of Research and Development, EPA/630/R-36/012 (1997).
[2] S. Pflieger-Bruss, H.C. Schuppe, W.B. Schill, Andrologia 36 (2004) 337.
[3] P. Nicolopoulou-Stamati, M.A. Pitsos, Hum. Reprod. Update 7 (2001) 323.
[4] F. Anca Caliman, M. Gavrilescu, Clean 37 (2009) 277.
[5] Y. Sui, N. Ai, S.H. Park, J. Rios-Pilier, J.T. Perkins, W.J. Welsh, C. Zhou, Environ. Health Persp. 120 (2012) 399.
[6] M. Furuya, K. Adachi, S. Kuwahara, K. Ogawa, Y. Tsukamoto, Life Sci. 78 (2006) 1767.
[7] S.K Khanal, B. Xie, M.L. Thompson, S.S. Sung, S.K. Ong, J. Van Leeuwen, Environ. Sci. Technol. 40 (2006) 6537.

ENV23

UHPLC-APPI-MS(/MS) FOR THE ANALYSIS OF FULLERENES AND C60-FULLERENE DERIVATIVES

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Fullerenes are cage-like molecules that are made solely by carbon. Since their discovery [1], this family of compounds have gained a prime role on scientific scene because of their interesting and unique characteristics. These compounds have many applications in different fields, especially in biomedicine (drug delivery systems, carriers for gene, diagnostic applications), cosmetics (antiaging formulations, sunscreen creams) as well as in industry (photovoltaics, electrical components, badminton and tennis rackets). Since these materials have entered into industrial and consumer use, they will become dispersed into the environment. For this reason there is a need of developing reliable analytical methods for their analysis.

Several methods have been developed for the determination of fullerenes mainly using liquid chromatography and liquid chromatography-mass spectrometry (LC-MS) [2,3], mostly in environmental waters [2-4]. Nevertheless, most of these works usually require long analysis times and are generally focused to the analysis of C60 and C70 fullerenes, neglecting fullerenes of higher molecular weight and also functionalized fullerenes. Moreover, there is a lack of reliable quantitative analytical methods for the extraction and analysis of fullerenes in more complex environmental matrices such as sediments and soils. Regarding LC-MS ionization techniques, atmospheric pressure photoionization (APPI) has recently been proposed as a good alternative to ESI and APCI for the analysis of fullerenes [5,6].

In this work an UHPLC-APPI-MS(/MS) method for the analysis of fullerene compounds using a TSQ Quantum Ultra AM (triple hyperbolic quadrupole instrument) which allows working at enhanced mass resolution was developed. Five fullerenes (C60, C70, C76, C78, and C84) together with three C60-fullerene derivatives (N-methyl fulleropyrrolidine, [6,6] Phenyl C61 butyric acid methyl ester and [6,6] Phenyl C61 butyric acid butyl ester) were selected for this study. The chromatographic separation of the 8 fullerene compounds was achieved in less than 5 minutes by gradient elution using a Hypersil Gold C18 (150 mm x 2.1 mm i.d., 1.9 µm) column (Thermo Fisher) and methanol-toluene as mobile phase at a flow rate of 500 µL/min. Negative APPI MS and MS/MS spectra were obtained in order to select quantitation and confirmation ions. As previously reported [6], no MS/MS fragmentation of the molecular ion $[M]^{-\bullet}$ was observed for pristine fullerenes because of their high molecular stability. So, for these compounds, highly-selective selected ion monitoring (H-SIM) mode working at enhanced resolution in Q3 (0.06 m/z units at full width half maximum, FWHM) and monitoring the isotopic cluster ions $[M]^{-\bullet}$ and $[M+1]^{-\bullet}$ was used. In contrast, for C60-fullerene derivatives, the loss of their functionalizing substituent was observed in the MS/MS spectra providing as product ion the pristine C60 fullerene. Thus, for these compounds, highly-selective selected reaction monitoring (H-SRM, Q1 at 0.06 m/z FWHM and Q3 at 0.7 *m/z* FWHM) was proposed by monitoring two SRM transitions (using the isotopic cluster ions $[M]^{-\bullet}$ and $[M+1]^{-\bullet}$ as precursor ions). Instrumental and method quality parameters were established and the applicability of the proposed UHPLC-APPI-MS(/MS) method was evaluated by analyzing fullerenes and C60-fullerene derivatives in several environmental samples.

[1] H.W. Kroto, J.R. Heath, S.C. O'Brien, R.F. Curl, R.E. Smalley RE (1985) C60: buckminsterfullerene. Nature (London) 318:162-163.
[2] D. Bouchard, X. Ma, J. Chromatogr. A 1203 (2008) 153.
[3] C.W. Isaacson, M. Kleber, J.A. Field, Environ. Sci. Technol. 43 (2009) 6463.
[4] Z. Chen, P. Westerhoff, P. Herckes, Environ. Toxicol. Chem. 27 (2008) 1852.
[5] H.C. Chen, W.H. Ding, J. Chromatogr. A 1223 (2012) 15.
[6] O. Núñez, H. Gallart-Ayala, C.P.B. Martins, E. Moyano, M.T. Galceran, Anal. Chem. (2012), *in press*. doi: 10.1021/ac300659h

ENV24

MULTIRESIDUE DETERMINATION OF CURRENTLY USED PESTICIDES IN SEDIMENT, WATER AND BIOTA. THE GUADALQUIVIR RIVER BASIN AS STUDY CASE.

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Pesticides are applied widely to protect plants from disease, weeds and insects damage. This usage becomes a real environmental pollution and human health risk because pesticides come into contact with water, sediments and biota. The aim of this work has been to develop a sensitive multi-residue method for the simultaneous target analysis of 41 pesticides, belonging to different chemical classes, in water, sediments and biota and it has been applied in real samples from Guadalquivir River.

Sediment and biota samples were freeze-dried prior to extraction. QuEchERS method was used for sediment, ethyl acetate extraction for biota and solid-phase extraction (SPE) with Oasis HLB for water samples.

The resulted extract was then analyzed by LC-MS/MS in positive ionization with an electrospray ionisation (ESI) source. Separation was carried out on a Luna C_{18} column (150 × 2.0 mm, 3 μm) using a gradient elution profile with mobile phase consisting of water-methanol both, 10 mM ammonium formate. The two most intense precursor ion → product ion transitions were monitored to obtain unambiguous confirmation of the compound identity.

In water samples, recoveries ranging from 48% to 70%, with relative standard deviations between 2%-19% were obtained; and low limits of quantification (0.2 to 6 ng/L) were achieved for all selected pesticides. For sediment samples, recoveries were between 40 and 105 % and, for biota between 35 and 98 %, relative standard deviation was in all cases below 20 % at the limits of quantification. These limits were 0.1-5.0 ng/g for sediments and 0.2-11.5 ng/g for biota.

The composition and spatial distribution of these pesticides were investigated in biota, sediments and water from the whole Gualdalquivir River Course (Spain). Twenty-five water and sediment samples were taken along the river. Fish samples were collected using electro-fishing by personnel of the ICMAN. Fish samples were only taken at five points. Collected fish included five Andalusian barbel (*Luciobarbus sclateri*) and one Comon carp (*Cyprinus carpus*). The levels of total pesticides in sediments and biota were low compared with the levels in water. The most ubiquitous pesticides were imidacloprid, malathion, dimethoate and the degradation product of atrazine, deethylatrazine. Pesticides in Guadalquivir River were contributed from soil runoff coming from the agricultural areas and from waste water treatment plants that dump their effluents to the river. In spite of concern on the adverse effect to humans posed by pesticide residues and their ubiquitous presence in water, edible biota from the examined water body were found to be safe for human consumption. However, periodic monitoring of these currently pesticides should be instituted so as to get information on the environmental quality of the Guadalquivir River water even if there seems, at present, to be no biological threat from their use.

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ENV25

GAS-CHROMATOGRAPHIC METHOD TO DETERMINATE THE EFFECT OF DRIED TREATMENT OF SAINFOIN (*ONOBRYCHIS VICIIFOLIA*) IN THE METHANE PRODUCTION

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Onobrychis viciifolia Scop. is a rustic forage legume adapted to multi calcareous soils located in cold and dry regions in the Iberian Peninsula. It has large production capacity, bromatological quality, palatability and consumption does not cause timpanism in cattle due to the presence of tannins in their cells. In addition, it is also excellent in regeneration of soil fertility.

Methane is one of the most important greenhouse gasses. Domestic ruminants are responsible for 18% of methane emissions. Methane production during fermentation represents a direct energy loss to the animal (up to 15%) [1]. Therefore, methane production is an ecological and economic problem. Reducing methane production by ruminants could improve their productivity, and reduce their contribution to greenhouse gas emissions.

However, before new strategies can be developed, it is essential to be able to measure methane emissions accurately to establish baseline values for current practices. Because there are inexpensive ways to test many different diets the *in vitro* techniques are the initial choice for investigating strategies for reducing methane production. Methane production can be measured in these simulated systems by sampling the gas and analysing its composition, using GC.

To compare the influence of dried treatment of sainfoin in the methane production, different treatments of dehydration and heating the plant were done. The treatments were: lyophilization, dried with natural air and dried at different temperatures (40, 60 and 80° C). Also, polyethylene glycol (PEG) was added to half the samples to facilitate the separation of tannins and proteins. Gas samples were obtained by digestion of plant with artificial saliva and rumen fluids.

GC parameter settings were: Methane detection was realized with flame ionization detector (FID, gas chromatograph HP-4890), equipped with a capillary column GS-DB-WAX (J&N Scientific), 30m, 0.25 mm id and 0.25 micron film thickness; The carrier gas was helium, and the flow rate was 2 ml/min.H_2 flow rate 35 ml/min, synthetic air flow rate 350 ml/min The temperature of the inlet and detector was maintained at 200 and 250º C respectively. Oven temperature was 70º C and the temperature program was isothermal (70º C temperature of column flow). Injection volume was 200 μl splitless and was realized with a Hamilton gas-tight syringe manually. Methane identification was based on retention times as compared with the standard methane. Analysis time was 2 min (included equilibration time). The standard curve established by plotting a linear regression of the standard quantities injected of each methane gas volume versus area of the peak obtained. The amount of gas in a sample was determined using the regression equation: Area=2420.3*amount ($\mu mol\ 10^{-14}$) + 168.7. Correlation: 0.99945. The results indicated that the addition of PEG, increased methane production (between 11 and 48%, depend of treatment). Air, 80° C, 60° C and 40° C PEG samples had similar value of CH_4 production and lower than the lyophilized samples. The samples with higher methane production were PEG lyophilized samples and natural air dried samples without the addition of PEG was lower methane production.

[1] H.P.S. Makkar and P.E. Vercoe (eds).(2007). *Measuring Methane Production from Ruminants*. Published by Springer FAO/IAEA. ISBN 978-1-4020-6132-5 (HB)

ENV26

ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY METHOD FOR THE ANALYSIS OF PERFLUORINATED ALKYL SUBSTANCES IN BIOTA.

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Perfluorinated alkyl substances (PFAs), which include perfluorooctane sulfonate (PFOS), constitute a diverse class of chemicals characterized by long, fully fluorinated carbon chains with different functional head groups. PFCs are used in products to resist grease, oil, stains, and water, and are also used in fire-fighting foam. Concern about their presence in the environment has increased since PFOS and related fluoroalkyl substances were detected in blood plasma of non-occupationally exposed humans and in animal tissues [1].
The objective of this study was to develop a sensitive and accurate screening method for PFCs in biota by using ultra high-performance liquid chromatography (UPLC-Q-TOF-MS) and to demonstrate its reliability in identifying target compounds at low levels in these complicated samples. This method was successfully applied to screen for PFAs in fish samples from four River Basins in Spain.

Fish samples from different species were treated by alkaline digestion with sodium hydroxide in methanol followed by solid-phase extraction.

Samples were processed using the XIC Manager and an XIC table containing the 21 targeted PFCs and about 28 non-targeted ones. A homemade MS/MS library was used to improve identification of target PFCs and high resolution MS to identify non-target ones, which were also fragmented and identified using the Formula Finder tool.

Quantitative method was developed using accurate mass measurements and known retention times for individual PFCs. For quantification of PFCs in the extracts, calibration curves for pure standards of each PFC in the range of 0.1 ng mL^{-1} to 100 ng mL^{-1} were generated using ABSciex MultiQuant Software. The concentration of target PFCs was calculated against these calibration curves using internal standards. The limits of detection and quantitation of the method were calculated by analysis of spiked biota with minimum concentrations of each individual compound at a signal-to-noise ratio of 3 and 10, respectively. They ranged between 0.1 and 8 ng g^{-1} and between 1 and 25 ng g^{-1}, respectively.

This method was successfully applied to material collected as part of the SCARCE project and provides evidence of the widespread presence of the PFCs in fish from the four largest rivers in the Spanish Mediterranean Rivers. It is clear that a great deal of work remains to be done to adequately describe the distribution of these materials in aquatic ecosystems and to estimate potential human exposures resulting from the consumption of fish from this region.

[1] Y. Picó, M. Llorca, M. Farré, D. Barceló, Crit. Rev. Food Sci Nut. 51 (2011) 605.

Acknowledgements: the section of mass spectrometry of SCSIE of the University of Valencia is deeply acknowledged for the use of the ABSciex triple TOFTM and particularly Dr. S. Galletero for the support. This work has been supported by the Spanish Ministry of Economy and Competitiveness through the projects CGL2011-29703-C02-02 and CSD2009-00065 as well as by the Research Programme on Health, Prevention and Prediction of the Illness of Conselleria de Sanitat of the Generalitat Valenciana (Project Nº 50/2011). DB acknowledges financial support from the Visiting Professor Program of the King Saud University, Riyadh, Saudi Arabia. This project has also been partly funded by King Saud University project number KSU-VPP-105. We also would like to thank the persons of the research groups from IDAEA and ICMAN for taking the samples.

ENV27

LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY TO DETERMINE SWEETENERS IN WATER SAMPLES AND SEWAGE SLUDGE

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Currently, sweeteners are an attractive alternative to replace the sugar; because they are not sources of calories to our body. Sweeteners are widely used in processed products that include tabletop sweeteners, baked goods, soft drinks, powdered drink mixes, candy, puddings, canned foods, jarms and jellies, dairy products. In addition, sweeteners are also added as excipients to some pharmaceuticals and personal oral care products. Recently, these additives have been suggested as potential organic contaminants because they are introduced in the environment by different pathways. After consumption, sweeteners are excreted and then enter to sewage treatment plants (STPs), where they can be absorbed to sludge generated during wastewater treatment [1-3].

In this work we develop two methods to determine eight sweeteners (saccharin, cyclamate, aspartame, acesulfame, neohesperidin dihydrochalcone, sucralose, stevioside and glycyrrhizic acid) in sewage, surface water and sewage sludge using solid phase extraction (SPE) or pressurized liquid extraction (PLE) followed by liquid chromatography − (electrospray) − tandem mass spectrometry (LC-(ESI)MS-MS).

The determination was performed using a rapid-resolution liquid chromatography coupled to a triple quadrupole tandem mass spectrometry with electrospray ionization (ESI). The chromatography column was an Ascentis Express reversed-phase amide (2.1 x 10 mm) with a 2.7 μm particle size. This column combines an embedded polar group stationary phase with the Fused-Core particles; this feature enhances the retention and selectivity for polar compounds, especially those that can act as hydrogen-bond donor such as sweeteners.

Optimal LC-(ESI)MS-MS conditions were performed using ESI in negative mode except for aspartame; data were acquired in multiple reaction monitoring (MRM) mode; the precursors ions chosen were $[M+H]^+$ or $[M-H]^-$ except for sucralose and stevioside ($[M+Cl^{35}]^-$), to enhance the sensitivity; confirmation of analytes was done by comparing the ratio between the two most abundant transitions.

In SPE and PLE several parameters were optimized several parameters in order to achieve the best extraction efficiency. Copolymeric hydrophilic-lipophilic balance HLB sorbent resulted to give superior efficiency to extract all compounds from aqueous samples. Different strategies such as deuterated compounds addition and matrix matched standards were taken into account to eliminate the matrix effect in LC-MS-MS.

This method was applied to several sewage and sewage sludge samples from two STPs and river water samples from different rivers located in Catalonia. Acesulfame, saccharine, cyclamate neohesperidin dihydrochalcone and sucralose were found in the samples analyzed.

[1] I.J. Buerge, M. Keller, H. Buser, M.D. Müller and T. Poiger, Environ. Sci. Technol. 45 (2011) 615.
[2] M. Scheurer, H. Brauch and F.T. Lange, Anal. Bioanal. Chem 394 (2009) 1585.
[3] R.N. Mead, J.B. Morgan, G.B. Avery, R.J. Kieber, A.M. Kirk, S.A. Skrabal, J.D.Willey, Mar. Chem. 116 (2009) 13.

ENV28

DEVELOPMENT OF A PDMS ROD-HPLC-DAD METHOD FOR THE DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN WATER SAMPLES

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Certain compounds have been found to cause disruption of the endocrine system, altering the development, growth, reproductive function and behaviour of organisms. These endocrine disrupting compounds (EDCs) include natural and synthetic chemicals, which are used in a great variety of applications, such as pesticides, fire retardants and plasticizers [1]. EDCs can reach the aquatic environment via wastewater treatment plant effluents, as conventional wastewater treatment processes are not specifically designed to remove these trace level organic contaminants [2]. Chlorinated phenols, alkylphenol polyethoxylates (APE) and bisphenol A (BPA) are EDCs. Chlorinated phenols are used in industry and agriculture and may also result from the degradation of phenoxy herbicides, organophosphorus pesticides and from the chlorination of humic substances. APE are non-ionic surfactants that have been used in cleaning products and industrial processes for more than 40 years. BPA is used in the manufacture of plastics and related products.

The polarity of these compounds and their low concentration in environmental samples makes it difficult to extract them from aqueous samples. In order to improve the sensitivity and therefore the detection limits, to comply with existing legislation, different extraction and preconcentration techniques have been developed. The most common techniques are solid phase extraction (SPE), which usually needs large sample volumes, and solid phase microextraction (SPME), which could be used in head space or immersion modes. Stir-bar sorptive extraction (SBSE) devices coated with polydimethylsiloxane (PDMS) have been used as an effective alternative [3]. Similarly, the use of PDMS rods allows the extraction and preconcentration of the compounds in a single step from water samples, avoiding the carryover problem related with SBSE and reducing costs [4, 5]. In the present study, we have developed an extraction technique based on the use of PDMS cord followed by LC with diode array detection (DAD) for the extraction of BPA, 4-nonylphenol (NP), 4-octylphenol (OP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) in water samples. Separation was achieved using a reverse phase column, with acetonitrile and MilliQ water with 0.5% acetic acid (40:60) as mobile phases in gradient mode. Various parameters affecting the extraction and desorption steps have been studied. The sample volume has been established in 50mL with enrichment factors of 5 for BPA, 80 for TCP and higher than 150 for PCP, NP and OP. Equilibrium was reached after 16 hours for BPA, TCP and PCP, and nearly as long for OP and NP. The addition of NaCl increases the recoveries, however, the addition of MeOH is not effective. Desorption parameters, such as solvent and time, were also studied, achieving good results with methanol for 45 minutes. The validation of the method was carried out with spiked water samples. Finally, the method was applied to the analysis of natural and waste water samples.

[1] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Mass Spectrometry Reviews, 29 (2010) 776.
[2] J. Xu, L. Wu, W. Chen, A.C. Chang, J. Chromatogr. A, 1202 (2008) 189-195.
[3] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1174 (2007) 27.
[4] A. Prieto, R. Rodil, J.B. Quintana, I. Rodríguez, R. Cela, M. Möder, Anal. Chim. Acta 716 (2012) 119.
[5] L. Montero, P. Popp, A. Paschke, J. Pawliszyn, J. Chromatogr. A 1025 (2004) 17.

Acknowledgments: this study has been financed by the Spanish Ministry of Science and Innovation; project CTM2011-28765-C02-02. C. Valls-Cantenys acknowledges a grant from the Spanish Ministry of Science and Innovation (Ref. BES-2009-024892).

ENV29

STRATEGIES FOR THE DETERMINATION OF CHLORINATED PARAFFINS BY GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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Chlorinated paraffins (CPs) are highly complex mixtures of chlorinated n-alkanes containing thousands of isomers, diastereomers and enantiomers. These mixtures have been produced on a large scale since 1930s and have been used for different industrial applications, such as additives in lubricant and cutting fluids for metalworking, plasticizers, adhesives, etc., due to their flame retardant properties [1]. According to their carbon chain length, CP mixtures can be classified into short (SCCPs, $C_{10\text{-}13}$), medium (MCCPs, $C_{14\text{-}17}$) and long chain chlorinated paraffins (LCCPs, $C_{18\text{-}30}$) [2], with a chlorine content varying from 30 to 70% by mass. Among them, SCCPs are of particular interest and concern because they are persistent in the environment, bioaccumulative through the food chain and toxic to aquatic organisms [3]. Therefore, some countries and organizations have imposed regulation on the production and use of these compounds [3]. SCCPs have been included on the OSPAR List of Chemicals for Priority Action and are classified under the European Community Water Framework Directive as Priority Hazardous substances. Recently, SCCPs have been selected as candidate to the list of persistent organic pollutants (POPs) by the Stockholm Convention [4]. Consequently, environmental levels of SCCPs should be monitored and reliable analytical methods are required.

Currently, CP mixtures are analysed by gas chromatography coupled to high (GC-HRMS) and low resolution mass spectrometry (GC-LRMS), operating in negative ion chemical ionization (NICI). GC-NICI-LRMS is by far the most frequently used technique for CP determination. ECNI-MS offers high sensitivity, but errors in the quantification (65-940%) have been observed due to strong differences on the response of congeners with different chlorination degree and unlike composition of the CP mixtures between samples and standards [5]. In the last years, many efforts have been devoted to dispose of suitable quantification methods for the determination of CPs, mainly using GC-LRMS [6], but so far there is no agreement on which method may be more reliable to be used as reference method.

In this work, an evaluation of strategies for the GC-NICI-MS analysis of chlorinated paraffins in environmental samples was performed using low resolution mass spectrometry with different mass analyser, such as ion-trap (IT), quadrupole (Q) and triple quadrupole (QqQ). In addition, the applicability of tandem mass spectrometry for the analysis of CPs, working in both electron ionization and negative ion chemical ionization modes, was also evaluated and the results were compared with those obtained using the GC-NICI-MS methods. Quality parameters, such as limits of detection (LODs) and quantification (LOQs), precision and accuracy, were determined for the studied GC-MS methods. In addition, several approaches were tested for the independent quantification of SCCPs and MCCPs in environmental samples. The results and conclusions achieved in this study are presented and discussed here.

[1] T.P. Rusina et al., Intern. J. Environ. Anal. Chem. 91, 4 (2011) 319.
[2] U.E. Friden et al., Environ. Int. 37 (2011) 1169.
[3] E. Sverko et al., Environ. Sci. Technol. 46 (2012) 4697.
[4] M.-L. Nilsson et al., Environ. Pollut. 163 (2012) 142.
[5] M. Coelhan, CLEAN – Soil, Air, Water, 38 (2010) 452.
[6] F.J. Santos et al., Anal Bioanal Chem 386 (2006) 837.

ENV30

SIMULTANEOUS DETERMINATION OF MICROPOLLUTANTS IN WATER SAMPLES BY HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Contamination of environmental waters by trace levels of organic substances is a subject of increasing concern in the majority of countries. Organic micropollutants in water are considered one of most the important kinds of organic contaminants because of their adverse environmental and health effects. This group includes any organic compounds that may be found at microgram per litre concentrations or lower in waters, such as pesticides, pharmaceutical residues, hormones, flame-retardants, plasticizers, perfluorinated compounds, among others [1]. The low levels of micropollutants in waters and the high complexity of water samples require the development of high sensitive and selective analytical methods that can simultaneously determine a broad range of these pollutants.

Therefore, this study focused on the developed of an analytical method, based on headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS), for the simultaneous characterization of 78 micropollutants in water samples. The selected target micropollutants include some volatile organic compounds (VOCs) (e.g. chlorobenzenes, naphthalene, chloroalkanes), endocrine disrupting compounds (EDCs) (e.g. bisphenol A and tributyl phosphate), odour compounds, (e.g. limonene, phenol, skatole), fragrance allergens (e.g. geraniol, cinnamyl alcohol, eugenol) and also some pesticides (e.g. heptachlor, terbutryn and dicofol). We have considered several variables affecting the chromatographic behaviour of the target compounds based on previous works [2-4], and investigated the experimental conditions affecting their extraction using HS-SPME. The variables considered in this study were: the type of fibre, the time and extraction temperature, and the ionic strength of the samples. Method validation showed good linearity, reproducibility, repeatability and low detection limits (at low ng/L levels).

The validated method has been used for the determination of the target organic micropollutants in different kinds of aqueous samples, such as permeates of reverse osmosis treatments, surface waters (Llobregat river, Barcelona), effluents of urban wastewater treatment plants (of EDAR, Vila-Seca) and sea waters (Mediterranean Sea). The optimized method showed good performance in the different types of waters studied.

[1] S. D. Richardson, Anal. Chem, 84 (2012) 747.
[2] A. Godayol, M. Alonso, E. Besalú, J. M. Sánchez, E. Anticó, J. of Chrom.A, 1218 (2011) 4863..
[3] E. Becerril, J. P. Lamas, L. Sanchez-Prado, M. Llompart, M. Lores, C. Garcia-Jares, Talanta 83 (2010) 464.
[4] N. Sadao, D. Shigeki, Anal. Chim. Acta 548 (2005) 79.

ENV31

ORGANIC NITROGEN COMPOUNDS IN INDOOR DUST SAMPLES BY COMPREHENSIVE GAS CHROMATROGRAPHY AND NITROGEN CHEMILUMINISCENCE DETECTION

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Settled dust particles act as a sink and repository of semi-volatile contaminants transported by the atmosphere. Since contaminants bound to indoor dust are more persistent than those outdoors, the composition of indoor dust is considered an indication of atmospheric pollution and is currently an important source of information when developing human exposure assessments [1]. Organic nitrogen (ON) compounds are a group of contaminants of special concern because of their known carcinogenic activity and their adverse environmental effects. Although the presence of ON compounds in atmospheric samples has been largely demonstrated [2,3], there is little information regarding the composition of ON in indoor dust.

The aim of this study is the determination of ON compounds in indoor dust samples. The high complexity of this matrix requires the use of highly selective extraction and high resolution techniques. The analytical method used here was based on in-cell clean up pressurized liquid extraction (PLE) of the dust samples followed by comprehensive two dimensional gas chromatography with nitrogen chemiluminiscence detection (GCxGC-NCD) [4]. The combination of these techniques results in a method for the analysis of ON in indoor dust samples, with higher specificity and sensitivity to traditional methods, that provided limits of detection and quantification at low ng g^{-1} for the identified ON compounds.

The method has been used for determining the ON content of indoor dust samples from smoking and non-smoking homes from Spain and the UK. Several ON compounds have been detected in the samples including nitriles, alkyl nitrocompounds, amides, nitro-PAHs, nitrosamines, nicotine and tobacco-specific nitrosamines. The presence of these compounds in the different kinds of samples and their health concern is also discussed.

[1] F. Mercier, P. Glorennec, O. Thomas, B. Le Bot, Environ. Sci. Technol. 45 (2011) 6716.
[2] M.Z. Özel, M.W. Ward, J.F. Hamilton, A.C. Lewis, T. Raventos-Duran, R.M. Harrison, Aerosol Sci. Technol. 44 (2010) 109.
[3] M.Z. Özel, J.F. Hamilton, A.C. Lewis, Environ. Sci. Technol. 45 (2011) 1497.
[4] N. Ramírez, M.Z. Ozel, A.C. Lewis, R.M. Marcé, F. Borrull, J.F. Hamilton, J. Chromatogr. A 1219 (2012) 180.

ENV32

BIOTIC AND ABIOTIC FATE OF THE ANTICONVULSANT LAMOTRIGINE IN THE AQUATIC ENVIRONMENT

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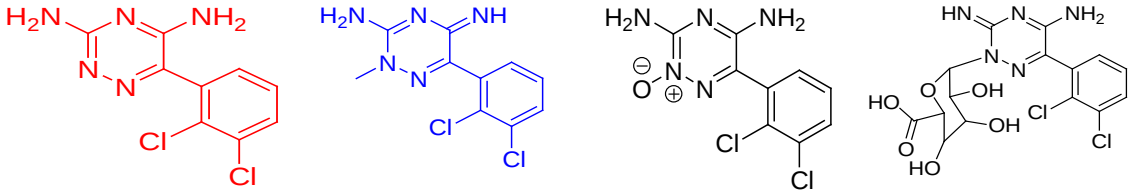
Several pharmaceuticals have been frequently detected in the aquatic environment. However, some drugs are not currently considered in current wastewater and aquatic environmental measurement programs. For instance, Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine) which is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder, chemically unrelated to any currently marketed antiepileptic drugs. It is extensively metabolized in humans to produce predominantly the N2-glucuronide and to a minor extent the N2-methyl-lamotrigine. The N2-methylated metabolite has been found to have cardio-active properties[1]. Recently, a U.S. study revealed the environmental occurrence of lamotrigine and its N2-glucuronide with mean concentrations in wastewater of 488 and 209 ng/L, respectively[2]. Less frequent detection in surface waters went along with occasional positive findings in groundwater samples.

In view of these findings, which suggest incomplete removal of lamotrigine and its glucuronide in sewage treatment plants (STP), we set out to assess the potential of biodegradation of the parent drug and its glucuronide by the bacterial community in mixed liquor from a municipal STP. The UPLC analysis of the samples from the aerated batch-reactor loaded with undiluted mixed liquor and spiked with lamotrogine showed the formation of a single metabolite. High resolution mass spectrometry (QToF) pointed towards conversion of lamotrigine through a methylation pathway. In ongoing studies we are currently investigating the degradation of the N-methylated lamotrigine in mixed liquor biorreactors.

A part for the identification of the TPs which can be formed in the WWTP, we also are currently studying the photolysis of lamotrigine and its metabolites in order to have a complete understanding of the fate of such molecule in the aquatic environment.

In a monitoring survey of surface waters from the river Rhine (Basel, Switzerland) and in effluent samples of Swiss STPs that discharge into this river, lamotrigine was detected along with its human metabolites and the proposed methylated TP using Orbitrap mass spectrometry.



Lamotrigine N2-methyl-Lamotrigine N2-oxide-Lamotrigine N2-glucuronide-Lamotrigine

Figure 1. Structures of Lamotrigine, its metabolites and its TP

[1] Hussein, Z et al. Br. J. Clin. Pharmacol. 43 (1997) 457
[2] Ferrer, I et al. Anal. Chem. 82 (2010) 8161

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ENV33

ASSESSMENT OF SUSPECTED ALLERGENS AND SYNTHETIC MUSKS IN WATERS BY SOLID-PHASE MICROEXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Personal care products (PCPs) are a class of emerging toxic substances which are composed of a wide variety of active ingredients, including preservatives, antimicrobials and fragrance components. The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has identified 26 of these components suspected to cause contact allergies (e.g., eugenol and citral) [1]. Due to their wide use in soaps, detergents, cosmetics, and other consumer products, fragrances can be found in domestic waters, so they are continuously released into wastewater treatment plants, where some of them cannot be completely eliminated during the different treatments. In the last years, increasing attention has been given to the determination of this group of substances. Analytical methods used for their quantification are mainly based on gas chromatography-mass spectrometry (GC-MS).

In the present work we have developed a methodology based on solid-phase microextraction (SPME) with GC-MS for the analysis of 16 common fragrance allergens and 2 synthetic musks in water samples. A preliminary evaluation of different fibres showed that the PDMS/DVB fibre was the most adequate for the extraction of the target compounds. A design of experiments with full factorial model has been applied to evaluate the effects of the experimental parameters affecting the extraction of the target compounds by HS-SPME (e.g., salt content, time and extraction temperature). After determining the optimum conditions (1.2 g NaCl, 45 min at 90ºC) the quality parameters of the proposed method have been evaluated. Finally, the method has been applied to the analysis of different water matrices such as wastewater, swimming pool water and bath water samples.

[1] SCCNFP/0017/98 Final FRAGRANCE ALLERGY IN CONSUMERS (December 1999).

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STUDY AND CHARACTERIZATION OF THE ORGANIC NON-VOLATILE FRACTION OF PRIMARY AND SECONDARY SUBMICRON AEROSOLS BY NEW SCREENING TECHNIQUES BASED ON HPLC-QTOF

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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 from sources such as biomass burning, incomplete combustion of fossil fuels, and wind-driven or traffic-related suspension of road, soil, mineral dust, sea salt, and biomass materials. Secondary particles are formed by gas-to-particle conversion in the atmosphere by nucleation and condensation of gaseous precursors, although primary particles may also be modified by single phase or multiphase reactions. Fine (<1 µm) and ultrafine particles (<0.1 µm) mainly stem from traffic emissions and are suspected to be hazardous to human health. Due to their small size they are able to pass through the respiratory tract thereby reaching the bloodstream.

Fine portions of aerosol samples were obtained in the city of Madrid (Spain) by using a Digitel High-Volume Autosampler DHA-80. 150 mm diameter 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, shortly, consisted in an ultrasound bath extraction with MeOH/DCM (2:1; v:v) for three times and a further clean up with activated alumina using hexane and dichloromethane as eluting solvents. The analysis was carried out in an Agilent 6520 Accurate Mass Q-TOF coupled to an Agilent 1200 Series Liquid Chromatograph. The extracts, diluted to 100 µL of methanol (Merck, HPLC grade), were analyzed in a flow of 100 µL of isocratic gradient with MeOH/H_2O (50/50: v/v) as a mobile phase in Electrospray (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, respectively. The acquisition was done in a wide mass range (25-1,700 m/z) in order to obtain a profile or screening of the aerosol samples.

We obtained a fingerprint of the main organic components of primary and secondary aerosol and they can be divided into those with anthropogenic and those with biogenic origin. In the first group, we found dicarboxylic acids (malic, glutaric or azelaic acids), petrogenic PAHs (phenanthrene, coronene or benz[a]anthracene), alkanals (nonanal), alkanoic acids (decanoic acid), and esters (iso-propil-palmitate). As biogenic compounds, we found diverse compounds originating from higher plants such as alkanes (tricosane to tritriacontane), monoterpenes (pinnic and pinonic acids or pinonaldehyde), isoprenes (2-methyl-treitol or methylglyceric acid) and sesquiterpenes (δ-cariofilinic acid) and carbohydrates (levoglucosan).

[1] Kavouras I.G. and Stephanou E.G., J. Geophys. Res. 107 (2002) 4069.
[2] Tuhkanen, T.A. and Beltran F.J., Chemosphere, 34 (1995) 2116.
[3] Williams, B.J., Goldstein A.H., Millet, D.B., Holzinger R., Kreisber N.M., Hering S.V., White, A.B., Worsnop D.R., Allan J.D. and Jimenez J.L. J. Geophys. Res. 112 (2007) 1.

ENV35

DETERMINATION OF ORGANIC POLLUTANTS IN WATER SAMPLES BY IN TUBE – SOLID PHASE MICROEXTRACTION COUPLED UPLC-TANDEM MASS SPECTROMETRY

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During the last decade, the use of many chemical substances without any control has caused a harmful effect in the ecosystems. Studies about these substances behaviour in the environment have become a focus of interest for the European Union (EU) [1]. Many of these contaminants are characterized by a strong persistence which explains their wide presence in the soil–water environment and their accumulation in fatty tissue. In this context, the EU adopted the WFD 2000/60/EC, whose objectives are to improve, protect and prevent further deterioration of water quality across Europe [2]. To comply with this regulation, a multiresidue analysis based on in-tube solid phase microextraction-ultra high liquid chromatography- tandem mass spectrometry (IT-SPME-UPLC-MS/MS) has been developed for 9 of the priority and preferent compounds analytes presenting different physicochemical properties included in the WFD.

IT-SPME coupling with UPLC-MS/MS was proposed by the first time in our knowledge and was used for on-line enrichment of analytes without any previous sample treatment. The device was equipped with a GC TRB-5 capillary column, used as pre-concentration loop, and two conventional six-port injection valves. Sample water volume and extracted volume for desorption of compounds were assayed and 4 ml of sample followed by 40 µl of methanol were selected as optimum conditions.

Separation was carried out on a UPLC C18 column (1.7 µm, 2.10 × 50 mm) using a gradient elution profile of mobile phase consisting of 10 mM ammonium formate in both methanol and water. The analytes were detected with a mass spectrometer after being ionised positively using an electrospray ionisation (ESI) source. The two most intense precursor ion→product ion transitions were monitored to obtain unambiguous confirmation of the compound identity, excepting for trifluoralin.

Analytical parameters of the proposed method were established. Selectivity, linearity, precision, recoveries and limits of detection (LODs) were studied. The method presents good linearity over the range assayed, 0.025-2.5 µg/L for chlorpyriphos and 0.025-25 µg/L for the other compounds. It also allowed reaching LODs between 0.025 µg/L to 2.5 µg/L and average recoveries ranged from 4.83 to 336.9 %. Precision was good, and the achieved intra and inter-day variation coefficients were < 26 and 31.6 % respectively. The results of the validation procedure confirmed that the method is suitable for the planned purpose.

The developed and validated method has been applied to several water samples from different sources and it has been demonstrated that it permits the on-line enrichment of the analytes with advantage of minimum sample manipulation, and the identification and quantification of some organic pollutants in water samples in the range of low parts-per-billion.

[1] Baugros, J. B., Giroud, B., Dessalces, G., Grenier-Loustalot, M. F., & Cren-Olivé, C., *Analytica Chimica Acta* 607(2008) 191.
[2] Barrek, S., Cren-Olivé, C., Wiest, L., Baudot, R., Arnaudguilhem, C., & Grenier-Loustalot, M. F., Talanta 79 (2009) 712.

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ENV36

ANALYSIS OF VOLATILE POLYFLUORINATED ALKYL SUBSTANCES IN WATER BY HEADSPACE SOLID-PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Polyfluorinated compounds (PFCs) comprise a diverse group of chemicals that have been widely used during fluoropolymer production and as surfactants in consumer applications for over 50 years. During production and usage, PFCs are released into the environment, where they have been found to be ubiquitous in water, air, sediment and biota. In recent years, several ionic PFCs, including perfluorocarboxylates (e.g., perfluorooctanoate, PFOA) and perfluoroalkyl sulfonates (e.g., perfluorooctane sulfonate, PFOS), have drawn a great interest because they are persistent in the environment, can be bioaccumulated through the food chain and some of them can be toxic to human and wildlife health [1,2]. Nevertheless, neutral PFC compounds, such as fluorotelomers alcohols (FTOHs) and perfluorooctane sulfonamides/ethanols (FOSAs/FOSEs), have received a limited attention despite they are the precursors of PFOA and PFOS and contribute to the presence of the ionic PFCs in the environment. FTOHs are important raw materials that are often used in many commercial and industrial products, including polymers, paints, and coating [3]. FOSAs and FOSEs are used in surface treatments for paper protection and in the production of fire-fighting foams, cleaners, personal care products, and in insecticides [3]. FTOHs and 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 [4]. Although information about their toxicity is still very limited, it is important to understand the occurrence and the distribution of these compounds in the environment.

The analysis of these compounds is difficult due to their volatility and the potential sources of background contamination. Nowadays, very few papers have been published regarding their presence in water samples. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) combined with gas chromatography-mass spectrometry (GC-MS) are the methods currently used for the analysis of these compounds in aqueous samples [5-7]. Nevertheless, these methods are time-consuming and require an intensive labour. Therefore, there is a great interest in fast and sensitive methods with enough selective for the determination of FTOHs and FOSAs/FOSEs in water samples. In this sense, headspace solid-phase microextraction can be an excellent alternative.

In this work, we have developed a new method for the analysis of FTOHs, FOSAs and FOSEs, in water samples at low concentration levels ($ng \cdot L^{-1}$) using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). Among the different SPME fibres evaluated, the polydimethylsiloxane-divinylbenzene (PDMS/DVB) stationary phase provided the best results. To achieve maximum sensitivity and selectivity, parameters affecting the efficiency of the SPME extraction and desorption of the target compounds were optimized. Moreover, different MS ionization modes, electron ionization (EI), positive (PCI) and negative ion chemical ionization (NICI), were evaluated to obtain the maximum sensitivity and selectivity in the determination of these compounds. Quality parameters, such as limits of detection and quantification, repeatability and long-time precision, were established and the proposed method was successfully applied to the analysis of FTOHs, FOSAs and FOSEs in water samples collected in the area of Barcelona.

[1] C. Liu, R.Y.H. Gin, V.W.C. Chang, B.P.L. Goh, M. Reinhard, Environ. Sci. Technol. 45 (2011) 9758.
[2] K. Kannan, Environ. Chem. 8 (2011) 333.
[3] N. Stock, F. Lau, D. Ellis, J. Martin, D. C. G. Muir, S. A. Mabury, Environ. Sci. Technol. 38 (2004) 991.
[4] C.M. Butt, U. Berger, R. Bossi, G. Tomy, Sci. Total Environ., 408 (2010) 2936.
[5] M. Plumlee, McNeill, K. Reinhard. Environ. Sci. Technol. 43 (2009) 3662.
[6] M. Mahmoud, A. Kärrman, S. Oono, K. Harada and A. Koizumi, Chemosphere, 74 (2009) 467.
[7] S.Taniyasu, K. Kannan, M. Ka So, A. Gulkowska, E. Sinclair, T. Okazawa, N. Yamashita. J. Chromatogr. A, 1093(2005) 89.

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MODELING SOIL-WATER SORPTION BY MEANS OF CHROMATOGRAPHIC SYSTEMS

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The uptake of organic compounds by soil from water plays an essential role in determining the transport and fate of contaminants in the environment. It is a process of great importance in the field of environmental protection and risk assessment.

The soil-water partition coefficient normalized to the organic carbon content of the soil, K_{oc}, is the most accepted constant for describing soil-water sorption independently of the type of soil considered. However, the experimental determination of K_{oc} data is often very expensive, time-consuming, and tedious, and tests involving soils are also subject to a variety of experimental difficulties and artifacts. As a consequence, estimation methods are required.

Chromatographic systems can provide practical and sustainable alternatives for the estimation of K_{oc} values. Their measurements are usually fast, economic, simple, and reliable. The key issue is finding which chromatographic systems model well the sorption of organic compounds by soil from water.

Recently, we have proposed a systematic procedure [1] for evaluating the goodness of chromatographic systems to model the sorption of neutral organic compounds by soil from water. It is based on the examination of the three sources of error that determine the overall variance obtained when soil-water partition coefficients are correlated against chromatographic retention factors: the variance of the soil-water sorption data, the variance of the chromatographic data, and the variance attributed to the dissimilarity between the two correlated systems. These contributions of variance can be easily predicted through the characterization of the systems by the solvation parameter model [2].

According to this procedure, several chromatographic systems of micellar electrokinetic chromatography and high-performance liquid chromatography, besides the reference octanol-water partition system, have been selected to finally test their performances by experimental correlations. The predicted variances agree with the experimental results. Both show that the high-performance liquid chromatography system based on an immobilized artificial membrane and the micellar electrokinetic chromatography systems of sodium dodecylsulfate and sodium taurocholate are the most suited for the estimation of K_{oc} values. They provide the most precise correlation models and have shown to predict well soil-water sorption coefficients of various tested herbicides of agrochemical interest [3].

[1] M. Hidalgo-Rodríguez, E. Fuguet, C. Ràfols, M. Rosés, Anal. Chem. 82 (2010) 10236.
[2] M. H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[3] M. Hidalgo-Rodríguez, E. Fuguet, C. Ràfols, M. Rosés, J. Chromatogr. A, doi:10.1016/j.chroma.2012.06.058.

ENV38

DETERMINATION OF PHARMACEUTICAL COMPOUNDS IN SEWAGE SLUDGE USING A STANDARD ADDITION METHOD APPROACH

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After the intake of pharmaceuticals, a significant fraction is excreted from the body with or without prior metabolism resulting in their arrival to wastewater treatment plants (WWTPs). Due to their incomplete removal in WWTPs, considerable amounts of these compounds remain in effluents and biosolids and so re-enter the environment. Therefore, the use of both reclaimed water and biosolids in fact introduces contaminants into the environment [1].

Different analytical methodologies have been developed to determine pharmaceutical compounds in sewage sludge based on the use of highly energetic extraction techniques such as microwave assisted extraction (MAE) or pressurized liquid extraction (PLE) [2]. However, due to the dissolved organic matter co-extracted from sewage sludge samples, ionic suppression is produced in most electrospray techniques used in LC-MS and so the use of isotope-labelled compounds is mandatory [3].

In this study we have developed an alternative fast and simple analytical methodology for the determination of nine pharmaceutical compounds –sulfamethazine, sulfapyridine, sulfamethoxazole, sulfathiazole, clofibric acid, naproxen, diclofenac and ibuprofen – in sewage sludge samples. A standard addition method based on ultrasonic solvent extraction (USE) followed by LC-MS/MS is used. This methodology has the advantage of not requiring the use of isotope-labelled compounds. The optimization of the extraction procedure led to a solution of 2 mL of MeOH:H_2O (1:1) being set for the extraction of the target compounds from 200 mg of freeze-dried sewage sludge. Extraction was performed for 15 min in an ultrasonic bath and then samples were subjected to centrifugation for 10 min at 4000 rpm. Extracts were collected and filtered (0.2 µm pore size) and then analysed by UHPLC-MS/MS (1290 Infinity LC System and 6430 Triple Quad, Agilent Technologies, USA) to determine the concentration of the target pharmaceuticals. Sample acquisition was performed in MRM mode by recording two MRM per compound. Standard addition method was applied in each sample adding a mixture of the nine pharmaceutical compounds ranging from 5 to 250 ng g^{-1} (n=6).

Analytical parameters such as recoveries and method detection limits (MDL) were determined by analysing blank samples (rabbit excrement). Recoveries of between 88% and 100% were obtained with repeatability at less than 16% and MDLs of between 1 and 12 ng g^{-1} with good linearity. These results are similar to those found using isotope-labelled compounds and HPLC-MS/MS [4, 5]. Finally, the analytical method developed was successfully employed for the determination of pharmaceutical compounds in real sludge samples. Although a seasonal dependence was observed for some compounds, ibuprofen, naproxen and sulfathiazole were the most abundant pharmaceuticals (from 30 to 1140 ng g^{-1}).

[1] M. Hörsing, A. Ledin, R. Grabic, J. Fick, M. Tysklind, J.C. Jansen, H.R. Andersen, Water Res. 45 (2011) 4470.
[2] A. Nieto, F. Borrull, E. Pocurull, R.M. Marcé, Trends Anal. Chem. 29 (2010) 752.
[3] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, H. Trufelli, Anal. Chem. 80 (2008) 9343.
[4] P. Vázquez-Roig, R. Segarra, C. Blasco, V. Andreu, Y. Picó, J. Chromatogr. A 1217 (2010) 2471.
[5] A. Jelic, M. Gros, A. Ginebreda, R. Cespedes-Sánchez, F. Ventura, M. Petrovic, D. Barceló, Water Res. 45 (2011) 1165.

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GROUPS OF POLYPHENOLS IN CORK BOILING WATER

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Cork oak landscapes extend over an area of almost 2.7 million hectares (ha), in seven countries: Portugal; Spain; Algeria; Morocco; Tunisia; Italy and France.Cork is composed of dead cells that accumulate on 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 (0,4-0,9 mg/L). Uncontrolled discharge of these untreated effluents will cause serious damages to the environment. Recovery of polyphenols can reduce pollution in these spills and on the other hand, it is a good opportunity to obtain useful by-products. This work characterized samples from cork boiling water in three factories and in several stages of the boiling cycle, searching for useful compounds.
The samples were analysed by HPLC on a 4.6 mm i.d. reversed-phase C18 column and Detection was carried out using a diode array and detector. Another samples fraction was analysed by HPLC-QTOF in order to confirm the target compounds.
Four known compounds which were previously reported in cork extract as low molecular weight polyphenols [1], were detected and quantified (gallic, ellagig, vanillic and protocatechiuc acids):

Identified low weight polyphenols (mg/L) per factory and boiling cycle

Boiling cycles								
Factories	**Factory 1**		**Factory 2**			**Factory 3**		
Days	**1**	**4**	**1**	**2**	**3**	**1**	**2**	**3**
Total polyphenols	484,65	943,32	502,02	802,29	892,29	243,37	365,39	463,61
Gallic acid	12,35	23,43	11,56	20,61	18,15	3,00	6,43	10,21
Ellagic acid	11,47	7,34	7,99	5,45	2,51	6,64	12,79	16,78
Vanillic acid	---	7,14	---	4,85	6,03	1,51	2,88	3,33
Protocatechiuc acid	6,8	12,12	0,99	4,28	9,86	2,11	1,71	4,9

On the other hand, traces of syringic and ferulic acids and vainillin were found.
In general, the amount of these compounds is proportional to the total polyphenols concentration during the boiling cycle but in some cases (factories 1-2), an alleged degradation of ellagic acid is likely due to a decrease of this concentration during the boiling cycle.
Identified polyphenols such as ellagic acid, have commercial value and their recovery can generate add-value for cork factories.
Four main compounds with relative abundance were detected and remain unidentified but may be derived from cork ellagitannins and currently their characterisation is in progress in order to find commercial applications.
At the same time, polyphenols extraction can reduce the pollution of these spills.
Margins: top and bottom: 2,5 cm; right and left: 3 cm.

[1] Elvira Conde et al., J. Agric. Food Chem., 45 (1997) 2695.

ENV40

FAST SIMULTANEOUS ANALYSIS OF MAJOR ANIONIC AND CATIONIC COMPOUNDS IN ATMOSPHERIC PARTICULATE MATTER BY CAPILLARY ELECTROPHORESIS WITH CONTACTLESS CONDUCTIVITY DETECTION

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Water soluble ionic compounds are major components of the atmospheric particulate matter (PM). This fraction includes inorganic anions (chloride, sulfate and nitrate), cations (ammonium, potassium, sodium, calcium and magnesium) [1] and a complex and poorly characterized mixture of organic compounds, among which dicarboxylic acids are the most commonly identified species.

The analysis of major ionic compounds present in the atmospheric particulate matter (PM) is mandated by the European Union to provide accurate information of PM composition in the background [2] and is also included as part of the PM2.5 National Ambient Air Quality Standards program from the US-EPA. Ion chromatography (IC) is the recommended and preferred technique for the analysis of inorganic anions and cations due to its robustness, sensitivity and separation power, however relatively long analysis times and two separate methods and columns for the analysis of anions and cations are commonly required. Capillary electrophoresis offers an attractive alternative to perform these analyses including low separation times, low reagent consumption and the possibility to analyze anions and cations simultaneously.

In this work a method for the simultaneous analysis of major inorganic and organic water soluble PM compounds based on the use of CE with dual opposite injection and contactless conductivity detection is described. Five different buffer compositions were tested to obtain a separation medium compatible with the analysis of anions, cations and low molecular weight carboxylic acids. Buffers were evaluated in terms of resolution, efficiency and sensitivity. Electroosmotic flow control and injection conditions were considered in order to achieve fast and reproducible separations. The minimum possible capillary length compatible with the commercial instrument was employed to minimize the separation time. Under the final selected conditions the separation of 11 ions was achieved in 70 seconds with limits of detection ranging between 0.010 and 0.1 mgL^{-1} (figure 1). The method was applied to the study of the distribution of water soluble compound in a PM filter. Observations showed an approximately homogeneous distribution of the analyzed compounds in the filter with RSDs around 10 %.

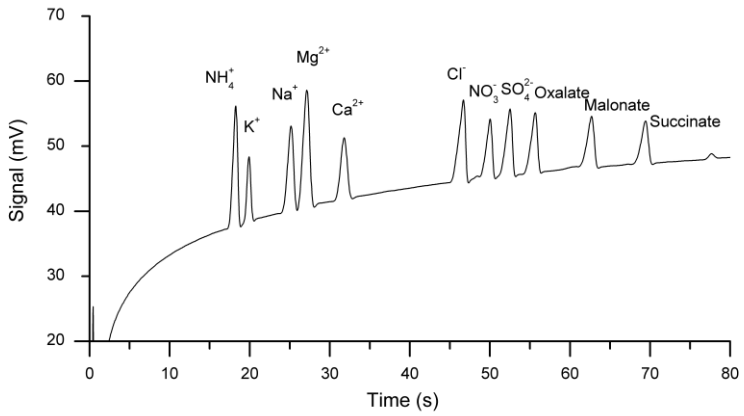

Figure 1. Electropherogram obtained from the analysis of a 5 mgL^{1-} standard mixture.

[1] Tsitouridou, R., Voutsa, D. and Kouimtzis, Th., Chemosphere. 52 (2003) 883.
[2] Directive 2008/50/CE of the European Parliament and the Council of 21 May of 2008.

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COUPLING OF THE IN-TUBE SOLID-PHASE MICROEXTRACTION AND CAPILLARY LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY FOR DETERMINATION OF CARBONYL COMPOUNDS IN ATMOSPHERIC PARTICULATE MATTER

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The instrumental approach proposed which is based on in-tube solid-phase microextraction (IT-SPME) coupled to capillary liquid chromatography/ mass spectrometry (µ-LC/MS) combines the advantages of a miniaturized system, along with the high sensitivity and selectivity of MS detection.

In a previous study [1], a miniaturized system based on coupling IT-SPME and capillary liquid chromatography using UV detection was used for the screening of carbonyl compounds derivatized with 2,4-dinitrophenylhydrazine (DNPH). In this work, other carbonyl compounds, 10 aliphatic aldehydes from formaldehyde to decyladehyde, unsaturated aldehyde of four carbon atom (crotonaldehyde) and a cyclic ketone (cyclohexanone) was tested using derivatization solution.

Gradient elution with water and acetonitrile and two capillary reversed-phase SiO_2 and TiO_2-based columns were employed. The titania-based columns have been used only in a few separations, since they have been recently introduced [2]. The results provided for the complex mixture of carbonyl compounds analyzed, using the reversed-phase (RP) TiO_2 column are comparable to those obtained with a RP SiO_2 column. The most remarkable difference between the two columns is that the peak broadening is lower in SiO_2-based column than in TiO_2-based one.

Concerning the optimization of MS conditions, differences between the semivolatile compounds (aliphatic aldehydes containing a number of carbon atom from 7 to 10, C_7- C_{10}) sensitivity and other compounds up to C_6, were found. The conditions were adjusted in order to achieve a maximum sensitivity for semivolatile compounds.

Parameters of IT-SPME such as sample volume and replacement volume were adjusted. The procedure of IT-SPME were compared to the direct inject using 2µl. The peak area normalized for the 12 carbonyl compounds tested, after concentration by IT-SPME is compared to peak area obtained using the direct injection. The semivolatile compounds were the carbonyl compounds that showed a greater concentration. Linear ranges from 0.005 to 5ng mL^{-1} of carbonyl compound derivatized were obtained. Inter-day precision (%RSD) was in the range from 5 to 18%. The method was applied to the screening of aqueous extracts of particulate matter PM_{10} and $PM_{2.5}$

[1] M.C. Prieto-Blanco, P. López-Mahía, P. Campíns-Falcó, J. Chromatogr. A 1218 (2011) 4834.
[2] M.C. Prieto-Blanco, Y. Moliner-Martínez, P. López-Mahía, P. Campíns-Falcó, J. Chromatogr. A 1248 (2012) 55.

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DETERMINATION OF ILLICIT DRUGS IN WATER SAMPLES BY COUPLING IN-LINE SOLID-PHASE EXTRACTION AND CAPILLARY ELECTROPHORESIS

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The abuse of illicit drugs has become a serious problem around the world. The residues of these substances and their metabolites that are excreted by humans flow into and through wastewater treatment plants. The fraction that is not removed remains in the environment, representing the main source of pollution in surface waters [1, 2] and even in drinking water [2, 3]. The study of the levels of these drugs in water samples can provide information about their consumption.

Capillary electrophoresis (CE) has been found to be a useful approach for the determination of these kinds of drugs in different matrices [4, 5]. One of the main drawbacks of CE is its poor sensitivity when is applied to the analysis of environmental samples. Therefore, in order that CE can be suitable for determining illicit drugs in water samples, where these drugs are usually present at low concentrations, it is necessary to develop strategies to reduce the LODs. Several approaches have been reported to solve this important issue in CE. One of them is the application of a preconcentration technique based on solid-phase extraction (SPE) [6-8]. Among the different strategies to combine SPE and CE, we have chosen the in-line coupling between both techniques to preconcentrate and separate cocaine (COC) and it major metabolite, benzoylecgonine (BE), in environmental water samples.

The SPE-CE device consisted of a short length of a capillary of 2 mm packed with Oasis MCX near to the inlet end of the separation capillary. Using this sorbent, higher retention of these kinds of analytes can be achieved because it can performance both hydrophobic and ionic interactions. A detailed study of different parameters affecting the in-line SPE performance, such as sample pH, volume of the elution plug and sample injection time have been studied. This approach has resulted efficient for the determination of the illicit drugs in environmental waters samples. Using this strategy is possible to obtain LODs at sub-ng/mL levels by the injection of much larger amount of sample than conventional CE analysis.

[1] M. Pedrouzo, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 34 (2011) 1091.
[2] I. Botello, F. Borrull, C. Aguilar, M. Calull, Electrophoresis 33 (2012) 528.
[3] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Environ. Sci. Technol. 42 (2008) 6809.
[4] C. Cruces-Blanco, A.M. García-Campaña, Trends Anal. Chem 31 (2012) 85.
[5] M. Janicka, A. Kot-Wasik, J. Namiesnik, Trends Anal. Chem 29 (2010) 209.
[6] P. Puig, F. Borrull, M. Calull, C. Aguilar, Trends Anal. Chem. 26 (2007) 664.
[7] R. Ramautar, G.W. Somsen, G.J de Jong, Electrophoresis 31 (2010) 44.
[8] P. Puig, F. Borrull, M. Calull, C. Aguilar, Anal. Chim. Acta 616 (2008) 1.

ENV43

MULTIVARIATE OPTIMIZATION OF PTV-GC-MS METHOD FOR SIMULTANEOUS DETERMINATION OF ORGANOMETALLIC COMPOUNDS

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Organomercury, organolead and organotin compounds are the organometallic species most often studied in the environment, and their presence has increased owing to anthropogenic activities. These compounds were recognized for the first time as being responsible of serious environmental contamination, and consequently the European Union listed them as priority pollutants. As an example, the Directive 2008/105/EC of the European Parliament and of the Council and more recently the Proposal for a directive of the European Parliament and of the Council, amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy 2011/0429, includes mercury and its compounds as well as butyltins in the list of 33 priority substances [1-3].

A simple chromatographic method was developed for the simultaneous determination of various organometallic compounds of mercury, lead and tin (inorganic tin, monobutyl-, dibutyl- and tributyltin, inorganic mercury, methylmercury, inorganic lead, trimethyl- and triethyllead) by using sodium tetra (N-propylborate) as derivatization reagent. In this work one of the most popular large-volume injectors, PTV-splitless (programmed temperature vaporization) injection, was optimized to determine 9 organometallic compounds at ultratrace levels. The injection conditions using an injection volume of 25µl were optimized applying experimental designs. Nine experimental factors that could affect the vaporization efficiency were studied (injector heating rate, injection speed, split vent flow, injector isothermal time, injector initial temperature, oven isothermal time, oven initial temperature, splitless time and a dummy factor). Then, the optimum values of the significant factors were found by a central composite design.

The chromatographic procedure developed was applied to a simple, reliable and rapid multielemental speciation method to determine various organometallic compounds in water samples. The procedure is based on one-step liquid-liquid derivatization/extraction with sodium tetrapropylborate ($NaBPr_4$) in the presence of a small volume of isooctane.

The organic extracts are analysed by GC-MS using tetrabutyltin (Bu_4Sn) as internal standard. Detection limits in the low $\mu g l^{-1}$ level, lineal range, repeatability and reproducibility in the range of 1.3-20% were achieved for all compounds under study. The accuracy of the method measured as the average percentage recovery of the compounds in water samples was satisfactory for all compounds studied. The whole method was successfully applied to the determination of organometallic compounds in real samples of different kind of waters.

[1] Directive 2000/60/EC of the European Parliament and of the Council L327 (2000)-1-
[2] Directive 2008/105/EC of the European Parliament and of the Council L384 (2008) 84.
[3] Proposal for a Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. 2011/0429

ENV44

STUDY OF PASSIVE SAMPLING FOR THE ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS IN AMBIENT AIR

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Monitoring programs of persistent organic pollutants, such as polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB) and polybrominated diphenylethers (PBDE), usually comprise the control of ambient air because global distribution occurs via atmospheric transport.

The determination of these compounds in ambient air comprises the following main steps: (a) sampling, (b) pollutant extraction, (c) extract clean-up (d) fractionation of the different families of compounds (e) instrumental analysis by GC-HRMS (f) quantitation by isotopic dilution method.

Sampling in the field is as important as analysis in the laboratory, because the final result of concentration in ambient air will depend on the amount of pollutants determined in the laboratory and on the total volume of sampled air. Two main techniques are usually used for air sampling: active and passive. Active sampling is based on the use of a high volume sampler that forces the air to pass through a filter and an adsorbent. Passive techniques are based on the diffusion of gaseous phase analyte molecules to the collecting medium (in this case a foam disk). They do not require the use of high volume samplers, allow to sample in remote places and to have integrated results. However, the two main disadvantages are (a) only vapour phase can be measured [1-3] and (b) they should be calibrated to know the volume sampled. Our work has focused on the comparison of both techniques for sampling of POPs in ambient air from Barcelona city.

Active sampling was performed by high volume samplers. Particle phase was retained by quartz-fiber filter, followed by a polyurethane foam (PUF) block for vapor phase absorption. Passive sampling consisted of polyurethane foam disks (140 mm diameter, 12 mm thickness), sheltered by two different size stainless steel housings (260 mm and 210 mm diameter, respectively).

First, the distribution of PCDD/F, PCB (DL-PCB and NDL-PCB) and PBDE between the particle phase and vapor phase was studied by active sampling and separate analysis of filter and PUF.

Secondly, two different procedures were studied to calibrate passive samplers: (a) native compound accumulation method, and (b) depuration compound method. The first one is based on the comparison among the amount of POP accumulated in the foam disk related to the concentration in air (determined by active sampling) to calculate the sampling rate (m^3/day). The depuration compound method is based on the loss of mass of certain compounds, added before the sampling, due to the diffusion to air.

Third, real samples collected by passive samplers for 60 days in Barcelona were analyzed and the results were compared to those obtained by active sampling during the same period.

Results showed quite good correspondence between passive and active sampling for those compounds that were mainly in vapor phase (PCB and some PBDE). The two different methods to calibrate passive samplers resulted in similar rates.

[1] M. Shoeib, T. Harner, Environ. Sci. Technol. 36 (2002), 4142.
[2] M. Mari, M. Schuhmacher, J. Feliubadaló, J.L. Domingo, Chemosphere 70 (2008), 1637.
[3] C. Moeckel, T. Harner, L. Nizzeto, B. Strandberg, A. Lindroth, K.C. Jones Environ. Sci. Technol. 43 (2009) 3227.

ENV45

DEVELOPMENT OF A GREEN ANALYTICAL METHOD FOR THE ANALYSIS OF ALKYLPHENOLS IN WATER SAMPLES BY MASE-LC-MS/MS

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Membranes assisted solvent extraction (MASE) was described at first by Hauser et al in 2001 [1]. This technique is based on the diffusion of organic compounds dissolved in an aqueous sample through a membrane bag into a small amount of organic solvent. MASE was satisfactory applied to the analysis of UV filters, VOCs, PAHs or polycyclic musk. However, to the best of our knowledge, no previous work based on the analysis of alkylphenols in waters by miniaturized MASE can be found in the literature.

Extraction solvent, temperature and extraction time were taking into account in the optimization of the MASE procedure. Briefly, 15 mL of water samples were placed in the 20 mL vial and extracted with 500 µL of hexane (filled in membrane bag). The vial was vigorously shaken at room temperature during 60 minutes at 750 rpm. After extraction, the extracts were evaporated to dryness under nitrogen stream, reconstituted in 200 µL of methanol and injected in LC-MS/MS system. The LC-MS/MS conditions were described in previous work [2].

The analytical methodology was validated for linearity, accuracy, precision, sensitivity for different kind of waters. The figures of merit were satisfactory: relative recoveries varied between 81-108% and repeatability and intermediate precision were <20% for all compounds. The quantitation limits of the method (MQLs) allow the determination of octyl- and nonylphenols at the levels established by the legislation in surface and seawater samples (Directive 2008/105/EC) [3]. For drinking water analysis, MQLs ranged between 0.02 and 0.04 µg L^{-1}.

The main advantages of the method are simplicity, no-labour and no-time consuming, free solvent and low cost. Furthermore, the method was successfully applied to the analysis of different types of water samples (seawater, surface water and drinking water) from A Coruña (NW Spain), in order to evaluate the water cycle of this area. Nonylphenol was detected in all surface and seawater samples at concentrations between 0.12 and 0.19 µg L^{-1}. In drinking water, NP was detected in all samples (<MQL) which showed that alkylphenols were eliminated during drinking water treatment.

[1] B. Hauser, M. Schellin, P. Popp, Anal. Chem. 76 (2004) 6029.
[2] N. Salgueiro-González, E. Concha-Graña, I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1223 (2012) 1
[3] Directive 2008/105/EC. Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and Amending Directive 2000/60/EC, European Parliament and the Council of the European Union, 2008.

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ENV46

CHARACTERIZATION AND DETERMINATION OF FATTY ALCOHOL ETHOXYLATES AND ALKYL ETHER SULFATES BY SCX SAMPLE CLEAN UP, AND SURFACTANT CLASS SEPARATION BY WAX AND HPLC – UV

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Fatty alcohol ethoxylates (FAE) and alkyl ether sulfates (AES) are widely used in cleaners. These molecules have in common a C12-C18 alkyl chain bound to an ethylene oxide (EO) chain, ending with a hydroxyl for FAE and a sulfate for AES. To analyze FAE and AES in cleaners and environmental samples is not an easy task owing to sample complexity, lack of chromophores, and broad ranges of polarity and volatility of the oligomers, as well as to easy hydrolysis of AES to yield FAE. Further, derivatization fails in the presence of triethanolamine (TEA) and other amines. In this work, a method for the characterization and determination of these two surfactant classes in cleaners is described. First, an SCX cartridge is used to remove TEA and other cations. Then, separation of FAE and AES in the eluate is achieved by anionic-exchange. In a previous work, a SAX cartridge was used to retain AES whereas FAE were eluted. However, a large concentration of HCl, followed by neutralization of the eluate with concentrated NH_3, was required to elute AES. In this work, the SAX cartridges have been substituted by WAX cartridges which make AES elution easier. Retention of both amines on the SCX cartridge and AES on the WAX cartridge was assured by adding HAcO to reach pH = 4. Elution of FAE from the WAX cartridge was achieved in two steps: 50% MeOH to elute a hydrophilic fraction, and 80% MeOH to complete the elution of the hydrophobic oligomers. Next, AES were eluted in three steps using 50, 80, and 95% MeOH, in all cases in the presence of concentrated NH_3; in this way, hydrophilic and hydrophobic AES oligomers were quantitatively eluted. Then, the FAE and AES fractions were independently derivatized with pthalic anhydride, and the solution containing the hemiesters of the alcohol and alcohol residues, respectively, were injected in the chromatograph. Separation was achieved by gradient elution on a C8 columm with acetonitrile – water in the presence of 0,1 % HAcO, and detected at 230 nm, as reported [1]. Other anionic surfactants such as linear alkylbenzene sulfates (LAS) appeared as broad bands on the AES chromatogram, but they did not interfere.

[1] M.Beneito-Cambra et al., J. Chromatogr. A 1218 (2011) 8511.

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ENV47

IDENTIFICATION OF TRANSFORMATION PRODUCTS OF SYNTHETIC ANALOGUES OF SILDENAFIL COMPOUNDS USING LC COUPLED TO THREE MASS SPECTROMETRIC ANALYZERS

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The incomplete removal of human pharmaceutical residues from the waste stream during sewage treatment has been widely documented in a large number of monitoring studies. One such class which was recently reported to occur in wastewater effluent at low levels are the so-called phosphodiesterase type V inhibitors (PDE-V). These drugs are prescribed for effective treatment of erectile. Sildenafil (Viagra®), tadalafil (Cialis®), and vardenafil (Levitra®) are the three drugs approved by the European Medicines Agency (EMEA). Although these drugs require prescription, some synthetic analogues of sildenafil are used to adulterate herbal medicines and cosmetic products that are available through Internet websites markets. Recently, the presence of all three erectile dysfunction (ED) treatment drugs has been reported in wastewater streams at very low concentrations. However, no data about the presence of the synthetic analogues is reported in the scientific literature. Once in the environment, the synthetic analogues of sildenafil can undergo different processes including photochemical reactions. Photolysis can be one of the dominant factors affecting the fate of the synthetic analogues of sildenafil. Suntest sunlight simulator apparatus was used to irradiate different aqueous samples (HPLC water, synthetic freshwater, and natural water, pH:7) containing undeclared drugs. Aliquots of the exposed solutions (1 mL) were taken at regular. Following chromatographic separation of the irradiated samples, number of TPS photoproducts in the Norneo samples, number of TPs photoproducts for homo and number of TPs hydroxihomo were detected. The combination of LC-ESI-QqQ-MS, LC-ESI-QqLIT-MS, UPLC-(+)ESI-QToF-MS and H/D-exchange experiments allowed to propose plausible chemical structures for the photoproducts taking into account the characteristic fragmentation patterns and the accurate mass measurements. The mass spectral fragmentation confirmed stepwise destruction of the piperazine ring. However for the compound norneosildenafil that does not bear a piperazine ring, only one photoproduct was formed from the hydrolysis of the sulfonamide bond which ultimately results in a sulfonic acid. The current work constitutes the first study on the photodegradation of undeclared erectile dysfunction drugs.

POSTERS

FOA

Food Analysis

FOA01

DETERMINATION OF POLYCHLORINATED BIPHENYLS IN HONEY SAMPLES FROM BRAZIL BY GC-MS/MS (QqQ)

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Honey is a natural product consumed by many people around the world. Its composition depends strongly on the plants species from which nectar, pollen and honeydew were collected, and other factors, such as environmental conditions and climate. Honeybees *(Apis mellifera*), perform the vital task of pollinating agricultural crops and native species and are important to the commercial product of honey and beeswax. Every day, 10,000-25,000 honeybee workers make an average of 10 journeys to explore roughly 7 km^2 in the area near the hive, gathering nectar, water, and pollen from flowers. During this process, various chemical products and particles, suspended in the air, are intercepted by these workers and retained in the hair of their body surface, or inhaled and attached to their trachea [1]. Thus, bees and honey have been used as indicators of environmental pollution in several studies [2,3], since honeybees are greatly affected by industrial chemicals and transport them to the colony, which ends as a contaminated honey [2].

Polychlorinated biphenyls (PCBs) are a family of toxic and persistent organic pollutants that are present in environmental samples at very low concentrations levels. Although they were banned in 1981 in Brazil, its use is still allowed in this country in old electronics, until its replacement by other PCB-free product. This has resulted in several use episodes, inadequate storage and disposal, contaminating the environment, wild-life, humans and all the food chain [4].

In this study, we present the results of the PCB levels found in some commercially available honeys collected in Brazil in 2011, to know the environmental pollution levels in the region of sample collection. Twenty PCB congeners (# 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 180, 189 and 194), including non-*ortho*, mono-*ortho*, and the most abundant PCBs were determined. The analytical procedure was based on liquid-liquid extraction with ethyl acetate followed by a clean-up step carried out using a multilayer column filled with neutral silica, silica modified with sulfuric acid and silica modified with potassium hydroxide. The final analytical determination was carried out by GC-MS/MS (QqQ), using the isotope dilution technique as quantification method.

The results show a low concentration level of PCBs, in the pg/g (fresh weight basis) range, indicating that there is not a significant contamination source for honey production in the studied areas from Brazil. The concentration and the PCB profile found in the different honeys investigated were very similar. The lowest chlorinated PCBs (# 28, 52 and 101) were the most abundant, in agreement with previously published studies [5,6].

[1] J. Devillers & M.H. Pham-Delegue (2002). In Taylor & Francis (Eds.), Honey bees: Estimating the environmental impact of chemicals. London: CRC Press.
[2] C. Blasco, P. Vazquez-Roig, M. Onghena, A. Masia & Y. Picó. J. Chromatogr. A 1218 (2011) 4892-4901.
[3] S.R. Rissato, M.S.Galhiane, M.V. de Almeida, M. Gerenutti & B.M. Apon. Food Chem. 101 (2007) 1719-1726.
[4] S. Mohr & I.H. Costabeber. Ciência Rural 42 (2012) 559-566.
[5] Ö. Erdogrul. Food Control 18 (2007) 866-871.
[6] A. Herrera, C. Pérez-Arquillué, P. Conchello, S. Bayarri, R. Lázaro, C. Yagüe & A. Ariño. Anal. Bioanal. Chem. 381 (2005) 695-701.

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FOA02

OCHRATOXINS LEVELS IN MEDITERRANEAN RED WINES

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Ochratoxins are toxic compounds produced by several filamentous fungi of *Aspergillus* and *Penicillium* genera. Ochratoxin A (OTA) has been the one most studied due to its toxicity and incidence. However, the simultaneous presence of OTA and its ethyl ester, ochratoxin C (OTC), in wines was described by Zimmerli and Dick [1]. The simultaneous presence of other ochratoxins, together with OTA, could change or even enhance the toxic potential [2, 3], but knowledge of their co-occurrence in food or their combined toxicological effects is still limited.

Wine is the second largest source of OTA intake for humans in Europe [4, 5]. The European Commission has established a maximum level of 2 μg L^{-1} for OTA in wine [6]. The presence of this mycotoxin in red wine has been reported in several countries, including the Mediterranean ones, but the presence or absence of its analogs is unknown.

In this study, a validated HPLC-FLD [7] method has been applied for the determination of the simultaneous presence of ochratoxin A and five analogs: ochratoxin B (OTB), OTC, methyl ester of OTA (MeOTA) and OTB (MeOTB) and the ethyl ester of OTB (EtOTB) in 96 red wine samples from Mediterranean countries.

Ochratoxins were extracted from red wine using immunoaffinity columns and their analyses were carried out by HPLC with fluorescence detection. Recovery for OTB, OTA, MeOTA and OTC (81.7, 93.5, 76.0, and 73.4% respectively), limits of detection (0.16, 0.32, 0.21 and 0.17 $ng \cdot L^{-1}$), and quantification (0.50 $ng \cdot L^{-1}$) values obtained during the validation process were used in this study. For confirmatory purposes, 10% of the red wine samples were reanalyzed by LC-MS (ionic-Trap) under the analytical conditions described in [7].

The presence of OTA, together with its five analogs, in red wine has been demonstrated for the first time in this study; the 6 ochratoxins appear simultaneously in 44.8% of the wines. OTA appears in 99% of the samples, although none of the wines exceeded the maximum OTA level established by the EU (2 μg L^{-1}). OTA co-occurs with OTB in all the samples and in 89.6% of the samples, OTC also appears. MeOTA, MeOTB and EtOTB have been found in 62.5%, 83.3% and 83.3% of the samples, respectively.

[1] Zimmerli, B., and Dick, R. *Food Addit. Contam.*13 (1996) 655.
[2] Knasmuller, S., Cavin, C., Chakraborty, A., Darroudi, F., Majer, B. J., Huber, W. W., and Ehrlich, V. A. *Nutr. Cancer* 50 (2004) 190.
[3] Pfohl-Leszkowicz, A., and Manderville, R. A. *Mol. Nutr. Food Res*, 51, (2007) 61
[4] JECFA (2007) *947*.
[5] SCOOP (2002). *task 3.2.7.*
[6] Commission Regulation (EC) 1881/2006. *Off. J. EU*, *L364*.(2006) 5
[7] Remiro, R., Ibañez-Vea, M., González-Peñas, E., and Lizarraga, E. *J. Chrom. A* 1217 (2010) 8249.

FOA03

DEVELOPMENT OF AN ANALYTICAL LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF MYCOTOXINS IN MILK

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Mycotoxins are toxic compounds produced by fungi that contaminate food and raw materials which can reach man or animals and affect their health. In order to determine the most frequent combinations of mycotoxins in food and be able to evaluate the health risks when low doses of these toxic compounds are ingested together over long periods of time, their simultaneous presence in foods must be studied.

The occurrence of mycotoxins in animal milk is a food safety-related problem since milk is an important food for adults and children [1]. The rumen flora can convert a number of mycotoxins (e.g. deoxynivalenol or zearalenone) into metabolites with low or no health risk to humans, so, the rumen of healthy animals is thus an important barrier [2], and it is expected that the mycotoxin levels in animal milk will be low. However, this barrier can be impaired due to various ruminant diseases, changes in animal diet, or through the use of contaminated feed for animals. For instance, the SCOOP 2002 study [3] and other researchers [4] sent out a warning regarding the possible presence of ochratoxin A (OTA) in milk. Moreover, other mycotoxins such as patulin, probably remains unchanged by the rumen metabolism [2]. For this reason, more investigation is needed [5].

The mycotoxins in milk that have been studied the most are OTA and Aflatoxin M1 (AFM1). AFM1 is the main aflatoxin B1 metabolite and it may be found in milk products obtained from livestock that have ingested contaminated feed [6]. It has been classified by the International Agency for Research on Cancer (IARC) in group 2B (possibly carcinogenic to humans) [7].

The objective of this study was to develop an analytical method using HPLC-MS/MS 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 and patulin in milk.

Chromatographic separation has been carried out in reversed-phase, adapting the [8] method. After ESI ionization, MS (triple quadrupole) detection was used in dynamic MRM. Mycotoxin extraction procedure from milk using different solvent combinations has been examined in order to combine the best recovery for mycotoxins and the less matrix effect in the detector. The effect of previous milk cleaning with nonpolar solvents, solvent extraction with acidified mixtures of water and polar solvents (acetone, methanol or acetonitrile) and SPE columns have been studied.

[1] F. Galvano, A Pietri, T Bertuzzi, L Gagliardi y col. *Mol. Nutr. Food Res*. 52 (2008) 496
[2] P. Kalac. *Food Chem.* 125 (2011) 307.
[3] SCOOP, Task 3.2.7, 2002 .
[4] D. Pattono, P.F. Gallo, T. Civer. *Food Chem*. 127 (2011) 374
[5] EFSA. The EFSA Journal 73 (2004) 1.
[6] Creppy EE. *Toxicol. Lett.* 127 (2002) 19
[7] IARC Monographs, 82 (2002)
[8] J. Rubert, K J James, J. Mañes, C. Solera. J. Chromat. A 1223 (2012) 84

FOA04

CHARACTERISATION OF PCBs AND PCDD/Fs IN FUNCTIONAL FOODS ENRICHED WITH OMEGA-3 BY GC-QqQ(MS/MS)

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Nowadays in food industry it is very common the use of additives to result in functional foods such as food enriched with omega-3. This enrichment occurs generally through the addition of fish oils to the food product since they are rich in long chain fatty acids omega-3 (EPA and DHA). However, it is well known that, fatty fishes, with high concentrations in omega-3, also present high concentrations of some priority pollutants (such as polychlorinated biphenyls, PCBs, polychlorinated dibenzo-*p*-dioxins, PCDDs, and polychlorinated dibenzo furans, PCDFs). The aim of the work was to characterise different types of functional foods enriched with omega-3 on the basis of their PCB and PCDD/F concentrations.

Five different types of commercially available functional foods enriched with omega-3, i.e. fish oil pills of different origin (n=11), milk (n=3), eggs (n=3), soy products (n=3) and biscuits (n=2), were purchased in different supermarkets from Madrid (Spain). The oil pill samples were extracted and purified according to the method previously described by Guzmán *et al.*, 2005 [1] using slight modifications, while for the remaining samples the treatment described by Bordajandi *et al.*, 2003 [2] was followed. The congeners selected for this study were the most toxic of the two families of compounds plus the most abundant PCBs in technical mixtures. In the case of PCBs, 20 endogenous PCBs were studied, 4 non-*ortho* substituted PCBs (Nos. 77, 81, 126 and 169) plus 8 mono-*ortho* substituted congeners (Nos. 105, 114, 118, 123, 156, 157, 167 and 169), all of them called dioxin-like-PCBs (DL-PCBs), and 8 major congeners in technical mixtures (Nos. 28, 52, 101, 138, 153, 170, 180 and 194), called indicator PCBs. On the other hand, in the case of PCDD/Fs, the seventeen 2,3,7,8-substituted congeners were chosen. For the final determination of these compounds a gas chromatograph (GC) coupled to a mass spectrometer (MS) with a triple quadrupole analyser (QqQ) (Thermo Fisher Scientific, Bremen, Germany) was used [3].

The highest PCB concentrations (expressed in pg/g lipid weight) were found in eggs samples, followed by milk, oil pills, soy products and cookies. PCBs profiles within each food group were similar. In general, in all the samples tested, PCBs 118 and 105 were the most abundant of the mono-*ortho* substituted congeners. Regarding indicator PCBs, congener number 153 and 138 were the most abundant in oil pills and eggs, whereas in milk, soy product and biscuit samples, PCBs 52 and 101 presented the highest contribution. In addition, it was also noted that, in all samples analysed, PCBs 77 and 126 were the most abundant non-*ortho* substituted congeners.

Regarding PCDD/Fs, the highest concentrations (pg/g lipid weight) were found in egg samples, followed by oil pills, biscuits, milks and soy products. The dioxin profiles within each food group were also similar. The most abundant congener was OCDD in all cases, followed by 1,2,3,4,6,7,8-HpCDD and OCDF for milk, eggs and soy products, and by 1,2,3,7,8,9-HxCDD and OCDF for oil pills. In the cookies samples, the second most abundant congeners were 1,2,3,4,6,7,8-HpCDD and 1,2,3,7,8-PeCDF .

[1] J. Guzmán*et al.*, Chemosphere 61 (2005) 398
[2] L.R. Bordajandi*et al.*, Chemosphere 53 (2003) 163
[3] A. García-Bermejo *et al.*, OrganohalogenCompounds 73 (2011) 653

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FOA05

ANALYSIS OF BLACKBERRY (*Rubus ulmifolius*) VOLATILES BY SPME FOLLOWED BY GC-MS. APPLICATION TO THE CHARACTERIZATION OF THE AROMA OF BLACKBERRIES FROM CALABRIA (ITALY).

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Blackberries (*Rubus ulmifolius*) are highly appreciated in the food industry, not only for their antioxidant properties, but also for their pleasant aroma and taste. They are used in the elaboration of different foodstuffs including marmalades, liquors, etc. Dehydrated, they are also employed as ingredients of breakfast cereals, yoghourts, etc.

Although food aroma is widely recognized as an important sensorial attribute and can also be considered as a quality index to characterize foods (geographical/botanical origin, processing and storage conditions, etc), few references have dealt so far with the analysis of blackberry volatiles [1-3].

The aim of this work was to develop a solid-phase microextraction (SPME)-based method for the GC-MS analysis of volatiles in freeze-dried blackberries. The effect of the most important operating factors (fibre coating, sample amount, extraction temperature, equilibrium time, extraction time, etc) on the fractionation of volatiles was taken into account for optimization. The highest total amount extracted and the lowest number of interferences were considered for selection of optimal conditions.

Under optimized conditions, a total of 30 volatiles of different functionality were identified and quantified (as percent relative data) in GC-MS chromatograms of blackberries. Major components included alcohols (*erythro*- and *threo*-2,3-butanediol, 1-octanol, 2-heptanol, 1-hexanol), aldehydes (nonanal, 2-hexenal), esters (ethyl 3-hydroxy-butanoate, ethyl dodecanoate, ethyl decanoate, ethyl octanoate) and terpenes (*p*-cymen-8-ol, myrtenol, borneol, limonene). Several of these compounds have previously been described to be relevant to blackberry aroma [1, 4-5].

As an example of application, this methodology has been used for the characterization of volatile composition of wild and cultivated blackberries from Calabria (Italy). The possibility of blackberry characterization from the analytical data obtained by the SPME method is discussed.

SPME followed by GC-MS is shown as an affordable, fast and solvent-free technique which can be performed with low sample amount and be easily implemented at the food industry for quality control purposes.

[1] E. Ibañez, S. López-Sebastián, E. Ramos, J. Taberna, G. Reglero, Food Chem. 63 (1998) 281.
[2] X.F. Du, A. Kurnianta, M. McDaniel, C.E. Finn, M.C. Qian, Food Chem. 121 (2010) 1080.
[3] M. Meret, P. Brat, C. Mertz, M. Lebrun, Z. Günata, Food Res. Int. 44 (2011) 54.
[4] R.A. Scanlan, D.D. Bills, L.M. Libbey, J. Agric. Food Chem. 18 (1970) 744.
[5] M.P. Gulan, M.H. Veek, R.A. Scanlan, L.M. Libbey J. Agric. Food Chem. 21 (1973) 741.

FOA06

HOLLOW-FIBER LIQUID PHASE MICROEXTRACTION FOR THE DETERMINATION OF NATURAL AND SYNTHETIC ESTROGENS IN MILK

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The study of estrogenic hormones exposure is an important topic since their involvement in endocrine disorders such as obesity, hyperactivity, diabetes, infertility etc. has been widely demonstrated. Besides, they may also be responsible of the development of certain types of cancer. These problems are usually associated with an excessive dietary intake, being bovine meat, milk and its derivatives the main sources, due to the administration of these hormones to keep milk production all year, even during lactating periods. That is why the determination of estrogens in these types of samples is of utmost importance.

In the last decade liquid-phase microextraction (LPME), based on classic liquid-liquid extraction (LLE), have come up as a novel alternative to previous extraction techniques, with the aim of miniaturizing and simplifying the sample pretreatment process. The different modes in which LPME can be developed, *i.e.* single-drop microextraction (SDME), hollow-fiber LPME (HF-LPME) and dispersive liquid-liquid microextraction (DLLME), make possible to carry out analyte extraction and preconcentration in a single step.

Current trends in sample preparation are focused in the analysis of samples containing complex mixtures, using very small amounts of solvents. In this sense, in HF-LPME, developed in 1999 by Bjergaard and Rasmussen [1], analytes are extracted into a supported liquid membrane sustained in the pores of a hydrophobic HF and then into an acceptor solution that is placed inside the lumen of the fiber. When the process is carried out in the so-called two-phase mode, the solvent that impregnates the membrane is the same to the one located inside the fiber. HF-LPME shows important advantages owing to the fact that the small pores on the walls of the fiber prevent macromolecules, such as proteins, to be co-extracted.

In this work, a group of ten estrogens, four of them being natural (estriol, 17β-estradiol, 17α-estradiol and estrone), four being synthetic (17α-etynylestradiol, diethylstibestrol, dienestrol and hexestrol) and one metabolite (2-hydroxyestradiol) have been extracted and preconcentrated from milk samples by HF-LPME using 1-octanol as extraction solvent, after protein precipitation with ACN containing acetic acid. Separation, determination and quantification was achieved by high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) and a fluorescence detector (FD) set in series. Parameters that affect the extraction efficiency (pH of the sample, ionic strength, extraction time, stirring speed and temperature) were investigated. Calibration, precision and accuracy studies were carried out to validate the methodology in Milli-Q water and in different types of milk samples.

[1] S.-P. Pjergaard, S., Rasmussen, K.E., Anal. Chem. 71 (1999) 2650.

FOA07

WIDE-SCOPE SCREENING BY UHPLC-QTOF MS AND GC-TOF MS FOR SEARCHING UNDESIRABLE ORGANIC COMPOUNDS IN AQUAFEEDS AND FISH

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Emerging feed-borne undesirables of novelty manufactured aquafeeds and fish exposed to these alternative feeds have been investigated. For this purpose, an advanced analytical strategy has been applied using gas chromatography (GC) and liquid chromatography (LC), both coupled to high-resolution mass spectrometry (HRMS) with time-of-flight mass analyzers (TOF MS). Generic sample treatment, based on the use of "universal" extracting solvents, allowed a non-selective sample preparation enlarging the scope of the screening for detection and identification of many undesirable compounds of very different polarities and volatilities.

UHPLC-QTOF MS has been selected for rapid qualitative screening to investigate the presence of around 1000 undesirable compounds in the samples. For this purpose, representative antibiotics, pesticides and mycotoxins were selected for a qualitative validation. Briefly, the methodology consists of solid-liquid extraction with acetonitrile followed by ultrasonic extraction and final centrifugation prior injection in the system. Blank and spiked samples (20µg/Kg and 100µg/Kg) were processed and later injected in LC-QTOF system. The screening detection limit (SDL) was established as the lowest concentration at which a compound was satisfactorily identified in all spiked samples tested (n=10) independently of their recovery and precision. The identification criterion was the presence of, at least, two m/z ions at the expected retention time, measured at their accurate mass (mass error lower than 2 mDa). When the methodology was applied to real samples, several compounds such as the ciprofloxacin antibiotic, or fumonisin B2 and zearalenone mycotoxins, were detected. A tentative identification was made for those compounds which reference standard was unavailable. When necessary, UHPLC-MS/MS with triple quadrupole mass analyzer was used for quantification and additional confirmation of the compounds detected.

In addition, GC-TOF MS has been applied for target screening of around 150 contaminants, and also for non-target screening by using the ChromaLynx Application Manager. The non-target methodology may be able to detect and identify a large number of GC-amenable compounds belonging to different chemical families without any kind of pre-selection of compounds so, unexpected undesirables may be discovered in an efficient way. The identity of detected components was established by accurate mass measurements and comparison of experimental full-acquisition spectra with theoretical MS libraries. When the reference standard was available, an unequivocal identification could be made. Samples were processed by QuEChERS and then injected in the GC-TOF MS system.

The combined use of UHPLC-QTOF MS and GC-TOF MS was found a powerful approach for the identification of a large number of undesirables in aquafeed and fish samples to assure fish health and food safety. However, the required sensitivity could not be achieved in some particular cases due to the complexity of these fatty matrices and the low concentrations in samples. In these cases, the use of MS/MS appears as an efficient alternative for a limited number of target analytes. New TOF instrument generations will surely provide improved sensitivity and will solve some of the sensitivity problems observed in this work.

FOA08

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD TO QUANTIFY VOLATILE COMPOUNDS IN RED WINE BY GC-MS

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Volatile compounds play an important role as they affect organoleptic properties of wine, such as colour, astringency and bitterness, giving a unique character and quality to each wine. These properties are largely caused by the presence of phenolic compounds, which are part of, along with alcohols, aldehydes and ketones, the volatile fraction of wine [1]. They also depend on, among other factors, the use of oak barrels during the aging process, owing to two mechanisms. Firstly, there is a direct transfer of volatile compounds responsible for certain aroma shades and secondly, there is an oxidation of some compounds because of the flow of atmospheric oxygen through the pores of the wood.

The aim of this work was based on the development and validation of an analytical method to determine and quantify a specific group of volatile compounds in red wine by gas chromatography coupled with mass spectrometry detection (GC-MS) with a ZB-WAX column [2,3]. The sample treatment consisted of a solid-phase extraction (SPE) [3] using polymeric reverse phase cartridges [4]. 46 volatile compounds were carefully selected due to its presence in young wines aged or not in oak barrels. All of them belonged to one of the following groups: aldehydes, ketones, alcohols, and phenol or furan derivatives [1].

The development of sample treatment was carried out by two different and consecutive designs of experiments (DoE). The first DoE consisted of a fractional factorial screening design (16 experiences) in an experimental wine which was not aged in oak barrels, employing five volatile compounds as response variables. Then, method optimization was performed with the most influential factors and a response surface design was carried out, concretely, a central composite design with three central points (30 experiments) [5], in order to obtain the combination of the factors at which that optimum is achieved.

Finally, analytical method validation was performed through the calibration intervals, regression data for each analyte, detection and quantification limits, analytes recoveries and, accuracy and precision of the method.

Once the method was validated, it would be applied to different red wine samples from different Spanish denominations of origin.

[1] N. Natali, F. Chinnici, C. Riponi. J. Agric. Food Chem. 54 (2006) 8190.

[2] V. Canuti, M. Conversano, M. Li Calzi, H. Heymann, M.A. Matthews, S.E. Ebeler. J. Chromatogr. A 1216 (2009) 3012.

[3] L. Castro-Vázquez, M. E. Alañón, E. Calvo, M. J. Cejudo, M. C. Díaz-Maroto, M. S. Pérez-Coello. J. Chromatogr. A 1218 (2011) 4910.

[4] M. González Álvarez, C. González-Barreiro, B. Cancho-Grande, J. Simal-Gándara. Food Chem. 129 (2011) 890.

[5] R. Bozalongo, J.D. Carrillo, M. A. Fernández Torroba, M.T Tena. J. Chromatogr. A 1173 (2007) 10.

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FOA09

DIRECT LC-MS/MS DETERMINATION OF GLYPHOSATE AND AMPA IN VEGETABLES

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Glyphosate (GLY) is claimed to be the world's biggest selling herbicide. The determination of this compound at low residue levels is very difficult due to its amphoteric character, low volatility, low mass and lack of chemical groups that might facilitate its detection. For this reason, most of methods developed until now, employ pre-column or post-column derivatization (mainly pre-column using FMOC) to form fluorescent derivatives and to reduce the polar character of the analytes facilitating its chromatographic retention.

The aim of this work is to develop a rapid and robust method for the direct determination of low concentrations of GLY and its principal degradation product, AMPA, in vegetables, based on the use of Hydrophilic Interaction Liquid Chromatography (HILIC) coupled to MS/MS. HILIC has been established as a valuable approach for the analysis of highly hydrophilic and polar compounds, and it is expected to facilitate the determination of GLY and AMPA without the need of derivatization. LC-MS/MS determination has been carried out in negative ionization mode, monitoring at least 3 transitions for each analyte to give more reliability to the identification/confirmation process and to minimize the possibility of reporting false positives or false negatives.

Different analytical columns have been tested (BEH amide, BEH HILIC, Obelisc R, Obelisc N), obtaining the best results with the Obelisc N. The modifiers added to both mobile phase and vial have resulted critical for enhancing sensitivity. The optimum conditions have been established with an isocratic gradient containing H_2O:acetonitrile (80:20), both 0.1% HCOOH, and 5 mM NH_4COOH/HCOOH in the sample injection vial. Other parameters such as the content of organic solvent in the vial or the effect of other additives have also been studied.

The method will be applied to the analysis of rice, maize and soybean samples, and different parameters like matrix effects, accuracy and precision, sensitivity and selectivity will be tested. The availability of both isotope-labelled GLY and AMPA will surely facilitate the correction of the important matrix effects expected in this type of samples.

FOA10

EASY, FAST AND ECONOMIC DETERMINATION METHOD OF LUTEIN, TOCOPHEROL ISOFORMS, TOCOPHEROL ACETATE AND Β-CAROTENE CONTENTS IN MEAT

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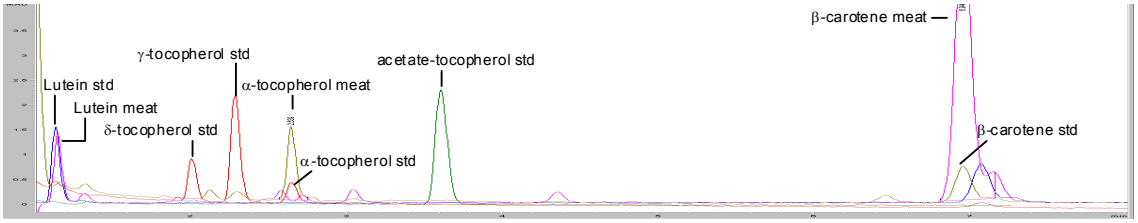
Lyophilized meat (0.1 g) was placed into a tube with 0.4 ml ethyl alcohol and vortexed. After the addition of 1 ml of hexane, the tube was vortexed for 15 min, then centrifuged at 3.500 rpm for 5 min. The lipophilic components were extracted twice. The upper layers were collected and evaporated under nitrogen. The dry residue was dissolved in 500 µl of acetonitrile–methanol–dichloromethane (75–15–10) and transferred into an amber vial. Finally, 40 µl were injected in the HPLC. Analyses were performed under yellow light at room temperature.

Chromatography was run on a HPLC 1100 Agilent equipped with a DAD scanning and ChemStation Agilent Software. HPLC separation used a 100×4.6 mm, RP C_{18}, 2.6-µm Kinetex column and krudkatcher ultra HPLC in-line filter (0.5 µm depth filter x 0.004 in id). The isocratic mobile phase was a mix of acetonitrile-methanol-dichloromethane-ammonium acetate 0.05 M in water 75–10–10–5. The flow rate applied was 1.5 ml/min and analysis was performed with controlled temperature (35 ºC) using a column oven. Analysis time was 8 minutes.

Tocopherol isoforms (δ, γ, α) were detected at 295 nm, α-tocopherol acetate was detected at 290 nm and lutein and β-carotene were detected at 450 nm. Both carotenoids and tocopherol isoforms were identified by comparison of retention times and spectral analyses with those of the pure standards. The concentration of the standards was determined by spectrophotometry.

Figure 1. Chromatogram of the carotenoids and tocopherol isomers standards (std) and content in meat.



The specificity, sensitivity and accuracy (recovery and precision) of the method were studied [1]

Table 1. Evaluation of the proposed method.

	Lineal range (µg ml^{-1})	Repeatability (%RSD)	Reproducibility (%RSD)	Recovery (%)	DL (µg ml^{-1})	QL (µg ml^{-1})
lutein	500 -0.02	7.2	12.3	92.5	0.01	0.02
β-carotene	1000 -0.02	5.8	9.1	95.3	0.01	0.02
δ, γ, α-tocopherol	500 -0.05	4.4	7.9	97.1	0.01	0.05
α-tocopherol acetate	1000 -0.02	5.0	8.4	91.6	0.01	0.02

RSD relative standard deviation, LD detection limit, QL quantification limit

The proposed method to determine β-carotene, lutein and tocopherol isoforms contents in meat is reliable, fast (analytical time around 24 h), easy and economic (cost for one hundred samples was lower than 1000€). The proposed method can be routinely performed in the laboratory.

[1] Food and Drug Administration of USA. (2000): Guideline for industry. Analytical Procedures and Methods Validation.

FOA11

INFLUENCE OF PULSED ELECTRIC FIELD TREATMENTS ON THE VOLATILE COMPOSITION OF DIFFERENT GRAPE VARIETIES

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Pulsed electric fields technology (PEF) has been successfully used in the extraction of phenolic compounds from grape skin. When examining the grape composition, volatile compounds are also important for its quality. Among them monoterpenoids, C_{13} norisoprenoids, esters, and benzenoid compounds stand out, due to their positive effect on the aroma; and C6 compounds, due to their negative influence on the quality. Monoterpenoids and C_{13} norisoprenoids are especially important to the aroma, as their perception threshold is low. This is commonly associated with flowers, honey and wax flavors, being the most important linalool, geraniol, citronellol, α-terpineol, β-damascenone, and β-ionone. Therefore, in this work the effect of PEF in the aromatic composition of three grape varieties was studied: Graciano, Tempranillo and Grenache. In order to do this, grapes were crushed and de-stemmed, and four different treatments were subsequently applied. Different control parameters for treatment time and total specific energy were adjusted. The process was carried out in continuous using a semi-industrial equipment. All treatments were performed in duplicate. Two samples of each variety were left untreated. The volatile compounds present in the different samples were analyzed in duplicate by SPME-GC-MS. The results showed that the content of monoterpenoid compounds decreased in the samples of Graciano variety, except for the highest energy input, which showed little differences with regard to the control. In the case of Tempranillo, minor differences were observed after PEF treatments, with the exception of total monoterpenoids, which increased the higher the energy input. However, in the case of Grenache variety the quantity of monoterpenoids was enhanced by the treatments, regardless of the energy input. As in the case of monoterpenoids, lower energy treatments had negative effects on C_{13} norisoprenoids for Graciano variety. In the case of Tempranillo, in general, all treatments had a negative effect on the presence of these compounds, whereas for Grenache variety a positive effect was observed in the amount of β-ionone. With regard to esters, the lower energy treatments favoured their presence in Graciano, intermediate energy treatments in Tempranillo and medium-high energy treatments in Grenache variety. The content of benzenoid compounds was slightly affected by the treatments for Graciano and Tempranillo varieties while PEF had a positive effect for Grenache variety. Finally, there were few differences between treatments for C6 compounds. Therefore, it can be stated that the effect of PEF was different depending on grape variety. Pulsed electric fields enhanced the volatile composition of Grenache variety while the volatile composition of Tempranillo and Graciano varieties was not favoured by the treatments applied. In conclusion, from the point of view of the volatile composition and taking into account the intensities used in this study, it can be concluded that PEF is an appropriate technology to improve the aromatic quality of musts and wines for Grenache grape variety.

Keywords: varietal compounds, PEF, must, Graciano, Tempranillo, Grenache

FOA12

CHARACTERIZATION OF FATTY ACID PROFILE BY GAS CHROMATOGRAPHY IN OILS AND BUTTERS USED NOWADAYS IN COOKING FROM DIFFERENT CULTURES

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Nowadays due to social habits changes different oils and butters has been introduced to population diet such as ghee, smen, tahin, argan oil or sesame oil [1-5]. For that reason, we have characterised fatty acid (FA) profile composition in several commercial oils (olive, pomace olive, refined olive, sunflower, corn, argan, palm, sesame, coconut, rapeseed) and butters (margarine, milk butter, lard, corn butter, ghee, tahin, smen), in order to compare saturated and unsaturated ratio of the FA as a nutritional quality factor.

FA profile was carried out by the analysis of FA methyl ester derivatives [6] from the oil and butter samples by GCMS on a Trace GC Ultra equipped with a split/splitless injector and an ISQ mass spectrometer detector. A fused silica capillary column was used with a 5% phenyl, 95% dimethylpolysiloxane as a stationary phase (30 m length, 0.25 mm internal diameter and 0.25 μm thickness) and programmed temperature (from 100ºC (0min) to 190ºC (10min) at 5ºC/min, to 200ºC (12min) at 10ºC/min), to 205ºC (15,5min) at 5ºC/min. The injector temperature was 250ºC, and the carrier gas was helium at 1,2mL/min and 60:1 split ratio. The detector conditions were: 250ºC interface temperature, 200ºC source temperature [7]. Components identification was based on comparison of its mass spectra with those of the US National Institute of Standards and Technology (NIST) database library of mass spectra. Complementary, standard grain fatty acid methyl esters mix from Sigma-Aldrich was used for the confirmation of the identifications.

FAME derivatives were prepared by duplicate, and two replicates were injected for each one. Statistics were carried out with XLSTAT Pro.

In general terms oils and butters analyzed have a great amount of oleic, linoleic, palmitic and stearic acid by this order. We have also found other minority FA in some of these samples like C11:0, C15:1, C19:0, C20:4, C20:3 and C22:0.

Between oils and butters, oleic and linolenic dominate in oils, and palmitic and miristic take importance in butters. In all samples unsaturated FA prevail over saturated FA except in milk butter, coconut oil, ghee and smen. In these last samples the prevalence of saturated fatty acids over unsaturated is considered to be negative from the nutritional point of view. In order to classify oils and butters a principal components analysis has also been done.

[1] C.Nasopoulou, Food Chemistry 129 (2011) 1108.
[2] N. Benkerroum, Food Microbiology 21 (2004) 399.
[3] M.Cherki, Nutrition, Metabolism & Cardiovascular Diseases (2005) 15, 352.
[4] S.Gharby Food Science and Technology 44 (2011) 1.
[5] M.Elleuch Food Chemistry 103 (2007) 641.
[6] AOAC International. Official Methods of Analysis (15th ed.) 969.33AOAC.
[7] N.Barniol, VI Congreso Internacional Alimentación, Nutrición y Dietética, Madrid (2008).

FOA13

PRELIMINARY STUDY OF THE USE OF ROOM TEMPERATURE IONIC LIQUIDS FOR THE PURIFICATION OF LACTULOSE

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Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is a synthetic disaccharide generated from lactose (4-O-β-D-galactopyranosyl-D-glucose) by heat treatment in basic media through the Lobry de Bruyn-Alberda van Ekenstein transformation. Its physiological action on the colonic motility pattern [1] and their ability to promote the selective growth of healthy intestinal bacteria in human gut has been extensively reported [2, 3]. Lactulose is mainly commercially available as a syrup containing about 80% solids with a lactulose content of 66%, including variable amounts of lactose and small contents of other sugars such as galactose, epilactose, tagatose and fructose. The presence of lactose in this product may be not desirable when dietary restrictions of this carbohydrate are prescribed. However, its purification from the reaction mixture is not straightforward.

Room temperature ionic liquids (RTILs) are non-molecular ionic solvents resulting from the combination of organic cations and different anions with melting points equal to or lower than room temperature. In general, RTILs are classified as environmentally friendly and provide a safe alternative to the use of traditional solvents which produce volatile organic compounds. Although solubility of lactose and lactulose in different alcohols (methanol, ethanol and propanol) have been previously reported [4], data about their solubility in RTILs is scarce [5].

In this work, solubility of lactose, lactulose, galactose, epilactose, tagatose and fructose in different RTIL at three temperatures (25, 45 and 75 ºC) has been evaluated. Effects of different percentages of water on the solubility of these sugars were also studied: quantitative values were determined by GC-MS after their derivatization to their corresponding trimethylsilyl ethers.

Differences in solubilities of the studied carbohydrates could allow their separation in complex mixtures, as well as the application of this method to different structural carbohydrates. These results constitute a preliminary study for the purification of lactulose using green solvents.

[1] C. Schumann, Eur. J. Nutr. 41 (2002) 1.
[2] K.M. Tuohy, C.J. Ziemer, A. Klinder, Y. Knobel, B.L. Pool-Zobel, G.R. Gibson, Microb. Ecol. Health Dis. 14 (2002) 165.
[3] V. De Preter, T. Vanhoutte, G. Huys, J. Swings, P. Rutgeerts, K. Verbeke, Aliment. Pharmacol. Ther. 23 (2006) 963.
[4] A. Olano, J. Food Sci. Technol. 16 (1979) 260.
[5] A.A. Rosatella, L. Branco, C.A.M. Afonso, Green Chem. 11 (2009) 1406.

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FOA14

DEVELOPMENT OF A METHOD TO QUANTIFY TOCOPHEROLS AND RETINOIDS IN COW, EWE AND GOAT MILK

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Tocopherols (vitamin E) and retinoids (vitamin A) are lipid-soluble micronutrients that play an important role in human health. Vitamin A is necessary for normal vision, cellular differentiation, growth, and reproduction. Tocopherols are well known for its antioxidant properties and for promoting inmunity. Consequently, it appears relevant to improve the nutritional quality of foods, such as milk and dairy products. Furthermore, fat-soluble vitamins could be used as tracer compounds in order to differentiate mountain dairy products.

Several studies suggest that the composition of the animal diet influences the composition of the milk fat-soluble micronutrient fraction, in particular, β-carotene, retinol and tocopherols. Carotenoids are precursors of vitamin A, mainly β-carotene. Vitamin A is found in dairy products as a mixture of ester compounds, retinlyl palmitate being the major ester. The content of free retinol in milk is very low [1].

The aim of this work was to optimize the analytical conditions to quantify tocopherols and retinoids in raw milk from different ruminants with a single extraction protocol. Four different extraction procedures were compared. A method including saponification was rejected because of saponification step caused considerable losses of tocopherols and retinoids, and converted the esters of retinoids in retinol. The other methods were adapted from a published procedure [2] by modifying the solvents used.

A simple and reproducible normal-phase (NP) high-performance liquid chromatography (HPLC) with fluorescence (FL) detection was developed for quantifying α-tocopherol acetate, α-, β-, γ-, and δ-tocopherol. Another NP-HPLC-FL method was developed for retinyl palmitate and retinol. NP was choosen because it allows a good resolution of β- and γ-tocopherol peaks. FL detector was chosen because of its sensitive and specific detection mode. Tocopherols are detected with a λ_{ex} of 290 nm and with a λ_{em} of 330 nm and retinoids are detected with a λ_{ex} of 325 nm and a λ_{em} of 475 nm.

Our method allows the quantification of tocopherols and retinoids in milk proceeding from different ruminants as sheep, goat and cow.

[1] M.C. Herrero-Barbudo et al., International Dairy J. 15 (2005) 521.
[2] C. Moltó-Puigmartí. et al., J. Chromatogr. A 1216 (2009) 4388.

FOA15

ANALYSIS OF QUALITY INDICATORS IN CRUNCHY VEGETABLE SNACKS

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Due to the present style of life, consumers are very interested in new and healthy products which provide, not only nutritive value, but also bioactivity and surprising sensorial properties. In this context, dehydrated vegetables are one of the foods that occupy a preponderant place. Although dehydration is one of the oldest preservation methods in food technology, during the last years it has suffered a huge evolution with the aim of solving the problems of conventional convective drying. As it is known, the high temperature of the drying process is an important cause for loss of quality, and by lowering this parameter, the operating time and the associated cost can become unacceptable. As an alternative, freeze-drying can better preserve the quality of foods; however, this technique is reserved for high added value products due to the expensive cost of water sublimation under vacuum. Nowadays, among the different commercial products that can be purchased by the consumers, the crunchy vegetable snacks can be highlighted. These attractive vegetables, presumably obtained by texturization by expanded micro-perforation, are offered in metal cans under modified atmosphere as healthy, tasty, surprising, environmentally friendly and with a wide range of applications, not only as snacks, but also as ingredients in several foodstuffs. However, to the best of our knowledge, no investigations have been published on the analysis of quality indicators in this type of products and on the evolution of the main chemical changes that can take place during their storage.

In this work, the determination by GC-FID of carbohydrates and by RP-HPLC of vitamin C and furosine (indicator of lysine loss due to the Maillard reaction evolution) has been carried out in crunchy pepper, tomato and onion commercial samples. For this purpose, three different cans from the same batch of each vegetable were purchased and immediately analysed after their acquisition and during their storage at ambient temperature (21-30ºC) and relative moisture conditions within the range 14-45% throughout their shelf-life. Once the cans were opened, a parallel storage, at ambient temperature and at 40°C, was carried out with the aim of simulating domestic conditions used by consumers and extreme conditions of storage.

The dry matter was high (84.8-90.4%), in agreement with the low values of water activity (0.25-0.27). As expected, the main carbohydrates in onion were sucrose (144 mg/g DM) and fructooligosaccharides (348 mg/g DM), while in tomato and pepper were fructose (155 and 286 mg/g DM, respectively) and glucose (97 and 190 mg/g DM). However, the most relevant result was the finding of sedoheptulose for the first time in tomato (3.7 mg/g DM) and pepper (2.4 mg/g DM).This higher-carbon monosaccharide can be used as starting material for the chemical synthesis of biologically active compounds [1]. Furosine was determined in pepper for the first time, in a higher amount (185.3 mg/100 g DM) than in tomato (96.5 mg/100 g DM) and onion (69.7 mg/100 g DM). Similar values had previously been reported for onion [2] and tomato [3]. An increase in furosine amount was observed during storage, particularly high under the most severe conditions. The highest vitamin C content was found in pepper (647 mg/100 g DM) and, according to the lower content of this vitamin in raw tomato, only 20 mg/100 g DM was quantified in the crunchy sample. No presence of vitamin C was detected in onion, since this compound has been reported as especially labile in this vegetable [4]. In pepper samples, the retention values of vitamin C at ambient temperature and 40ºC were 34 and 5%, respectively, after 90 days of storage when the modified atmosphere was removed. According to the quality parameters studied in the present work, it is possible to conclude that crunchy vegetable snacks obtained by this process are safe and keep their quality and bioactivity if the samples are stored under the conditions recommended by the manufacturer.

[1] A.Yokota, J. Ferment. Bioeng. 75 (1993) 409.
[2] A. Cardelle-Cobas et al., J. Agric. Food Chem. 53 (2005) 9078.
[3] J.A. Rufián-Henares et al., Eur. Food Res. Technol. 228 (2008) 249.
[4] C.L. Mota et al., Food Bioproducts Process. 88 (2010) 115.

FOA16

DETERMINATION OF TERPENOIDS IN MILK: COMPARISON OF TWO ANALYTICAL METHODS

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Terpenes are a vast group of plant secondary metabolites derived from the isoprene unit (C5). Monoterpenes (C10), sesquiterpenes (C15) and carotenoids (C40) pass from the rumen to the milk with some minor alterations. They become a part of dairy products at levels highly dependent on their intake from feeds. These non-polar or slightly polar compounds are characterized by specific flavours, which provide an added value to the product. Several investigators have proposed that some of them, particularly sesquiterpenoids abundant in dicotyledoneae, could be used as markers of mountain pastures with a variety of species. The purpose of this work was to optimize the SPME-GC-MS analytical conditions to determine terpenoids in milk from flocks allowed to graze in mountain pastures.

Solid-phase microextraction (SPME) is now widely applied to determine terpenoids in milk. Two methods were compared for the determination of terpenoids in milk. In the first method, terpene analysis was conducted on the fat fraction of milk. A two-step centrifugation was done to extract the terpene-containing milk fat from the whole milk. In the second method, terpenoid compounds were analyzed directly without previous fat extraction. In both methods the effect of extraction temperature was studied. The separation of terpenoid compounds by GC-MS was done according to the method of Abilleira et al. [1].

The analytical conditions finally chosen allow the extraction and detection of the largest amount of compounds while avoiding the destruction of thermolabile ones and guaranteeing a good reproducibility of the results.

[1] Abilleira, E. et al., Food Chem. 120, (2010) 1162.

FOA17

OPTIMIZATION OF PLANT PROANTHOCYANIDINS ANALYSIS BY DIRECT INJECTION-ELECTROSPRAY-QTOF MASS SPECTROMETRY

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Proanthocyanidins or condensed tannins are polymers of flavan-3-ol that are widely distributed in the plant kingdom and are among the most abundant polyphenols in our diet. Besides their participation in food quality attributes such as astringency, bitterness, aroma and colour stabilization, proanthocyanidin consumption has been associated with numerous health benefits due their antioxidant, anti-carcinogenic, cardioprotective, antimicrobial and neuro-protective activities.

The biological activity of plant proanthocyanidins depends on their chemical structure and concentration. However, owing to their structural diversity and complexity, the qualitative and quantitative analysis of proanthocyanidins is a difficult task. In this regard, mass spectrometry has enabled great advances, with the prevalence of MALDI-TOF instruments due to the soft ionization of the MALDI source and the broad mass range of the TOF mass analyzer. Nevertheless, the sample preparation procedure for MALDI-TOF analysis usually requires a careful optimization (i.e., matrix, concentration, solvents and crystallization conditions) that often leads to less reproducible results than in electrospray ionization (ESI) analysis. Therefore, it would be very useful to be able to carry out the direct injection (DI) analysis of proanthocyanidins by mass spectrometry using ESI, with good resolution, high sensitivity and low mass discrimination. Among the mass instruments commercially available today, the most appropriate to fulfil the mentioned requirements probably are ESI-QTOF designs.

The aim of this work was to evaluate the possibility of analyzing proanthocyanidins by DI-ESI-QTOF mass spectrometry. To reach this goal, an exhaustive optimization of the different working and instrumental variables involved in the analysis, such as eluent flow and composition, flow of ionization source gases, ion extraction and ion optic voltages was carried out. Special consideration was given to those variables affecting sensitivity, mass spectra integrity and mass range detection.

Peanut and almond skin extracts containing proanthocyanidins of great diversity (type-A and type-B) were chosen for method optimization. Although commercial ESI-QTOF instruments are usually devoted and factory preconfigured for the analysis of small molecules, mass spectra of high mass compounds can be successfully obtained. It is only necessary to have a deep knowledge of the different variables involved in the analysis, and to carry out a careful manual tune of the instrument. Following these premises, once evaluated and optimized the working variables, reproducible mass spectra of proanthocyanidins in several nut skin extracts were obtained by DI-ESI-QTOF MS, showing prevalence of monocharged ions, with no significant degradation, very good resolution and mass ranges up to 5000 u.

As a conclusion, ESI-QTOF instruments can be used to obtain reliable mass spectra of proanthocyanidins by direct injection analysis, even overtaking MALDI-TOF instruments, mainly due to its better reproducibility and easier sample preparation procedures.

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FOA18

SIMULTANEOUS DETERMINATION OF ANTIOXIDANTS IN COMPLEX FOODS: TOMATO SAUCES

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The present study shows the optimization of the analytical method for the simultaneous determination of antioxidants of different nature (phenols and carotenoids) present in complex foods, such as tomato sauces. The antioxidant compounds hydroxytyrosol from the virgin olive oil, quercetin and its derivates, from the onion, and quercetin-rutinoside (rutin) and lycopene (*cis*-and *trans*) from the tomato. These antioxidant were simultaneously extracted by liquid-liquid extraction (LLE) with the extraction solution hexane/acetone/ethanol (50/25/25, v/v/v). Then, the phenolic compounds (hydroxytyrosol, quercetin and derivates and rutin) were analyzed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), and the analytical column was BEH C_{18} with 1.7 µm as the size particle. On the other hand, the carotenoide lycopene, in its forms *cis* and *trans*, was analyzed by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD), and the analytical column was YMC carotenoid with 3 µm as the size particle. The two developed methods were validated in terms of linearity, calibration curves, precision, accuracy, recoveries, sensitivity and matrix effect. Then, these methods were applied for the analysis of different tomato sauces.

FOA19

CONTRIBUTION TO ISOLATION, PURIFICATION AND CERTIFICATION OF A NEW CERTIFIED REFERENCE MATERIAL FOR HOMOYESSOTOXIN BY CHROMATOGRAPHIC AND MASS SPECTROMETRIC METHODS

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Yessotoxins (YTXs) are lipophilic marine toxins characterized by sulphate moieties and a ladder-shape polyether backbone. YTXs are regulated by the European Union with a maximum permitted level of 1 mg YTX equivalents/kg meat in shellfish products. The regulation applies to the sum of yessotoxin (YTX), homoyessotoxin (hYTX), and their corresponding hydroxylated metabolites at C-45 (45-OHYTX & 45-OHhYTX) [1]. For routine laboratories working under quality standards such as that of ISO 17025, certificate reference materials (CRMs) are needed in order to provide accurate results. High quality standards are also important to provide an estimate of the uncertainty of measurements. The Measurement Science and Standard division (formerly CRMP Program) of the National Research Council of Canada (NRCC) is a well-established international supplier of CRMs for marine toxins [2]. Because of the wide range of marine toxin CRMs needed by laboratories internationally NRCC frequently collaborates with other research groups around the world in order to meet requirements. Until now only a CRM for YTX was available, and the quantitation of other YTX analogs was performed with mass spectrometric detection assuming an equi-molar response. However, the assumption of equi-molar responses may not always be correct, especially when working with selected reaction monitoring transitions in LC-MS. Alterations in the molecular structure may give different fragmentation pathways, different fragment ions, or different relative abundances among product ions. Previous studies performed at IRTA identified a strain of the marine dinoflagellate *P. reticulatum,* isolated from the Ebro Delta (South Catalonia, Mediterranean Sea), that produced large amounts of hYTX [3]. A collaboration between NRCC and IRTA was commenced aimed at producing a new CRM for hYTX. First, large-scale cultures of the *P. reticulatum* were grown and the particulate fraction (69% of total toxin) was harvested by filtration. The dissolved fraction remaining in the culture media was recovered by dispersion of the sorbent resin DIAON HP20 for 24h (31% of total toxin). Subsequently, consecutive orthogonal chromatographic fractionation protocols based on size-exclusion chromatography and reversed-phase chromatography were applied on preparative and semi-preparative scales, using LC-MS/MS for monitoring the target analyte as well as interferences. Approximately 5 mg of hYTX was eventually isolated, and purity was established through LC-MS and qNMR methods. From this material a CRM was produced with an assigned certified value and uncertainty. CRM-hYTX has been released in 2012 [4] and is now available for all routine and research laboratories around the world.

[1] European Commission. Regulation (EC) Nº 853/2004. *Official Journal of the European Union* L 139 of 30 April 2004.
[2] http://www.nrc-cnrc.gc.ca/eng/programs/imb/crmp.html
[3] M. Campàs, P. de la Iglesia, M. Fernández-Tejedor, J. Diogène, *Anal Bioanal Chem* 396 (2010) 2321.
[4] P. de la Iglesia, S.D. Giddings, S. Craig, P. Leblanc, M.A. Quilliam and P. McCarron (2012). CRMP Technical Report. CRM-hYTX-20111102, National Research Council Canada, Halifax. NRCC publication #50498.

FOA20

VALIDATION OF THE PRE-COLUMN OXIDATION HPLC-FLD METHOD FOR SCREENING OF PARALYTIC SHELLFISH POISONING TOXINS IN SHELLFISH

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Paralytic shellfish poisoning (PSP) toxins are highly hydrophilic alkaloids produced by marine dinoflagellates (genera A*lexandrium*, *Pyrodinium* and *Gymnodinium*), and freshwater cyanobacteria. This group of toxins comprises more than 20 saxitoxin analogs [1] with a common backbone consisting on a tri-cyclic perhydropurine. The PSP toxins are potent neurotoxins acting on voltage-gated Na^+ channels of excitable cells and consequently, they are able to specifically block the excitation current in nerve and muscle cells, resulting in paralysis [1]. Therefore, the accumulation of PSP toxins in shellfish is a serious issue for health authorities, and the commercialization of seafood products with PSP toxins above 800µg eqSTX/kg shellfish meat is forbidden in the EU [2]. The AOAC mouse bioassay (MBA), Official Method 959.08 [3] has been the 'gold standard' control method applied for decades that protected public health. However, the use of animals for routine analysis implies strong ethical concerns. Moreover, the EU supports the total replacement of animal-based methods when alternative methods are available [4]. In 2005, the AOAC approved the Official Method 2005.06 as an alternative method based on pre-column oxidation followed by chromatographic analysis and fluorescence detection [3], also accepted in the EU [5]. In this method the PSP toxins are converted into the corresponding fluorescent imino purine derivatives and resolved in reversed-phase HPLC. Unfortunately, the method has not been successfully implemented by most monitoring programs since it is tedious and time-consuming.

In this work we have adopted the strategy of the CEFAS (UK) [6], which refined and extended the method validation, and simplified the workflow to increase the sample throughput. Thus, in-house validation of the simplified procedure is currently on-going at IRTA aimed at accreditation under the ISO 17025 quality standard. The scope includes carbamoyl (STX, NEO, GTX1,4 and GTX2,3), N-sulfocarbamoyl (C1,2 and B1) and decarbamoyl (dcSTX) toxins. The OMA 2005.06 was only applied for the extraction, SPE clean-up, derivatized with periodate oxidation and analyzed by HPLC-FLD. The results obtained have shown extremely reliable detection capabilities with quantitation limits ranging from 10 to 160 µgSTX eq./kg for all toxins except GTX1,4 (280 µgSTX eq./kg), in mussels (*Mytilus galloprovincialis*) and Pacific oysters (*Crassostreas gigas*). Toxin recoveries obtained were similar to those reported in the AOAC 2005.06 method and also those validated by the CEFAS. Recoveries ranged between 60-110% for all toxins, presenting variability among the different toxins and concentration levels. Preliminary data on method validation shows the fit-for-purpose performance of the HPLC-FLD with pre-column oxidation method for screening of samples. This method will provide a sensitive and fast response with a limit of quantification even lower than that provided by the MBA which is ca. 350 µgSTX eq./kg, and reasonable recoveries considering the complexity of the method. This simplification makes the HPLC-FLD feasible to perform the screening of PSP toxins in samples. It is expected that application of this screening will reduce very significantly the use of the MBA. The MBA will be still used to confirm and quantify PSP toxins when these aill be present. Within the monitoring program carried out in Catalonia, where *Alexandrium minutum* and *Alexandrium catenela* toxins basically consist on GTX1,4 & GTX2,3, it seems to be feasible to replace the MBA with the instrumental analytical assuring an equivalent level of public health protection.

[1] M. Kodama, Aqua-BioScience Monographs 3(1) (2010) 1.
[2] Anon. Regulation (EC) Nº 853/2004. Off. J. Eur. Union, L 139, (2004).
[3] Anon. Official Methods of Analysis of the AOAC on-line (http://www.eoma.aoac.org/), (2012).
[4] Anon. Directive 2010/63/EU. Off. J. Eur. Union, L 276, (2010), 33.
[5] Anon. Regulation (EC) Nº 1664/2006. Off. J. Eur. Union, L 320, (2006), 13.
[6] Anon. Refinement and in-house validation of the AOAC HPLC method (2005.06): the determination of paralytic shellfish poisoning toxins in mussels by liquid chromatography and fluorescence detection. Final report. CEFAS (http://www.food.gov.uk/multimedia/pdfs/aoachplc.pdf), (2008).

FOA21

VALIDATION OF ENZYMATIC METHODS AGAINST A REFERENCE HPLC METHOD: APPLICATION TO OENOLOGICAL PARAMETERS

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Rapid identification and quantitation of organic acids and sugars in wines are very important for wine technology and quality control because of the influence of these components on organoleptic properties of wines. Furthermore, organic acids have a major influence on the biological stability of wines and the determination of sugar content is necessary to monitor the fermentation process. Since oenological practices in the cellar many times depend on the values of these parameters, their fast and easy determination is essential. Enzymatic methods are very useful for this purpose because they offer high specificity, considerable time saving, minimal sample preparation and simple instrumentation over other traditional methods. However, to ensure the reliability of the results provided by enzymatic methods, a rigorous validation step is necessary.

The aim of this study was to evaluate the reliability of the results provided by enzymatic methods to determine glucose and fructose, malic, lactic and acetic acids. Validation was performed against a reference HPLC method, which in turn was also internally validated.

The HPLC reference method was applied using two detectors: a diode array detector (DAD) for organic acids and a refraction index detector for sugars. The method allows the simultaneous quantification of glucose and fructose, and malic, lactic and acetic acids, with any sample pre-treatment. Figures of merit such as accuracy, precision, linearity and limits of detection and quantification were determined and showed suitable values.

Then, several wine samples of different varieties (Garnatxa blanca, Xarel·lo, Garnatxa negra, Trepat, Syrah and Samsó) were analysed by both methods and statistically compared. From the comparative study, we concluded both methods provide results with no significant differences at the probability level studied when working with acids. For sugars, the results were comparable for white wines. However, when the red wines were analysed by HPLC, these showed a matrix effect that should be solved by a clan-up of the sample previous to their analysis.

[1] M. Castellari, A.Versari, U. Spinabelli, S. Galassi, A. Amati, J. Liquid Cromatogr. 23 (2000) 2047.
[2] U. Regmi, M. Palma, C.G. Barroso, Anal Chim. Acta 732 (2012) 137.
[3] International Organisation of Vine and Wine. Compendium of International Methods of Analysis of Wines and Musts, Edition 2012, Vol 2.

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FOA22

ANALYSIS OF UV INK PHOTOINITIATORS IN FOOD. PREVENTING FALSE NEGATIVES BY IMPROVING LC SEPARATION

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Ink photoinitaitors are low molecular weight compounds added to UV inks to print food packaging materials (plastic, cardboard, etc.). Due to different processes these compounds may migrate into foodstuffs. EU Directives [1] have established specific migration limits (SML) being of 0.6 mg kg^{-1} for benzophenone, one of the photoinitiators most frequently used nowadays. For other UV ink photoinitiators SML values have not been established and only it is advised that these compounds should not come into contact with food to avoid endangering human health or changing the characteristics of the product [2]. Typically, the quantitation of UV ink photoinitiators is carried out by LC-MS/MS [3,4] using selected reaction monitoring (SRM) and two transitions per compound to fulfil the EU Directive [4], which establishes as a confirmation criteria the ratio between these two transitions. Nevertheless, interfering compounds coeluting with the analytes could affect the ion ratio providing false negatives. This problem was found when analysing eleven photoinitiators in food samples [5] due to the presence of Harman, an heterocyclic amine that interfere in the determination of benzophenone [6]. In this case, the use of ultra-high resolution mass spectrometry (mass resolving power higher than 50,000 FWHM) to prevent the false negatives was proposed [6].

In this work, the strategy proposed to avoid the interference of harman is the improvement of the chromatographic separation. An UHPLC-MS/MS method has been developed increasing the chromatographic resolution between Harman and benzophenone and allowing the determination of fifteen UV ink photoinitiators in packed food. A chromatographic resolution better than 1.5 was achieved using a Thermo Accucore PFPP column of 150 mm x 2.1 mm I.D., 2.7 µm (Thermo Fisher Scientific) and gradient elution with methanol and 25mM acetic acid-ammonium acetate buffer at pH 3.7. Electrospray in positive mode and a triple quadruple mass analyser (TSQ Quantum Ultra AM, Thermo Fished Scientific) working in low resolution selected reaction monitoring (SRM) was used to acquire the data. The analysis of spiked food samples demonstrated the prevention of false negatives since ion ratio, in the presence of Harman, now agree with that observed for standards. The UHPLC-MS/MS method proposed was applied to the analysis of food samples after a simple sample treatment based on a QuEChERS procedure. Method quality parameters and recoveries were established and limits of detection down to ppb levels were achieved for the UV ink photoinitiators. The analysis of several food samples obtained from commercial markets was performed and no false negatives were detected.

[1] Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs, Off. J. European. Commun. L220 (2002) 18.
[2] Commission Regulation (EC) No. 2023/2006 of 22 December 2006 on goodmanufacturing practice for materilas and articles intended to come into contact with food. Off. J. Eur. Union (2006) L384/75.
[3] H. Gallart-Ayala, E. Moyano, M.T. Galceran, J. Chromatogr. A 1208 (2008) 182.
[4] European Commission (2002) Commission Decision of 12 August, 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. European Commission, Brussels.
[4] G. Sagritini, G. Caprioli, G. Cristally, D. Giardiná, M. Ricciutelli, R. Volpini, Y. Zuo, S. Vittori, J. Chromatogr. A 1194 (2008) 213.
[5] H. Gallart-Ayala, O. Nuñez, E. Moyano, M.T. Galceran, J. Chromatogr. A 1218 (2011) 459.
[6] H. Gallart-Ayala, O. Nuñez, E. Moyano, M.T. Galceran, C.P.B. Martins, Rapid Commun. Mass Spectrom. 25 (2011) 3161.

FOA23

SPECIFIC DETERMINATION OF SINGLE PYRETHRINS IN VEGETABLES AND FRUITS BY LC-MS/MS

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Pirethrins are natural insecticides derived from chrysanthemum flowers widely used for insect control, containing a mixture of six components: pyrethrin I, cinerin I, jasmolin I, pyrethrin II, cinerin II, and jasmolin II. Pyrethrins can be analyzed by GC or HPLC, however, most of analytical methods reported until now are focused on individual determination of pyrethrins components using liquid chromatography (LC) coupled to a low selective detection such as UV. The aim of this work is to develop a rapid, efficient and sensitive LC-MS/MS method for the individual quantification and confirmation of pyrethrin components in vegetable and fruit samples by the monitoring of two specific transitions for each pyrethrin component working in Selected Reaction Monitoring (SRM) mode. The full scan mass spectrum showed the abundant presence of $[M+Na]^+$adducts, so the use of HCOOH and ammonium acetate as additives were tested. In presence of HCOOH no change in the mass spectrum was observed, while for ammonium acetate sodiated adducts practically disappeared resulting in the formation of $[M+H]^+$ as base peak.

Different extraction solvents have been tested (methanol, acetonitrile, acetone/water (70:30) and acetone). Finally, vegetable samples have been extracted with acetone/water or acetone and raw extracts were directly injected in the LC-MS/MS system.

Method validation was carried out evaluating linearity, accuracy, precision, specificity, limit of quantification (LOQ) and limit of detection (LOD) in eight types of fruit and vegetable samples (lettuce, cucumber, tomato, pepper, strawberry, potato, rice and pistachio) at 0.05 mg/Kg and 0.5 mg/Kg (referred to the sum of all pyrethrins) using both acetone/water (70:30) and acetone as extraction solvents.The method based on acetone/water (70:30) extraction led to satisfactory recoveries between 70-110% and good precision (below 14%) for all pyrethrin components in lettuce, pepper, strawberry and potato. The method based on acetone (100%) allowed satisfactory recoveries for lettuce, cucumber, tomato and rice samples obtaining in all matrices recoveries between 71-107% and RSD values below 15%. In pistachio samples satisfactory results were obtained only for pyr II, cin II and jas II. All validation parameters were evaluated for both specific transitions (quantification and confirmation) selected for each analyte. LOQ was 0.05 mg/Kg for all validated matrices and LODs were between 0.001 and 0.00002 mg/Kg.

Validated methods have been applied to the analysis of vegetable and fruit samples taken from local markets.

FOA24

ANALYSIS OF QUINOLONE RESIDUES IN PIG MUSCLE USING LIQUID CHROMATOGRAPHY WITH FLUORESCENCE, MASS SPECTROMETRY AND TANDEM MASS SPECTROMETRY DETECTION. COMPARISON OF THREE ANALYTICAL METHODS

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The research in the field of contamination in foods has extended in the last years beyond classical contaminants (polyaromatic hydrocarbons, dioxins or polychlorinated biphenyls, pesticides or biocides) to other compounds such as pharmaceuticals or personal care products [1]. Antibiotics and their degradation metabolites rank among the most used drugs in human and veterinary medicine. Resistance to antibiotics and other anti–infective agents constitutes a major threat to public health and ought to be recognized as such more widely than it is at present [2].

One of the most important groups of antibiotics is quinolones. They are a family of highly potent antibiotics with a broad spectrum of activity against both Gram–negative and Gram–positive pathogens [3]. They are widely used in human and veterinary medicine in the treatment of infections and represent an expanding class of broad–spectrum antibacterials. Antibiotics have become an integral part of the livestock production industry and can be used therapeutically to treat disease or to prevent it as well as for promoting growth [4]. Their use in veterinary applications can result in the appearance of residues of the compounds and metabolites in edible animal meats and may give rise to public health concerns, including development of resistant bacterial strains, toxic effects or allergic hypersensitivity [5]. In this context, it is of a crucial importance to develope more analytical methodology to quantify and indentify these compounds in food–producing animal.

This work presents a comparison between three analytical methods developed for the simultaneous determination of 8 quinolones regulated by the European Union (marbofloxacin, ciprofloxacin, danofloxacin, enrofloxacin, difloxacin, sarafloxacin, oxolinic acid and flumequine) in pig muscle, using liquid chromatography with fluorescence detection (LC–FD), liquid chromatography-mass spectrometry (LC–MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS). Norfloxacin was used as surrogate. The procedures involve an extraction of the quinolones from the tissues, a step for clean–up and preconcentration of the analytes by solid–phase extraction (SPE) and a subsequent liquid chromatographic analysis. All methods give satisfactory results in terms of linearity, precision, accuracy and limits of quantification. The limits of detection of the methods ranged from 0.1 to 2 ng g^{-1} using LC–FD, from 0.3 to 2 using LC–MS and from 0.2 to 0.3 using LC–MS/MS, while inter- and intra-day variability was under 15 % in all cases. Most of those data are notably lower than the maximum residue limits (MRLs) established by the European Union for quinolones in pig tissues. The methods were satisfactorily applied for the determination of quinolones in six different commercial pig muscle samples purchased in different supermarkets located in the city of Granada (Spain).

[1] T.A. Ternes, A. Joss, H. Siegrist, Environ. Sci. Technol. 38 (2004) 392A.
[2] K. Kümmerer, J. Antimicrob. Chemother. 52 (2003) 5.
[3] J.A. Hernández–Arteseros, J. Barbosa, R. Campañó, M.D. Prat, J. Chromatogr. A 945 (2002) 1.
[4] M. Ramos, A. Aranda, E. García, T. Reuvers, H. Hooghuis, J. Chromatogr. B 789 (2003) 373.
[5] M. Martínez, P. McDermott, R. Walker, R., Vet. J. 172 (2006) 10.

FOA25

IDENTIFICATION OF BIOACTIVE PEPTIDES IN HYPOALLERGENIC INFANT MILK FORMULAS BY CE-TOF-MS ASSISTED BY SEMIEMPIRICAL MODELS OF MIGRATION BEHAVIOUR

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Hypoallergenic infant milk formulas (IF) are manufactured by hydrolysis of milk proteins in order to diminish the risk of protein allergy and can contain up to hundreds of different peptides at different levels of concentration. Some of these peptides, which are inactive within the sequence of the parent proteins, possess biological activity or can be activated after being released by enzymatic hydrolysis during gastrointestinal digestion. This potential to generate a positive impact on body functions or conditions have been a subject of growing interest of food and pharmaceutical companies, which are interested in bioactive peptides from protein hydrolysates as ingredients in functional dairy foods, drugs and cosmetics [1-3].

In this study we used capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS) for separation and identification of bioactive peptides in three IFs. An appropriate sample clean-up using a citrate buffer with dithiothreitol and urea followed by solid-phase extraction (SPE) with C18 and StrataX cartridges allowed detection of a large number of low-molecular-mass bioactive peptides. This preliminary identification was solely based on the measured experimental monoisotopic molecular mass values (M_{exp}) [2]. Later, we evaluated the classical semiempirical relationships between electrophoretic mobility and charge-to-mass ratio (m_e versus q/M^{α}, α=1/2 for the classical polymer model) to describe their migration behaviour. The assistance of migration prediction proved to be useful to improve reliability of the identification, avoiding misinterpretations and solving some identity conflicts [3].

After revision, the identity of 24, 30 and 38 bioactive peptides was confirmed in each of the three IFs. A significant number of these peptides were reported as inhibitors of angiotensin converting enzyme (ACE), however, the presence of sequences with other biological activities such as antihypertensive, antithrombotic, hypocholesterolemic, immunomodulation, cytotoxicity, antioxidant, antimicrobial, antigenic or opioid was also confirmed.

[1] Hartmann, R., Meisel, H., Curr. Opin. Biotech., 18 (2007) 163.
[2] B. Hernández-Ledesma, A. Quirós, L. Amigo, I. Recio, Int. Dairy J., 17 (2007) 42.
[3] P. B. Kunda, F. Benavente, S. Catala-Clariana, E. Gimenez, J. Barbosa, V. Sanz-Nebot, J. Chromatogr. A, 1229 (2012) 121.

FOA26

SYNTHESIS OF OLIGOSACCHARIDES DERIVED FROM STACHYOSE HYDROLYSIS BY PECTINEX ULTRA SP-L

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α-Galactosides are derivatives of sucrose that consist of galactose residues linked by α-(1-6) linkages to the glucose moiety. These oligosaccharides are found abundantly in grain legumes and their consumption is associated with the production of flatulence. However, they might be used as a prebiotic growth substrate for intestinal bacteria because they pass undigested to the colon [1]. Several studies provide convincing evidence that α-galactosides have beneficial effects on the survival of different bifidobacteria strains [2,3]. Raffinose and stachyose are industrially available in large amounts as a byproduct from the production of soy protein isolate, and they seem to be a promising raw material to manufacture of new oligosaccharides. The relatively inexpensive commercial enzyme preparation Pectinex Ultra SP-L (Pectinex), produced by *Aspergillus aculeatus*, has been shown to contain fructosyltransferase activity [4] and therefore, it could be used as a catalyst in the large-scale production of stachyose-derived oligosaccharides with improved prebiotic properties. The objective of this work has been to investigate in more detail the fructosyltransferase activity from *A. aculeatus* of the commercial enzymatic preparation Pectinex during stachyose hydrolysis.

Enzymatic synthesis of oligosaccharides from stachyose using Pectinex was carried out under different reaction conditions such as temperature (50, 60, and 70 ºC), pH (3.5, 4.5, 5.5, 6.5, and 7.5), stachyose concentration (100, 300, and 600 g/L), enzyme concentration (17, 34, and 78 U/mL), and time up to 24 h. Purification of the reaction mixtures was performed following the method described by Morales et al. [5]. The enriched fraction was characterized by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Mass spectra were obtained over the *m/z* range 100-1500. Analysis of carbohydrates was performed by gas chromatography (GC) using a flame ionization as detector (FID). The trimethylsilyl oximes of mono-, di-, and oligosaccharides were resolved using a 8 m × 0.25 mm × 0.25 μm film fused silica capillary column coated with CP-SIL 5CB (methyl silicone). The oven temperature was programmed from 150 to 165 ºC at 3 ºC/min, then at 5 ºC/min to 340 ºC and held at this temperature for 10 min. Quantitative analysis was carried out by the internal standard method. The amount of remaining stachyose and the yield of oligosaccharides in the reaction mixtures were expressed as weight percentage of total carbohydrate content.

Different oligosaccharides from DP2 to DP8 were formed during stachyose hydrolysis by fructosyltransferase activity of *A. aculeatus*. Galactosyl-melibiose (DP3) was synthesized as a result of fructosidase activity, whereas fructosyl-stachyose (DP5) and difructosyl-stachyose (DP6) were formed as a consequence of the fructosyltransferase activity of the enzyme preparation. Temperature, pH, substrate and enzyme concentrations were varied to select the optimum conditions for tri- or oligosaccharide synthesis. The optimal reaction conditions for the synthesis of penta- and hexasaccharides were 60 ºC, pH 5.5, 600 mg/mL of stachyose, and 34 U/mL of enzyme during 3h. However, to obtain the maximum yield of galactosyl-melibiose (67%), the assays should be carried out at 60 ºC and pH 5.5, using 100 mg/mL of stachyose and 34 U/mL of enzyme during 24 h since it was a very stable to hydrolysis.

In conclusion, stachyose could be used as a substrate for the enzymatic synthesis of new oligosaccharides that may open new opportunities in the development of future prebiotics.

[1] L.AM. Van den Broek, et al, Lait 85 (2005) 125.
[2] I. Trojanova, E. Vlkova, V. Rada, M. Marcunek.Folia Microbiol. 51 (2006) 320.
[3] C. Martínez-Villaluenga, R. Gómez. Int. Dairy J. 17 (2007) 116.
[4] A. Montilla, N. Corzo, A. Olano, M.L. Jimeno, J. Agric. Food Chem. 57 (2009) 5007.
[5] V. Morales, M.L Sanz, A. Olano, N. Corzo, Chromatographia 64 (2006) 233.

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SYNTHESIS OF PREBIOTIC OLIGOSACCHARIDES DERIVED FROM LACTOSE AND LACTULOSE USING IMMOBILIZED β-GALACTOSIDASE FROM *Lactobacillus plantarum*

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In the food industry an increasing attention is being paid to the synthesis of prebiotic new oligosaccharides derived from lactose (GOS) or with improved prebiotics properties using β-galactosidases since their chemical production is very tedious. β-Galactosidases from different sources are commercially available, however, in many cases their use is limited by a low specific activity, low thermostability or high inhibition by products [1]. Therefore, the search and characterization of new and improved β-galactosidases in GRAS microorganisms is a mandatory subject. *Lactobacillus plantarum,* a very interesting source for the production of new enzymes in food, has been described to have tanase activity [2]. However, to the best of our knowledge, its β-galactosidase activity has not been studied yet.

Thus, the aim of this work was to evaluate the activity of a β-galactosidase isolated from *L. plantarum* (LPG) previously purified, characterized and immobilized in our laboratory. Lactose and lactulose were used as substracts and two different pHs were assayed for production of GOS and oligosaccharides derived from lactulose (OsLu) along the incubation time.

A β-galactosidase from *L. plantarum* (LPG) was over-expressed in *E. coli* and purified via a single chromatographic step by using lowly activated IMAC supports as previously described [3]. Production of GOS and OsLu was carried out using 300 g/L of lactose or 450 g/L of lactulose in 50 mM of sodium phosphate and sodium acetate buffer at two different pHs (5 and 7) and 1 g of glyoxyl-LPG during incubation at 45ºC up to 24 h. Samples of 200 µL were withdrawn from the reaction mixtures at different times. GOS and OsLu were determined by HPAEC-PAD on a CarboPac PA-1 column (250 × 4 mm) connected to a CarboPac PA-1 (50 × 4 mm) guard column. The elution, at a flow rate of 1 mL min^{-1}, was in gradient using 100% of 100 mM NaOH solution from 0 to 20 min and 0-100% of 100 mM NaOH and 50 mM NaOAc solution from 20 to 70 min. After each run, the column was washed for 10 min with 100% 100 mM NaOH and 1 M NaOAc. Quantification of carbohydrates was performed by external calibration and the remaining amount of lactose and lactulose and the yield of GOS and OsLu were expressed as % by weight of the total carbohydrate content in the reaction mixtures. All experiments were performed in duplicate.

Different GOS were formed by transgalactosylation during lactose hydrolysis, 6-galactobiose, 3'- and 6'-galactosyl-lactose being identified. Both pH values tested (5 and 7) gave similar results. It was observed that synthesis of trisaccharides predominated over other oligosaccharides. Among the GOS formed, the main trisaccharide was 3'- galactosyl-lactose, showing a maximum value of 10 g/100g of total carbohydrates after 3 h of incubation at pH5. Total GOS formation gave a maximum value of 34 and 32 g/100g of total carbohydrates for 4h of incubation at pH 7 and 5, respectively. Formation of OsLu was also observed by the LPG tested, 6-galactobiose, 6'-galactosyl-lactulose, 1- galactosyl-lactulose being found in the reaction mixture. An unknown trisaccharide was also detected and was tentatively assigned as 3'- galactosyl-lactulose. This trisaccharide showed a maximum formation at 3h of incubation (9 g/100g) for both pH studied. The highest yield of OsLu was produced after 24 h of reaction at pH 5 and pH 7, reaching values of 32 and 24 g/100g of total carbohydrates, respectively.

The present study shows the feasibility of β-galactosidases *L. plantarum* strains to hydrolyze and transglycosylate lactose and lactulose producing high yields of prebiotic oligosaccharides.

[1] M. Ladero, A. Santos, F. Garcia-Ochoa, Enzyme Microb. Technol. 38 (2006) 1. [2] J.A. Curiel, L. Betancor, B. De Las Rivas, R. Muñoz, J.M. Guisan, G.J. Fernández-Lorente, Agric. Food Chem. 58 (2010) 6403. [3] I. Acebrón, J.A. Curiel, B. de las Rivas, R. Muñoz, J.M. Mancheño, Protein Expression Purif. 68 (2009) 177.

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FOA28

EVALUATION OF RISK OF CONTAMINATION BY PERFLUORINATED FOODS FOR INFANTS AND TODDLERS OF VALENCIAN COMMUNITY

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Perfluorinated compounds (PFCs) comprise a large group of man-made fluorinated organic chemicals. They have been produced since the 1950s and are used for various industrial and consumer- related applications, such as food packaging materials, protective coatings for textiles, carpets, papers, and surfactants. As a result of their widespread use and resistance to degradation, PFCs are now globally distributed in the environment, and some are routinely measured at the ng/mL level in the blood of people living in industrialized nations Animal studies have demonstrated different chronic and subchronic effects from PFASs including, hepatotoxicity, developmental toxicity, immunotoxicity, and carcinogenicity as well as hormonal effects from PFASs [1].

This work describes the development and validation of an analytical methodology to determine 18 perfluorinated compounds (PFCs), using 8 isotopically labeled internal standards, in foods for infants and toddlers using solid-phase extraction (SPE) with an ion-exchanger as extraction and pre-concentration procedures, followed by liquid chromatography–triple quadrupole-mass spectrometry (LC–QqQ–MS) equipped with an electrospray source in negative ionization mode and related possible side effects on human health have been reported, particularly in the case of an exposure during the early stages of development, (notably the perinatal period) [1].

The method was validated for the analysis of human breast milk. The average recoveries of the different matrices range of 48 to 120% with a relative standard deviation (RSD) lower than 21% and method limits of detection (MLOD) ranging from 6 to 100 ng/L for the different compounds in breast milk.

The proposed method was applied to a first set of 30 breast milk samples from Valencian Community women. The main PFCs detected in all these samples were PFOS and PFOA with respective median values of 25.2 (range from 20 to 502) and 15 (range from 30 to 102.4) ng/L, respectively. These exposure data appeared in the same range as other reported values for other Spanish cities as Barcelona as well as other European countries.

[1] Y. Picó, M. Llorca, M. Farré, D. Barceló, *Crit. Rev. Food Sci. Nut.* 51 (2011) 605.

Acknowledgements: Research Programme on Health, Prevention and Prediction of the Ilness of Conselleria de Sanitat of the Generalitat Valenciana (Project Nº 50/2011)Spanish Ministry of Economy and Competitiveness through the programs Consolider-Ingenio 2010 (Project No. CSD2009), and fundamental research (Project No. CGL2011-29703-C02-02) DB acknowledges financial support from the Visiting Professor Program of the King Saud University, Riyadh, Saudi Arabia

FOA29

STRATEGIES TO IDENTIFY AND DETERMINE METABOLITES AND TRANSFORMATION PRODUCTS OF AMOXICILLIN IN ANIMAL TISSUES USING HIGH RESOLUTION MASS SPECTROMETRY

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Amoxicillin (AMX) is the antibiotic belonging to the β-lactamic family most added to medicated feeds due to its high antimicrobial activity. However, it has been described that AMX presents stability and sensitivity problems and, thus, its determination in samples from animal origin results in a difficult issue. To ensure safety to consumers, the development of rapid and sensitive methods to determine the presence of AMX, main metabolites and transformation products in biological tissues is increasingly demanded.

In our work, ultra high-performance liquid chromatography coupled to high-resolution Orbitrap mass spectrometry detection has been applied to the analysis of AMX in animal tissues. Interferences from the complex matrix of animal tissues have been separated, thus minimizing the matrix effect as well as improving the LOQ to be fully compatible with the MRL established by European Community for AMX in this kind of samples. AMX, metabolites and transformation products occurring in medicated chicken samples have been extracted by SPE. In order to try to identify new compounds generated by the metabolism, Principal Component Analysis (PCA) and related chemometric techniques have been applied. Original raw data resulting from the injection of extracts of positive chicken muscle matrices and blank biological samples have been transformed first into mzXML files for further analysis. For each sample, the significant MS features characterized by their retention time and m/z values have been subsequently taken to construct a data set focused on the differentiation of positive and blank samples. Then, the identification of discriminant components has been studied by PCA to try to find patterns dealing with AMX, metabolites and transformation products.

[1] Commission Regulation 37/2010. Off. J. Eur. Commun. 1 (2010), L15.
[2] C.A. Macarov, L. Tong, M. Martínez-Huélamo, M.P. Hermo, E. Chirila, Y.X. Wang, D. Barrón, J. Barbosa. Food Chem. *accepted* (2012).
[3] P. Gómez-Rodríguez, M.P. Hermo, J. Barbosa, D. Barrón. Anal. Bioanal. Chem., *submitted* (2012).

FOA30

IMPROVEMENT OF PRECISION IN THE SPME FRACTIONATION OF BLACKBERRY (*Rubus ulmifolius*) VOLATILES

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Analysis of blackberry (*Rubus ulmifolius*) volatiles provides a valuable approach, not only to study its flavor, but also for its objective characterization, as volatile concentration depends on different factors such as blackberry type, origin or processing. Whereas the gas chromatography-mass spectrometry coupling (GC-MS) is the technique of choice for their determination, the required volatile fractionation step can be carried out by different procedures [1-2].

Solid Phase Microextraction (SPME) is a fast, simple, affordable and solvent-free technique which provides a fraction suitable for GC-MS analysis. Although SPME has been widely used for the qualitative analysis of different fruit volatiles [3], it has scarcely been applied to the study of blackberry aroma [1-3]. Furthermore, the quantitative performance of SPME is sometimes questioned, as the variable recovery towards different compounds and its lack of robustness result in low precision data.

Previous studies on application of statistical analysis to SPME data carried out in our laboratory [4] showed the presence of compound-depending trends in the dispersion of the results, which could be used to improve their precision.

The aim of this work is to evaluate if this methodology could be used to improve the precision in the quantitative analysis by SPME followed by GC-MS of blackberry volatiles.

As a first step, a model system of blackberry juice consisting of water, sugars and 30 volatile compounds relevant to blackberry aroma (Figure 1) was prepared. Dispersion results from 10 replicates of the analysis of this model system were then analyzed by simple regression and principal component analysis. From these results, compounds with similar fractionation behaviour were selected. The use of concentration ratios between these compounds showed a lower dispersion than that of single relative data (percentage of total volatile composition). Validation of this methodology was done by applying the same procedure to data from a blackberry juice.

The methodology here proposed can be applied to improve the precision of GC quantitative data from SPME volatile fractionation with characterization purposes.

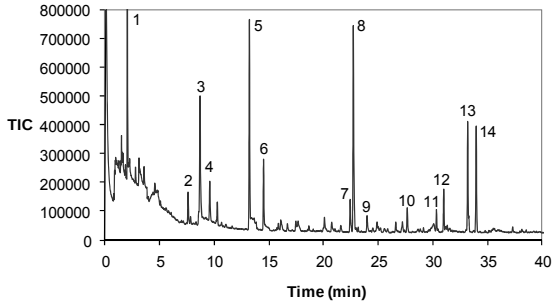

Figure 1. GC-MS profile of blackberry volatiles fractionated by SPME. (1) Ethanol, (2) 2-heptanone, (3) unknown, (4) ethyl hexanoate, (5) 2-heptanol, (6) 1-hexanol, (7) α-terpinolene, (8) 1-octanol, (9) α-terpineol, (10) endo-borneol, (11) 1-decanol, (12) myrtenol, (13) *p*-cymen-8-ol, (14) trimethyl-pentan-1,3-dioldiisobutyrate.

[1] M. Meret, P. Brat, C. Mertz, M. Lebrun, Z. Günata, Food Res. Int. 44 (2011) 54.
[2] X.F. Du, A. Kurnianta, M. McDaniel, C.E. Finn, M.C. Qian, Food Chem. 121 (2010) 1080.
[3] E. Ibáñez, S. López-Sebastián, E. Ramos, J. Tabera, G. Reglero, Food Chem. 63 (1998) 281.
[4] A.C. Soria, M.A. García, I. Martínez-Castro, J. Sanz, J. Chromatogr. A 1008 (2003) 105.

Acknowledgements: authors thank the Ministerio de Ciencia e Innovation of Spain (project AGL2009-11909), and the Comunidad Autónoma of Madrid (Spain) and European funding from FEDER program (project S2009/AGR-1464, ANALISYC-II). Ana C. Soria thanks Ministerio de Economía y Competitividad for a RyC contract.

FOA31

BIOACTIVE POLYPHENOLS AND OTHER OENOLOGICAL PARAMETERS IN GALICIAN MONOVARIETAL WHITE WINES

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Recently, the potential of phenolic compound analysis for the differentiation of wines by grape variety, age or geographical origin in the context of food authentication, food security and anti-fraud has been recognized [1,2].

32 white wines belonging to 2010 and 2011 vintages and vinified from 12 different varieties of *Vitis vinifera* were directly analyzed by means of a simple and fast reversed-phase HPLC-DAD method in order to get their qualitative and quantitative polyphenolic profile. The identity of each particular phenolic compound was verified by means of LC-MS-MS. The total polyphenolic index (TP), a very useful parameter for comparative purposes, and the antioxidant activity (AA) were also measured.

In this survey, 2 public research centers (the Enological Station of Leiro-Ribadumia, Pontevedra & the Agroforestry Training and Experimentation Center of Guísamo, Coruña) and 21 wineries belonging to the 5 Galician Appellations of Origin (AO) have been collaborated. Among wineries, 18 are small and medium enterprises (SMEs) and the other 3 belongs to the category of “Vino de Autor” (Signature Wines). Therefore, wine samples represent fairly well the wine map of the Galician Autonomous Community.

Among the white grape varieties studied, some are the most commercially important and are associated in some way to a certain AO: Albariño (Rias Baixas & Ribeira Sacra), Treixadura and Torrontés (Ribeiro), Godello (Valdeorras) and Doña Blanca (Monterrei), although some wines are made mixing different percentages of them. Other varieties are growing in minority crops and some are even autochthonous varieties in recovery. This study aims to contribute to the characterization of them all.

Finally, the evaluation of the data obtained by means of chromatographic analysis showed that the phenolic pattern can support the classification based on origin (AO) and grape variety of authentic Galician wines. This fact has a great potential for fraud control, which is particularly important in the marketing of these white wines, as they correspond to products of medium to high price.

[1] H. Otteneder, R. Marx, & M. Zimmer, Australian J. Grape & Wine Res. 10 (2004) 3
[2] L. Jaitz, K. Siegl, R. Eder, G Rak, L. Abranko, G. Koellensperger, S. Hann, Food Chem., 122 (2010) 366

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FOA32

DETERMINATION OF FREE FATTY ACIDS IN TERUEL CHEESE BY GC-FID WITH SEMI-AUTOMATED SPE SYSTEM

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Lipolysis during cheese ripening is usually evaluated by determining the concentration of free fatty acids (FFA). FFA have a direct impact on the flavour of many cheese varieties, in particular, C4-C10 acids due to their characteristic flavour. The concentration of the short- and medium-chain FFA profile has been suggested as an index for characterising cheeses over the ripening period [1].

FFA changes during ripening were studied in artisan Teruel cheese, a hard ewe's raw milk cheese. This cheese has been produced with in new eight-lobed mold. Samples were analysed at eight different stages of ripening after 1, 15, 30, 60, 90, 120, 180 and 240 days.

The lipid extract was fractionated and triacylglycerols were separated from the FFA on an aminopropyl-bonded column (Sec-Pack Vac, Waters, USA) using a semi-automated solid phase extraction (SPE) system ASPEC GX-271 (Gilson Villiers-le-Bel, France). The extract was analysed by Gas Chromatography (GC) (Hewlett-Packard 6890 HP Series GC System, Spain) equipped with a flame ionization detector (FID) (Hewlett-Packard). FFA were analysed according to the modified method of Chávarri et al. (1997) [2].

The total amount of free fatty acids increased, although not at a constant rate, from cheesemaking to the end of ripening. Long chain fatty acids (LCFA) (C16 to C18) were the most abundant and kept increasing up to day 180, whereas medium chain fatty acids (MCFA) (C10 to C14) and short chain fatty acids (SCFA) (C4 to C8) increased up to day 240, although these compounds were found in smaller quantities.

Semi-automated SPE extraction showed good linearity and precision. Complete extraction of the standard solution using SPE was carried out to obtain calibration curves. *n*-Pentanoic (C5), *n*-heptanoic (C7) and *n*-nonanoic (C9) acids were added as internal standards. Intra-day and inter-day precision were evaluated. Standard curves for all fatty acids were linear between 10 and 800 mg/L except those for C16:1, C18:1 and C18:2 which were linear only up to 600 mg/L. In all cases correlation coefficient values were greater than 0.995.

[1] A.H. Woo, J. Dairy Sci. 67 (1984) 960.
[2] F. Chávarri, J. Dairy Res. 64 (1997) 445.

FOA33

CHARACTERIZATION OF DIHYDROQUERCETIN-3-*O*-XYLOSIDE IN MUSTS OF CV. ALBARIÑO (VITIS VINIFERA L.)

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Phenolic compounds play important functions on plants and represent a remarkable example of metabolite plasticity. They provide to plant products organoleptic characteristics (colour, taste, flavour,...) and are frequently used as chemical markers in chemotaxonomy. Moreover, some phenolics are antioxidants contributing to a reduction in the risk of cardiovascular diseases, hyperthension and cancer [1]. Initially antioxidant activity was principally associated with stilbenes, but flavonoids and their derivatives are similarly actives. Dihydroflavonols are an important group of flavonoid derivatives in white musts and wines and Baderschneider and Winterhalter [2] reported that the radical scavenging capacity of these derivatives does not differ significantly from that of other phenolic antioxidants. However, and in spite of the importance of these phenolic compounds, there is little information about these compounds in musts; it is especially true for musts from Galician white cultivars.

In this study we report the characterization of dihydroquercetin-3-*O*-xyloside in musts of the white cv Albariño, the most important white cultivar growing in Galicia (northwest Spain). This compound was previously reported in white German wines but, as far as we know, it was identified here for the first time in white musts.

The extraction of phenolic compounds was carried out according to the procedure described previously [3] and the methanolic extracts were used for preparative PC on 3MM Whatman paper using Water as mobile phase. The dark UV-absorbent band (R_f 0.41-0.46) that did not change with ammonia vapours was eluted in methanol and repeatedly injected (twenty times) in reversed-phase HPLC-DAD. Peak of retention time 17.6 (that showed an UV spectrum with an absorbance maximum in 290 nm and a shoulder in 340 nm that suggest a dihydroflavonol derivative) was collected using a Waters WFC III fraction collector and characterized by spectrophotometric and chromatographic methods by comparison with dihydroflavonol standards. Further evidence for the structure of the compound was by analyzing the products of the acid hydrolysis [4] and by LC-MS/MS. Dihydroquercetin-3-*O*-xyloside was confirmed by its *m/z* 435 $[M-H]^-$ ion, yielding characteristics ions at *m/z* 303 and 285 upon dissociation. Aglycone was identified as dihydroquercetin (taxifolin) by comparison with an authentic standard and sugar as xylose according to Markham [4].

[1] Li, H.; Wang, X.; Li, P. and Wang, H. Food Chem. 112 (2009) 454.
[2] Baderschneider, B. and Winterhalter, P. J. Agric. Food Chem. 49 (2001) 2788.
[3] Masa, A.; Vilanova, M. And Pomar, F. J. Chromatogr. A 1164 (2007) 291.
[4] Markham, K.R. Techniques of Flavonoid Identification. Academic Press, London (1982).

FOA36

CHARACTERIZATION VIA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY OF CAROTENOIDS IN PRESSURIZED EXTRACTS OF NEOCHLORIS OLEOABUNDANS

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Nowadays, one of the main interests in Food Science and Technology is the extraction and characterization of new bioactive compounds of natural origin that can be used as functional ingredients able to promote our health. In this sense, considering the huge biodiversity and the fact that many microalgae species remain unknown it is easy to understand that microalgae represent one of the most important biological sources for novel biological active compounds. In fact, it has been suggested that secondary metabolites produced by these organisms, when submitted to extreme conditions (changes of salinity, temperature, nutrients, UV-vis irradiation), provide unique structures with important activities for human health such as antioxidant, antiviral, antimicrobial, or anticarcinogenic, etc. For instance, *Dunaliella salina* and *Haematococcus pluvialis* (two green microalgae) have received considerable attention in recent years as natural sources of carotenoids (β-carotene and astaxanthin respectively).

Another important aspect to be considered when dealing with new bioactives from microalgae, is the development of appropriate, fast, cost-effective and environmental-friendly extraction processes able to isolate the compounds of interest.

The aim of this work was to carry out the chemical characterization, by means of high-performance liquid chromatography-tandem mass spectrometry, of different carotenoids in pressurized liquid extracts from *Neochloris oleoabundans* demonstrating for the first time the potential of this innovative green microalga. The different pressurized liquid extraction conditions were optimized using an experimental design with two variables at three levels: temperature at 40, 100 and 160 ºC, and percentage of limonene in ethanol at 0, 50 and 100 %. The response variables selected were extraction yield (%) and total content of carotenoids (expressed as mg β-carotene/g extract). Under the different extraction conditions, *N. oleoabundans* is able to accumulate different amount of carotenoids, mainly lutein, cantaxanthin, zeaxanthin, or monoester and diester of astaxanthin, among other. Comparing the chromatographic profile obtained for each pressurized extract it is possible to observe differences both in the quantity and the identity of the carotenoids present.

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FOA37

DEVELOPMENT AND VALIDATION OF A NEW METHOD OF IODIDE AND THIOCYANATE QUANTIFICATION IN COMMERCIAL MILK BY HPLC-IC-PAD USING HYDROXIDE-SELECTIVE ANION-EXCHANGE COLUMN

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Electrochemical analysis of low concentrations of iodide and thiocyanate has been of great importance in environmental, food and clinical samples [1]. However, in all non-chromatographic methods, interferences from other analytes or from matrix constituents affect the quality of results. The combination of chromatography and pulsed amperometric detection on a silver electrode resulted in improved selectivity and increased sensitivity.

Thiocyanate is, after perchlorate, a potent inhibitor of iodide uptake by the thyroid but may be more concentrated than perchlorate in some food items such as milk products. Because thiocyanate in cow's milk usually is 1000 times higher than median level in breast milk, bottle-fed infants may be exposed to considerably higher levels of thiocyanate in milk-based infant formula, increasing the risk that iodide transport into the infant's thyroid gland will be reduced [2].

An anion-exchange chromatography method in combination with pulsed amperometric detection (PAD) with silver electrode for the analysis of iodide and thiocyanate in commercial dry powder milk and infant formula using an hydroxide-selective anion-exchange column Dionex IonPac AS16 (250x2mm I.D.) was developed.

A dried milk sample was suspended in water and diluted with acetic acid (3% v/v). The mixture was finally centrifugated to achieve precipitation and the supernatant phase was filtered and diluted 1:2 for injection in the ICS-5000 ion chromatography system.

This rapid ion chromatographic method (10 min), with isocratic separation (mobile phase: 30 mM NaOH) exhibits correct linearity and an adequate limit of detection.

Five different types of milk samples were used to successfully validate the developed method and no problems related with the complex matrix were observed. Analyzing samples containing 50 μgL^{-1} of iodide and thiocyanate good precision values were obtained (2,02% RSD for reproducibility and 3,11% RSD for repetibility) and good recovery percentages (97-109%) where achieved.

This work affords a new application of the well-know hydroxide-selective anion-exchange column with adequate analytical parameters for the development of future industrial quality control application.

[1] J. Cheng, P. Jandik, N. Avdalovic, Analytica Chimica Acta, 536 (2005) 267-274.
[2] R.A. Niemann, D.L. Anderson, Journal of Chromatography A, 1200 (2008) 193-197.

FOA38

CLASSIFICATION OF OLIVE OILS ACCORDING TO THEIR GENETICAL ORIGIN USING PROTEIN PROFILES ESTABLISHED BY SDS-PAGE

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Olive oils have exceptional organoleptic characteristics, which vary depending on the genetic variety. It is therefore important to have methods of analysis to discriminate between genetic varieties. Proteins allow to classify the oil because its content depends only on the genetic and botanical variety, being independent of soil type, crop or weather. Proteins present in olive oil arise from seeds, fruit and leaves. However, the isolation and characterization of protein fraction derived from leaves have been scarcely studied. In this work, a simple and rapid assay to evaluate the extraction yield of proteins occurred in olive leaves was performed. The isolation of proteins was optimized in terms of cellulase enzyme, temperature, and organic extractant content. The total protein content was measured using the standard Bradford assay and the proteic fractions present in the extract were analyzed by SDS-PAGE. The addition of cellulase led to an increase in the yield of the proteins extraction due to the lysis of cell walls. Several genetic varieties of olive leaf were also analyzed by SDS-PAGE. Different protein profiles were clearly distinguished according to genetic origin of olive leaf.

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FOA39

CHARACTERIZATION OF OLIVE OILS USING PROTEIN PROFILES OF OLIVE LEAF ESTABLISHED BY CAPILLARY ELECTROPHORESIS WITH UV DETECTION

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Authenticity of edible oils is a very important aspect from the point of view of marketing and quality, also having a significant influence in human health and economy. Among different constituents in olive oil, proteins are less subjected to the environmental conditions, harvesting time, fruit ripening, extraction technology, etc. Then, they could constitute excellent markers to a to discriminate between different genetic varieties of olive oils in order to detect possible adulterations. During the elaboration process, proteins contained in olive leaf are transferred to the oil, being important to establish an analytical methodology to characterize its main proteins and peptides. In this work, the development of a capillary electrophoretic method to separate the main constituents of proteic fraction in leaf oil is described. After the extraction of protein fraction from leaf oil samples were dissolved in 60 µM cacodylic acid (CACO) and 2-amino-2-methyl-1,3-propanediol (AMPD) CACO-AMPD buffer containing 1% dodecyl sulfate (SDS) and 1% 2-mercaptoethanol. Next, the separation of SDS-protein complexes was carried out using poly(vinyl alcohol) (PVA) as a dynamic sieving matrix. Several extracts of olive leaf belong to different genetical varieties were characterized, giving distinct fingerprints, which could be suitable for classification purposes.

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FOA40

STUDY OF THE USE OF ENZYMES IN THE EXTRACTION OF THE PROTEINS PRESENT IN THE OLIVE FRUIT

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Olive oils are obtained from fruit pressing processes, being the proteins present in seeds and pulp transferred to the oil in small amounts. Proteins, raised from the genetic material, may constitute a valuable tool to distinguish the genetic variety of olives and possible adulterations. However, very few studies related to the composition of proteins and peptides present in the seed and pulp have been done. This fact is probably due to the difficulty of working with a lipid matrix and the low content of the proteins present. Therefore, it is required the development of efficient protein extraction methods. In this way, the use of enzymes that break down the association protein-lipids as well as other tissue cells, which could constitute an interesting approach to improve the protein extraction. In this work, the use of several enzymes in the extraction of proteins contained in seed and pulp has been studied. The type of enzyme and different extraction conditions were critically compared and its influence on the recovery protein was examined. For this purpose, the Bradford assay and SDS-PAGE technique for the separation of proteic fractions were employed. An important number of bands in the 10 to 150 kDa region were also distinguished, giving different profiles between seed and pulp.

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CHARACTERIZATION OF VOLATILE ORGANIC COMPOUNDS IN THE TRUFFLE *Tuber Brumale* BY HS-SPME AND GC-MS ANALYSIS

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As it is well-known, the culinary and commercial value of truffles is mainly due to their sensorial properties such as their aroma, the quality of which clearly gives economic value to this edible fungi[1]. Centelles, a small town in our area (Osona,Catalonia, North-eastern Spain), has for many years been an important focus for the wild truffle market in Spain. The aim of this study is the characterization of the volatile organic compounds in the winter truffle, *T. Brumale*. Samples were collected, *via* well trained dogs, on February 2012 from naturally growing truffle areas, located in two fields of hazels (watered and unirrigated, respectively). Samples were stored at 4ºC immediately after collection and the analysis was done in 24-48h and after 1, 2 or 3 weeks in order to assess the effect of storage time on the quality of the aroma. Two replicates were extracted and injected for each sample. In our study, volatile organic compounds (VOCs) have been identified via solid-phase micro extraction (SPME) - gas chromatography-mass spectrometry (GC/MS) analysis[1-6]. An extraction method based on static headspace SPME with a 75 µm carboxen/polydimethylsiloxane coating (CAR-PDMS) fibre was applied. Extraction was carried out in a 10 mL glass vial with a PTFE/Silicone septa vial with 1g of sample, at 55ºC, 6 min equilibrium and 30 min extraction. GC-MS analysis was performed with a Teknochroma TRB-WAX capillary column (60m x 0.25mm x 0.25µm) and oven programmed temperature. The SPME-extracted volatiles were directly desorbed (5 min) into the split-splitless injector at 250ºC, with Helium constant flow (1.2 mL/min), split injection ratio 50:1.The mass spectrometer operated in EI^+ mode, filament emission current 150µA and source temperature 200ºC. Xcalibur software and NIST'08 database library were used for acquisition data and mass spectra identification of the *Tuber Brumale* VOC's. Several groups of compound appeared. We found, according to their relative abundance, methoxybenzene- (13)*, thiazole- and pyridine- (9), ketone- (8), alcohol- (8), ester- (7), sulfide-, sulfone- and sulfoxi- (3), saturated hydrocarbon (3), acid- (10), aldehyde- (2), phenol (6), unsaturated hydrocarbon (2), polyaromatic- (1) and furan- (1) derivatives. These groups are in agreement with those found in the literature [2,7] .

* number of compounds in each group

[1] A.M. Gioacchini, Rapid Comm. Mass Spectr. 19 (2005) 2365.
[2] T. Talou, Flavour and Fragrance Journal, 4 (1989)109.
[3] G. Mauriello, *J. Chromatogr. Sci.* 42 (2004) 299.
[4] R.E. March, Int. J. Mass Spectrom. 249 (2006) 60.
[5] P.Díaz, LWT – Food Sci. Technol. 42 (2009) 1253.
[6] L. Culleré, Food Chem. 122 (2010) 300.
[7] *M. Kiss,* J. Appl. Botany Food Quality 84 (2011) 102.

FOA42

DETERMINATION OF 15 MYCOTOXINS IN *SILYBUM MARIANUM* BY UHPLC-MS/MS USING ALTERNATIVE SAMPLE TREATMENTS

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In recent years, the interest in food supplements has been increasing and some are used daily by consumers for various reasons. Many food supplements have botanicals as main ingredient, which can be contaminated by fungi in the field, during harvesting and storage. Some of these fungi (as *Fusarium*, *Aspergillus* and *Penicillium* genera) produce mycotoxins, natural secondary metabolites with high toxicity and serious effects on humans and animals.

European Commission has established maximum permissible levels of mycotoxins in different foods (Directive (EC) 1881/2006 and subsequent amendments), but not in food supplements; on the other hand, regulatory limits for mycotoxins in botanicals (also used for medicinal purposed) are being discussed at the European Pharmacopoeia level, although only aflatoxins are currently legislated by the different monographs of herbal products used in the pharmaceutical industry, allowing a maximum level of 2 $\mu g \cdot Kg^{-1}$ for aflatoxin B1 and 4 $\mu g \cdot Kg^{-1}$ for the sum of aflatoxin B1, B2, G2 and G1. Therefore, analytical methods for the determination of mycotoxins in these plants and supplements are required, in order to study their occurrence.
The methods usually used for the preparation and cleaning up of the sample are based on immunoaffinity columns, which are not suitable for multiresidue purpose, being also expensive. Thus, it is necessary to develop multiresidue, cheap efficient and environmentaly friendly extraction systems, in line with the new criteria of Green Chemistry. QuEChERS and dispersive liquid-liquid microextraction (DLLME) are examples of new procedures following this trend.

In this work a method based on UHPLC-MS/MS has been developed for the determination of 15 mycotoxins [aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), fumonisin B1 (FB1), fumonisin B2 (FB2), nivalenol (NIV), deoxynivalenol (DON), fusarenon-X (F-X), HT-2 toxin (HT-2), toxin T-2 (T-2), ochratoxin A (OTA), citrinin (CIT), sterigmatocystin (STE) and zearalenone (ZON)] in extract and seeds of milk thistle (*Silybum marianum*), an herbal product recommended in the treatment of liver problems.

A modified method based on QuEChERS procedure has been used for the extraction of FB1, FB2, NIV, DON and F-X, while a subsequent DLLME clean-up step was required for the determination of the rest of mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, T-2, HT-2, STE, CIT and ZON). Calibration curves were established in the presence of matrix (seeds). Low LODs (3xS/N) and LOQs (10xS/N) were obtained. Thus, the method allows the quantification of aflatoxins at concentrations lower than the maximum level established by Pharmacopeia in botanicals. Moreover, the rest of mycotoxins can be determined at concentrations lower than their usual established limits in different foodstuff. The precision (repeatability and intermediate precision) was less than 10% in all cases, and the recoveries obtained were between 63.3% and 97.3%, except for ZON (approx. 47%).

Finally, the method was also applied for studying the occurrence of these mycotoxins in several market samples: 6 seed samples and 1 extract. Two of the seed samples (purchased in bulk) gave a positive result for T-2 and TH-2, with contents above 363.0 and 826.9 $\mu g \cdot Kg^{-1}$, respectively, and in one of them ZON was detected at a concentration below its LOQs.

The results show the suitability of this procedure for the monitoring of mycotoxins in herbal products.

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FOA44

ADVANTAGES OF HIGH RESOLUTION MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETRY FOR RAPID AND COMPREHENSIVE PESTICIDE SCREENING IN FOOD ABSTRACTS

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High resolution mass spectrometry has grown appreciably in use in the recent past with availability of new instrumentation being a significant contributor to that growth. Recognition of the value of accurate mass data, ultra high resolving power, and accurate isotope abundance in compound identification and formula determination has become more prominent. Simultaneously, the need for accurate global analysis of regulated compounds in complex matrices has created pressure to apply better tools in the analysis of these compounds. A high resolution TOF on a novel Folded Flight Path ™ platform, serves as the framework for the analysis of diverse compounds of environmental interest. The ability to simultaneously detect compounds of interest (targeted) and survey other possible contaminants offers substantial opportunity in environmental and food safety efforts. Plant, vegetable and food extractions were performed by the method of Mol, et al (Anal. Chem. 80 (2008), p 9450). These extracts were spiked at various levels with a mixture of 210 compounds, covering the range of 0.3 through 300 ng/mL. Of the 210 compounds (pesticides, mycotoxins and other exogenous chemicals) 181 were amenable to analysis by positive mode electrospray ionization. Samples were diluted and analyzed by UHPLC system interfaced to an high-resolution time-of-flight mass spectrometer. The mass spectrometer rwas set to a resolution mode of 50,000 at full mass range (50 to 2,500 m/z).Pulsed post-source collision induced dissociation (MSc^2) was used to acquire alternating parent and product ion spectra that were recorded on separate data channels. The analysis of trace level analytes in complex matrices has been investigated using high performance time-of-flight mass spectrometry. Speed of acquisition and high performance capabilities have led to the detection and confident identification of over 200 analytes in complex food-based matrices including tomato, feed grain and egg. Analysis times below 3 minutes were achieved by providing full-spectrum data collection up to 200 spectra / sec (if needed) with no compromise to the quality of the analytical data. Sub-ppm mass accuracy and isotope abundance accuracy can be used to provide unambiguous molecular formula confirmation of targeted and non-targeted analytes.Resolution and mass accuracy also lend to a more robust deconvolution. Additional structural confirmation can be achieved using MS with comprehensive CID.

POSTERS

FMD

Fundamentals

FDM01

COMPARISON OF COMERCIALLY AVAILABLE PERFLUORINATED AND n-ALKYL STATIONARY PHASES FOR HPLC ANALYSIS OF LUOTONINS

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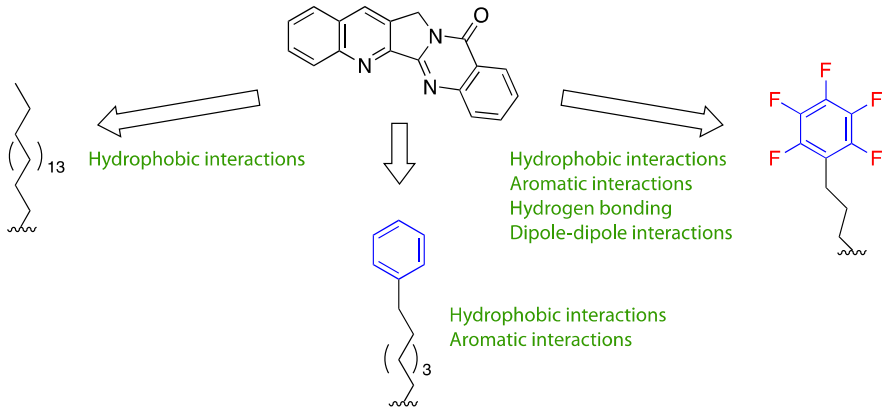
Nowadays, a great variety of stationary phases are commercially available possessing different chemistries and, therefore, different modes of interaction compared to traditional alkyl bonded phases. Thus, a significant increase in the selectivity and efficiency can be reached by choosing a stationary phase adequate for solving a particular analytical problem. Perfluorinated packings offer stronger retention and higher selectivity [1] in the case of isomers, halogenated, polar and non-polar compounds. The complex retention behaviour is consequence of the nature of different interactions (hydrogen bonding, dipole-dipole, aromatic (π-π) and hydrophobic). In the present study we compare the chromatographic behaviour of luotonin A and six newly synthesized derivatives using conventional C18 and pentafluorophenylpropyl-silane (PFP) stationary phases. Due to the nature of the interactions in both stationary phases, the elution order of the studied alkaloids was significantly altered. In order to elucidate the kind of interactions in both columns, the retention factors were correlated to the chemical structures and the lipophilicity of the compounds.



With the aim to design the most efficient separation, the influence of different experimental parameters was considered. Isocratic and gradient elution modes were assayed, and the gradient elution showed to be more convenient. The influence of the nature of the organic solvent in the mobile phase was also studied. Thus, the most frequent acetonitrile and methanol were assayed. The proportion of organic solvent in the mobile phase became a keystone to achieve a good separation. Different temperatures were assayed (25, 35 and 45 ºC). In good agreement with the results described for PFP stationary phases [2], different behaviours were found at higher and lower percentages of organic solvents, due to the change in the chromatographic retention. The changes in the retention time, selectivity, efficiency, resolution and base line are also discussed for the analytical procedure proposed.

[1] L. Havlíková, L. Matysová, R. Hájková, D. Satínsky, P. Solich, Talanta 76 (2008) 597.
[2] I. Regos, D. Treutter, J. Chromatogr. A 1217 (2010) 6169.

FDM02

EVALUATING THE SELECTIVITY OF COMMERCIAL IONIC LIQUID COLUMNS FOR CAPILLARY GAS CHROMATOGRAPHY BY THE SOLVATION PARAMETER MODEL

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Over the past decade, *ionic liquids* (ILs) have generated a great interest because of their unique and tuneable physicochemical properties and their versatility for various applications. They are organic salts with melting points below 100 °C and, typically, they possess negligible vapor pressure, wide liquid ranges, high viscosity and good thermal stability; these properties making them ideal candidates as stationary phases (SPs) in GC. Moreover, they are able to separate both polar and nonpolar compounds due to their dual-nature selectivity, which can be modified by changing the cation/anion combination [1]. So far, only six different ILs have been commercially used to prepare capillary columns (Supelco™). As main advantages, they offer different separation properties over traditional columns prepared with polysiloxanes and poly(ethylene glycols), lower column bleed, longer life, and higher thermal stability and resistance to damage from moisture and oxygen. Although they have a high polarity, this fact does not explain their different retention properties.

The *solvation parameter model* (SPM) has been extensively used to evaluate the retention characteristics of a variety of GC stationary phases, including ILs. This model is described for GC by the Abraham's equation:

$$\log k = c + eE + sS + aA + bB + lL$$

where k is the retention factor of a solute on a given SP at a specific temperature; c is the model intercept; letters E, S, A, B, and L represent the solute descriptors that are probe-specific parameters determined for many molecules; and letters e, s, a, b, and l are the system constants in which all information concerning the solvation properties of the stationary phase is represented. Therefore, they are used to characterize the strength of each type of interaction. Specifically, e defines the capability of the SP for π-π and n-π interactions and s for dipole-type interactions; while a and b, are the hydrogen-bond basicity and acidity of the SP, respectively; and l describes the overall dispersive-type interactions.

Consequently, the aim of this work was to characterize the retention properties of three commercial ionic liquid columns (IL59, IL76 and IL82) by the solvation parameter model, studying the effect of temperature on selectivity, and comparing their separation characteristics to the classical SPs in capillary GC. For this study, more than 70 solutes with varied functional groups were injected in each IL column at four temperatures between 100 and 160 °C. The system constants were obtained by multiple linear regression analysis of their five solute descriptors and their log k values.

The results revealed that dominant contributions to retention are the dipolar-type and hydrogen-bond basic interactions, being π-π and n-π interactions barely significant. All the ILs studied can be considered highly cohesive and weakly hydrogen-bond acid SPs. An increase of the system constants with the SP polarity was found, except for the l constant. A principal component analysis for the commonly used SPs in GC clearly showed that these columns fill an empty area of the available selectivity space.

[1] J.L. Anderson, in: M. Koel (Ed.), Ionic liquids in Chemical Analysis, CRC Press, Boca Raton, 2009, pp. 139.

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FDM03

ENTHALPY OF VAPORIZATION OF IONIC LIQUID 1-BUTYL-3-METHYLIMIDAZOLIUM BIS(TRIFLUOROMETHYLSULFONYL)IMIDE MEASURED BY CAPILLARY INVERSE GAS CHROMATOGRAPHY

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Ionic liquids (ILs) are organic salts, with melting points below 100 °C, which comprise cations such as substituted imidazoliums or pyridiniums, paired with anions such as halides, sulfates, phosphates and others (e.g., bis[trifluoromethylsulfonyl]imide, NTf_2). There are potentially millions of ILs with different physico-chemical properties which can be engineered to optimize temperature, substrate solubility, yield and selectivity in chemical reactions and separations processes. Due to their extremely low vapor pressures, ILs do not release harmful vapors to the environment. Therefore, it would be advantageous to replace the traditional volatile solvents by these substances, that could lead to a removal of a major source of environmental pollution. This lack of volatility has been assumed to be common to all ionic liquids that do not undergo thermal decomposition, but recent experiments have challenged this assumption. Therefore, the vaporization properties of all new ILs should be experimentally checked.

Gas Chromatography (GC), apart from its proven ability to resolve complex mixtures of compounds, allows to obtain the Flory-Huggins interaction parameter and the Gibbs free energy of mixing, directly related to the solute-stationary phase (SP) system. In addition, other magnitudes related to one of the two system components, namely, the Hildebrand solubility parameter and the vaporization enthalpy of the solute, or the solubility parameter of the SP can also be estimated. This variant of the GC is called Inverse Gas Chromatography (IGC).

In most of the IGC studies, packed columns are used for the determination of the specific retention volume (V_g), the fundamental retention parameter of a substance in IGC. Capillary columns are very popular nowadays, but scarcely used in IGC due to the difficulty in measuring the SP amount in the column and the carrier gas flow-rate, both essential to obtain reliable V_g values. However, some of their inherent qualities, such as the faster achievement of solute-SP equilibrium and improvements in the methods of suppression of the residual activity of the column walls, make them ideal for this purpose. Several years ago, we developed a new equation that overcame the aforementioned drawbacks and allowed the accurate calculation of V_g values in capillary columns.

Consequently, the objective of this work was to evaluate the possibility of obtaining vaporization enthalpies ($\Delta_{vap}H_2^o$) of ionic liquids through their solubility parameters (δ_2) by IGC using capillary columns. To achieve this, several capillary columns of different film thicknesses were prepared by the static method using the ionic liquid $[C_4MIM][NTf_2]$ as stationary phase. More than 60 organic compounds covering a wide range of functional groups were used as probe solutes and injected in the capillary columns at five temperatures between 313.15 and 393.15 K. The independence of the solute V_g values on the amount injected, the carrier gas flow-rate and the SP film thickness was carefully checked.

The δ_2 and $\Delta_{vap}H_2^o$ values at 298.15 K were compared with data taken from literature. A good agreement was achieved in both cases. Therefore, IGC seems to be appropriate to obtain reliable vaporization enthalpies of ionic liquids through their solubility parameters using capillary columns. It is only necessary to know the concentration of the solution employed to prepare the chromatographic column, inject very small amounts of different groups of substances and be sure that adsorption phenomena are absent.

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FDM04

TESTING CHROMATOGRAPHIC MODELS TO PREDICT RETENTION OF ACID-BASE ANALYTES UNDER GRADIENT ELUTION USING METHANOL AS ORGANIC MODIFIER

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Modeling the retention of acid-base analytes under RP-HPLC gradient elution is quite difficult. Gradient elution implies the change of the mobile phase composition during the measurement so the pK_a of the analytes and the pH of the mobile phase, which depend on the mobile phase composition, also change during the elution process. Since both parameters have a strong effect on retention, including them in the chromatographic model becomes critical in order to perform good predictions.

Three different chromatographic models that predicted the retention of acid-base analytes were previously developed[1]. These models combined three different approaches to the prediction of the retention of neutral compounds under gradient elution that used one, two and three adjustable parameters respectively[2,3], with a model that described the isocratic retention of ionizable analytes[4]. All models showed a good agreement between the experimental results and the calculated predictions when acetonitrile was used as the organic modifier, being the two-parameter model the one that gave the best relationship between accurate results and previous experimental work to be done[1].

On the basis of these results, the three chromatographic prediction models have been tested using methanol as the organic modifier. The 12-compound set used in the previous work has now been expanded to 22 substances with more structural complexity and pharmaceutical interest.

To model the pK_a variation of the tested compounds with the composition of the mobile phase, a key factor in understanding retention of acid-base analytes under gradient elution, an empirical equation has been used (Eq. 1)[5]:

$$ {}^{s}_{w}pK_a = a_S\, {}^{w}_{w}pK_a + b_S + \delta \qquad (1) $$

${}^{s}_{w}$pK_a is the pK_a in the mobile phase (hidroorganic mixture) referred to water calibration, ${}^{w}_{w}$pK_a is the pK_a in pure water, a_S and b_S are parameters that depend on the functional group of the analyte and □ is a parameter that depends on the organic fraction of the mobile phase. Another way of modeling the ${}^{s}_{w}$pK_a variation would be by determining experimental ${}^{s}_{w}$pK_a values for several mobile phase compositions and then fitting them to a second grade equation, a method that has been previously used[1]. These two methods have been compared in order to find out whether the calculated values can replace the experimental ones when modeling the ${}^{s}_{w}$pK_a variation without losing accuracy in the retention prediction models.

Analogously, there are two paths to model the ${}^{s}_{w}$pH of the mobile phase but calculating the ${}^{s}_{w}$pH variation with the mobile phase composition using empirical equations does not offer a clear advantage in time savings over determining it experimentally. Thus, for the ${}^{s}_{w}$pH variation, the experimental method has been used in this work.

[1] A. Andrés, A. Téllez, M. Rosés, E. Bosch. J. Chromatogr. A 1247 (2012) 71.
[2] P. Nikitas, A. Pappa-Louisi. Anal. Chem. 77 (2005), 5670.
[3] A. Téllez, M. Rosés, E. Bosch. Anal. Chem. 81 (2009) 9135.
[4] D. Bolliet, C.F. Poole, M. Rosés. Anal. Chim. Acta 368 (1998) 129.
[5] X. Subirats, M. Rosés, E. Bosch. Sep. Purif. Rev. 36 (2007) 231.

FDMO5

BASIC DRUGS AND MOBILE PHASE BUFFERS: CATION EXCHANGE AS ADDITIONAL RETENTION MECHANISM

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In a previous work [1], it was found that some basic drugs containing amino and hydroxyl groups in adjacent carbons (β-blockers) showed different retention times when using mobile phases of the same pH (measured in the mobile phase) and organic modifier composition but prepared from different buffers. In order to elucidate the responsible mechanism of this chromatographic behavior, several basic drugs are systematically studied using different reversed-phase columns and mobile phases containing tris(hydroxymethyl)aminomethane (Tris), ethanolamine and dihydrogenphosphate as buffering species, and both acetonitrile and methanol as organic modifiers. In all cases, the studied drugs are more retained than expected in the presence of cationic buffers (Tris and ethanolamine), suggesting a combined retention mechanism based on the hydrophobic interaction of the analytes with the stationary phase together with an ion exchange mechanism [2]. Based on these mixed retention mechanism investigated in a previous work for a polymeric column [3], the observed extra retention times are attributed to a cation-exchange process between the positively charged conjugate acids of both the analyte and the buffer, being the latter anchored on the negatively charged silanol sites present in the stationary phase.

In addition, a complementary study about buffer-analyte interactions in free solution is carried out by means of isothermal titration calorimetry. Results confirm the lack of interaction between the studied β-blockers and Tris buffer out of the chromatographic system and, therefore, the role of the stationary phase in the proposed retention mechanism.

[1] L.G. Gagliardi, C.B. Castells, C. Ràfols, M. Rosés, E. Bosch, J. Sep. Sci. 31 (2008) 969.
[2] U.D. Neue, K. Tran, A. Méndez, P.W. Carr, J. Chromatogr. A 1063 (2005) 35.
[3] R. Ruiz, M.J. Ruiz-Angel, M.C. García-Álvarez-Coque, C. Ràfols, M. Rosés, E. Bosch, J. Chromatogr. A 1028 (2004) 139.

POSTERS

1PP

Industrial Processes and Products

IPP01

CHARACTERIZATION AND DETERMINATION OF THE ALCOHOL FRACTION OF ESSENTIAL OILS BY CHROMOGENIC DERIVATIZATION AND RP-HPLC-UV

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Essential oils, which are obtained from plant parts by steam distillation, are used in a variety of applications, mainly in cosmetics and natural medicine. Essential oils are commonly characterized by GC-FID and GC-MS. Owing to the low UV absorptivity of most components, HPLC has been scarcely used. Alcohols and multifunctional compounds containing alcohol groups are an important part of both natural and synthetic essential oils. In this work, the alcohol fraction of essential oils is characterized by RP-HPLC-UV with previous derivatization with a symmetric cyclic anhydride (phthalic anhydride). On the other hand, computer-assisted HPLC method development was used to facilitate the optimization of the chromatographic separation optimization. For this purpose, the DryLab® software for chromatography modeling (Molnár-Institute, Berlin, Germany) was used. Separation of the derivatives was carried out on a C8 column (fused-core Ascentis-Express, 2.7 µm, 15 cm x 4.6 mm ID, Supelco, Bellefonte, PA, USA) using gradient elution with water-acetonitrile. The method was applied to the essential oils of *Mentha arvensis* and *Mentha piperita*, and two commercial samples probably constituted by synthetic surrogates of essential oils of mentha and rose. The chromatograms showed the peaks of a series of alcohols and esters, including menthol, geraniol and β-citronellol, as well as other non-identified components, with very low limits of detection. For all the samples, mobile phase composition and column temperature were both optimized using DryLab®. In all cases, excellent resolution between all the peaks pairs was achieved using initial sets of four chromatograms. Optimal separations were achieved by increasing acetonitrile concentration from 30% to 80% in 45 min. Optimal column temperature was 6-8ºC. Limits of detection and other figures of merit were compared to those obtained by GC-MS.

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IPP02

IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS AS BIOMARKERS OF MICROBIAL CONTAMINATION IN COSMETIC PRODUCTS BY SPME-GC/MS

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Most of cosmetic formulations, due to their composition and high water content, are products suitable of biological degradation by microorganisms. Microbial contamination represents an important risk for consumer health, since it can lead to irritations or infection, especially when these products are applied to damaged skin, eyes or to babies [1]. Several outbreaks of microbial infection caused by contaminated cosmetics have been reported, being the most typical contaminants the genus *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., and *Pseudomonas* spp., the most frequently responsible and the main problem of cosmetic industry [2]. For this reason, cosmetic companies are required to maintain optimal preservation of their commercial products. Nevertheless, a recent review of cosmetic microbiology highlights how important is for industry to have faster methods for microorganisms detection than traditional plating, which is still in use nowadays [3].

The production of microbial volatile organic compounds (MVOCs) during metabolic processes, which occur in the presence of different bacteria cultures, have been well reported and reviewed [4]. In these surveys, solid-phase microextraction (SPME) has been widely used for MVOCs sampling from bacterial cultures or infected samples.

In this work, an alternative methodology for a quick detection of microbial contamination in cosmetic products based on SPME, to collect headspace volatiles emitted from contaminated cosmetic samples is proposed. The subsequent analysis of the volatile fraction was done using gas chromatography-mass spectrometry (GC/MS) for the separation and the identification of the collected MVOCs. Thus, different cosmetic samples, either rinse-of or leave-on, were inoculated with the bacteria most commonly found in contaminated cosmetics. The analysis of the volatile fraction from pure bacterial cultures and inoculated samples revealed the presence of characteristic MVOCs (e.g. 1-undecene, 2-nonanone, 2-undecanone and 2-tridecanone) most probably related with microbial growth. Many of these odd carbon alkenes and methyl ketones have already been reported as MVOCs characteristic of the microbial genus studied. A kinetic study confirms the applicability of this method for monitoring the microbial contaminated samples over time.

The results obtained in this “proof-of-concept” study suggest the suitability of the SPME-GC/MS as a promising methodology for simple and rapid detection of microbial contamination in cosmetic products.

[1] Leranoz, S., Dermofarmacia, 21 (2002) 74.
[2] Lundov, M.D. and C. Zachariae, Int J Cosmet Sci, 130 (2008) 471.
[3] Orus, P. and S. Leranoz, Int. Microbiol., 8 (2005) 77.
[4] Schulz S, Dickschat JS., Nat. Prod. Rep. 24 (2007) 814.

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IPP03

INFLUENCE OF REACTION PRESSURE IN THE ENZYMATIC SYNTHESIS OF POTENTIAL PREBIOTIC OLIGOSACCHARIDES

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In the present work, we have investigated the production, via enzymatic synthesis, of a bioactive oligosaccharide (2-α-D-glucopyranosyl-lactose) studying the influence of the reaction pressure (up to 100 bar) and comparing the performance to conventional atmospheric conditions.

This new oligosaccharide may possess high interest due to its potential prebiotic nature. This potential bioactivity is attributed to the formation of the α1-2 linkage in its structure, elucidated by nuclear magnetic resonance (NMR) as Gal β1-4 – [Glu α1-2] - Glu. This type of linkage is characterized by high resistance to the gastro intestinal digestion. Consequently, this compound could effectively stimulate the selective growth of bacteria that are beneficial to the large intestine, mainly bifidobacteria and lactobacilli.

Once the optimized reaction conditions for the synthesis of 2-α-D-glucopyranosyl-lactose were attempted in previous assays carried out in our research group [1], we have focused our attention in this investigation on the study of the potential effect of reaction pressure in the high-yield synthesis of the trisaccharide which could favor the feasibility of scaling up the process.

The transglycosylation reactions for trisaccharide synthesis, based on transfer of the glucose unit of the sucrose to lactose by *Leuconostoc mesenteroides* B512-F dextransucrase, were carried out in a reactor system equipped with a 100 ml stainless steel cell, a high-pressure pump for the delivery of the gas into the system and a controller which allows to automatically monitoring the speed of agitation and the reaction temperature. Dextransucrase (0.8 U mL^{-1}) was added to the sugars solution (250 g L^{-1} of sucrose and 250 g L^{-1} of lactose in 20 mM sodium acetate buffer, pH 5.2, supplemented with 0.34 mM $CaCl_2$). The reaction mixture was introduced into the reactor vessel that was incubated in a temperature-controlled bath at 30-35ºC under shaking conditions (300 rpm) for 24 hours. Samples of the reaction mixture were removed at regular time intervals and immediately immersed in a boiling water bath for 5 min to inactivate the enzyme. Monitoring of the process was carried out by liquid chromatography with refraction index detector (LC-RID), using an Agilent Technologies system with a NH_2 column (250 x 4.6 mm, 5 µm particle size) and acetonitrile / water 75:25 (v:v) as mobile phase in isocratic mode at a flow rate of 1 ml min^{-1}.

The results showed a slight influence of pressure on the final yield of 2-α-D-glucopyranosyl-lactose obtained, according to the parameters studied, including the gas used to pressurize the system (N_2 or CO_2), as well as the pressure applied. In addition, the solubility of the starting substrates in pressurized conditions will be further studied in order to increase the yield of the trisaccharide throughout the enzymatic process.

In conclusion, under the experimental conditions used in this work, the production under pressure via enzymatic synthesis of 2-α-D-glucopyranosyl-lactose has been achieved. Thus, this investigation can be used as a starting point for addressing the synthesis of new bioactive oligosaccharides under pressure conditions as an alternative reaction medium for enzyme catalyzed reactions.

[1] Díez-Municio, M., Montilla, A., Jimeno, M. L., Corzo, N., Olano, A., Moreno, F. J., J. Agric. Food Chem. 60 (2012), 1945.

POSTERS

NDE

New Developments in Sample Treatment and Instrumentation

NDE01

DEVELOPMENT AND OPTIMIZATION OF A GRAS METHODOLOGY TO EXTRACT GLUCOSINOLATES FROM BROCCOLI LEAVES

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Broccoli belongs to the Brassicaceae family (*Cruciferae*) and it is a potential source of health promoting compounds like glucosinolates, polyphenolic compounds, etc. Intensive broccoli cultivars are associated with the production of considerable by-products, meanly leaves that are usually discarded even though they may have a similar composition to the edible parts of the plant. These by-products could be used as complements in animal feeding or as nutraceutical reservoirs, which could contribute to reduce their environmental impact and at the same time give them certain economic value. For this reason, attention is now being paid to the possible alternative use of such residues as source of glucosinolates, which, in preliminary research, are potent inducers of cytoprotective enzymes and inhibitors of carcinogenesis.

In order to obtain the highest amount of glucosinolates as possible from broccoli leaves in an economic, generally recognized as safe (GRAS), fast and efficient way, we have decided to check the effectiveness of two different extraction procedures based on the deactivation of myrosinase, a class of enzymes that catalyzes the hydrolysis (breakdown) of glucosinolates by heating the sample (microwave and oven), and using water as GRAS solvent to extract the glucosinolates. A Box–Behnken design was chosen to optimize the extraction conditions in both procedures, three parameters were studied for the microwave treatment (water volume, time and sample amount) and four in the oven treatment (temperature, water volume, time and sample volume). After the optimization procedure, the microwave based treatment was proposed as it provided slightly better results in terms of the amount of extracted glucosinolates in a shorter period of time.

Moreover, a new LC-DAD-MS/MS method was developed to identify and separate the extracted glucosinolates. It has been used a analytical column, Gemini 3µ C_{18} (150 x 4.60 mm), and gradient elution mode of the mobile phase, which was composed by formic acid in water and formic acid in acetonitrile, in order to decrease as much as possible the chromatographic run and obtain the best resolution between peaks. Finally, the LC-DAD-MS/MS method was fully validated.

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NDE02

DESIGN OF POROUS POLYMER SORBENTS FOR MICROPOLLUTANT EXTRACTION

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Functionalization of crosslinked porous polymer resins leads to materials with numerous applications in many areas including ion exchangers, chromatographic packings, polymer-supported catalysts, solid-phase extraction (SPE) adsorbents. SPE is a well-known and efficient method for sample treatment allowing preconcentration and interferents elimination.

SPE using ion exchange or chelating materials offers many advantages for metal ions separation since it simplifies the separation process and reduces the disposal costs as well as the solvent uses and exposure.

This talk will focus on the preparation of functional macroporous resins containing various organic ligands as active groups. The aim is to design functional materials able to complex metallic ions for an application in solid-phase extraction. Several processes will be discussed here.

The simplest method consisting of grafting some organic ligands on commercial sorbents will be first described. Grafting is an interesting method since the properties of the initial resins are well-suited for SPE applications. Some results obtained by grafting on Amberlite® XAD-4 will be presented[1].

However, grafting is time-consuming and presents some drawbacks. Thereafter, another approach is the direct copolymerization of a functional monomer with a crosslinker (for example divinylbenzene). Suspension copolymerization with control of the porosity enables to prepare resin beads adapted to SPE[2]. Grafting and direct copolymerization will be compared in the case of the catechol ligand[3].

Nevertheless, even if selectivity is a challenging concern, it is difficult to achieve with the previously mentioned materials. When selective recognition is desired, adding an imprinting effect to those porous systems is a good alternative. Imprinted polymers are prepared by copolymerization of a functional monomer and a crosslinker in the presence of a template. The removal of the template generates inside the polymer network binding sites with high affinity and selectivity towards the template. This technique has been applied to the design of nickel imprinted polymers[4] and some organic pollutants.

[1] *(a)* P.Vanloot, C.Branger, A.Margaillan, C.Brach-Papa, J.L.Boudenne, B.Coulomb, Anal. Bioanal. Chem., 389 (2007) 1595-1602; *(b)* S.Boussetta, C.Branger, A.Margaillan, J.L.Boudenne, B.Coulomb, A.Margaillan, React. Funct. Polym., 68 (2008) 775-786; *(c)* G.Depecker, C.Branger, A.Margaillan, T.Pigot, S.Blanc, F.Robert-Peillard, B.Coulomb, J.L.Boudenne, React. Funct. Polym., 69 (2009) 877-883.
[2] J.Bernard, C.Branger, I.Beurroies, R.Denoyel, S.Blanc, A.Margaillan, Polymer 51 (2010) 2472-2478.
[3] J.Bernard, C.Branger, I.Beurroies, R.Denoyel, A.Margaillan, React. Funct. Polym., 72 (2012) 98-106.
[4] W.Meouche, C.Branger, I.Beurroies, R.Denoyel, A.Margaillan, Macromol. Rapid Commun. 33(2012)928−932.

NDE03

CHARACTERIZATION OF STEROLS BY HIGH TEMPERATURE LIQUID CHROMATOGRAPHY USING A POROUS GRAPHITIC CARBON STATIONARY PHASE

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Phytosterols, unsaponifiable components of vegetable oils and other foods, reduce blood cholesterol levels by inhibiting its intestinal absorption. In addition, these compounds show anti-inflammatory, antibacterial and antioxidant activities [1]. Official methods involved saponifications, extractions of the unsaponifiable fraction, isolation by thin-layer chromatography and derivatization followed by gas chromatography [2-4]. On the other hand, most of HPLC separations are done at temperatures between 25 and 40ºC; however, along the last years, high temperature liquid chromatography (HTLC) up to 200ºC has been made possible by the development of new stationary phases with outstanding thermal stability [5-6]. In comparison to conventional HPLC, HTLC provides lower backpressures at high flow rates, shorter analysis times, better efficiencies and different selectivities. In this work, an HTLC method for the characterization of phytosterols by direct injection of vegetable oils after a simple dilution with mobile phase is proposed. For this purpose, the use of porous graphitic carbon columns like Hypercarb (Thermo Fisher Sientific) and Zirchrom-CARB (Zirchrom) was investigated. The solvent gradient composition using methanol, ethanol and chloroform was optimized in combination with temperature programing. The two porous graphitic carbon columns were comparively discussed. At 100-140ºC, the substitution of methanol by a “green chemistry” solvent like ethanol did not increase the backpressure. It was observed that an increase of 2ºC in the column temperature was equivalent to an increase of *ca.* 1% of $CHCl_3$ in the mobile phase composition. Using flow rates of 2 mL min^{-1}, the time analysis was substantially reduced, giving chromatograms of analytes in less than 15 min. Efficiency was also largely improved by increasing the column temperature.

[1] J.S. Perona, J. Nutr. Biochem., 17 (2006) 429.
[2] L. Cercaci, Food Chem., 102 (2007) 66.
[3] M.J. Lerma-García, Electrophoresis, 29 (2008) 4603.
[4] T. Greibrokk, J. Sep. Sci., 24 (2001) 899.
[5] J. Dolan, J. Chromatogr. A, 965 (2002) 195.
[6] G. Vanhoenacker, J. Chromatogr. A, 1082 (2005) 193.

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NDE04

NEW CHEMILUMINESCENT DERIVATIZATION METHODOLOGY FOR PEPTIDES AND GLYCOPROTEINS DETECTION IN CAPILLARY ELECTROPHORESIS.

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The study of glycoforms of glycoproteins is a relevant approach to the early detection of some diseases. In fact, the relation between some illnesses and a change in the glycosylation pattern of some glycoproteins is under intensive research. However, in-depth studies of glycosylation changes are a challenge due to: i) the structural similarity between glycoforms, and ii) the low concentration in biological fluids of many of the glycoproteins of interest as biomarkers.

Capillary electrophoresis (CE) is an appropriate technique for achieving separation of isoforms (electrophoretic peaks containing one or more glycoforms), of some of the glycoproteins used as biomarkers. However, for the analysis of minor glycoproteins in biological samples, this separation technique must be coupled to a very sensitive detection technique. Chemiluminescence (CL) is a very convenient detection technique in terms of sensitivity, even though derivatization of the great majority of analytes is needed. A derivatization reaction through thiol groups of the proteins (after a reduction step of disulfide bridges) is of interest in some cases, because it is less prone to the formation of multiple reaction products than when the reaction is carried out through amino groups.

In this work, a methodology applicable to peptides and proteins for selective derivatization with a chemiluminescent tag through thiol groups in the presence of amino groups is proposed. The luminol derivative chloroacetyl-luminol, synthesized in our laboratory, was employed as labelling agent. As a proof-of-concept, oxidized glutathione was employed as a polypeptide model. Reduction and derivatization conditions for different initial concentrations of oxidized glutathione were optimized using mass spectrometry and CE with UV detection. Under the best conditions, a good derivatization yield of glutathione derivatized only through thiol groups was obtained when the concentration of this peptide in the sample was 10^{-5} M.

A home-made CE instrument with CL detection was employed. It includes a sheath-flow cuvette used as a post-column reactor that allows the oxidation of the derivatized glutathione with sodium hypobromite at the exit of the separation capillary. The light produced by this reaction is measured by a photomultiplier tube (PMT). Several detection parameters, such as PMT voltage and positioning, oxidant concentration and flow rate through the post-column reactor, and signal processing were studied. Under the best conditions achieved up to now, the CE-CL instrumental limit of detection (LOD), measured injecting luminol, was satisfactory ($5x10^{-6}$ M). This system has allowed the detection of derivatized glutathione with a LOD only hampered by the slow derivatization rate of low concentrations of analyte.

The ratio of isoforms separated by CE of alpha-1-acid glycoprotein (AGP) can be used as a biomarker of different diseases. In this work we show a first attempt to use the methodology developed for CE analysis of isoforms of AGP using CL detection.

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NDE05

ANALYSIS OF ANABOLIC STEROIDS IN URINE BY GC-APGC-MS/MS (QqQ AND QTOF). POTENTIAL USE FOR DOPING CONTROL

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Exogenous androgenic anabolic steroids (AAS) are synthetic derivatives of testosterone with a common structure which contains four rings. The use of AAS can stimulate the formation of muscle cells, increasing the muscle growth. For this reason, they are widely used for athletic performance. Since their first prohibition in 1976 [1], they remain as the most frequently detected group of substances in doping control analyses [2]. Therefore, doping control laboratories have to develop adequate analytical approaches for the detection of AAS misuse. Most of AAS are quickly metabolized after human administration. Thus, AAS metabolites are the most suitable biomarkers for the screening of AAS.

Due to the fact that their use by athletes is prohibited at any time, the mere presence of one of their metabolites in urine is enough to declare an adverse analytical finding. Consequently, any improvement in the detection of AAS is important for the doping control field. Analytical methodologies using chromatographic techniques coupled to tandem mass spectrometry with triple quadrupole (QqQ) are the most adequate approaches for the determination of AAS or their metabolites in urine samples due to their excellent sensitivity and selectivity. At the moment, doping control laboratories are using two strategies for the detection of AAS: methods based on LC-MS using atmospheric pressure interface (API) and GC-MS methods using electron ionization (EI). LC-API-MS methods allow for the reduction of sample treatment and the possibility of detecting thermolabile compounds. However, only those compounds with an ionisable centre can be detected and important AAS biomarkers such as totally reduced metabolites cannot be detected by this technique. On the other hand, besides the drawback of the compulsory derivatization step, GC-EI-MS methodologies have the limitation of the high fragmentation of the compounds in the source which can hamper the selection of an adequate precursor ion in MS/MS strategies.

In this work, a new atmospheric pressure interface with softer ionization, developed for using in gas chromatography (APGC) [3], is investigated for detection of target AAS with GC-APGC-QqQ and GC-APGC-QTOFMS. Firstly, derivatized [4] and underivatized AAS were tested, in order to evaluate the need to apply this time-consuming sample preparation step and to check the extra structural information which can be extracted from this step. Next, the fragmentation behaviour of representative AAS was investigated by QqQ and QTOFMS, in order to be extended to other steroidal structure compounds.

This interface promotes ionization with very little fragmentation for underivatized analytes, with the result of $[M+H]^+$ or $M^{\cdot+}$ ions (depending on the presence or not of water in the interface) as the base peak of the spectra, similar to those obtained by LC-MS. The reduced fragmentation observed by using this new source can have a significant impact on target analysis at trace levels. In the case of trimethylsilyl (TMS) derivatives, a slightly higher fragmentation was observed but also related with OTMS losses.

In both approaches, with and without derivatization, the reduced fragmentation in the full scan spectrum given by the APGC source facilitates the selection of abundant and/or more specific precursor ions in tandem MS experiments, allowing for the development of more efficient tandem MS methods, which would increase the selectivity and the sensitivity of the method.

[1] World Anti-Doping Agency (WADA) http://list.wada-ama.org/ (*last accessed* 8/6/12).
[2] WADA accredited laboratories statistics. http://www.wada-ama.org/Documents/Resources/Statistics/Laboratory_Statistics/WADA_2010_Laboratory_Statistics_Report.pdf.
[3] T. Portolés, J. V. Sancho, F. Hernández, A. Newton, P. Hancock, J. Mass Spectr. 45 (2010) 926.
[4] J. Segura, R. Ventura, C. Jurado, J. Chromatogr. B. 713 (1998) 61.

NDE06

DEVELOPMENT OF A METHOD BASED ON HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF IODINATED X-RAY CONTRAST MEDIA IN ENVIRONMENTAL WATERS

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Hydrophilic interaction liquid chromatography (HILIC) has emerged as an alternative to reverse phase liquid chromatography (RPLC) and normal phase liquid chromatography (NPLC) modes for the determination of polar and hydrophilic compounds [1, 2]. This chromatographic mode is characterized for the use of a NP stationary phase in combination with a RP mobile phase, containing a high percentage of organic solvent. The popularity of HILIC has arisen in the last years due to the growing demands for the determination of polar drugs, metabolites and biologically related analytes [2]. Iodinated x-ray contrast media (ICM) are the most widely administered intravascular polar pharmaceuticals used in X-ray diagnostic procedures [3, 4]. Considering that RPLC methods applied for this analytes have been not satisfactory enough due to the polar and hydrophilic nature of the ICM, an HILIC approach was studied as an alternative for the determination of these compounds.

For the method development the most important parameters affecting the chromatographic separation were optimized. These variables included: 1) type of organic solvent in the mobile phase, 2) type of aqueous phase (including the acid additive, pH and ionic force), 3) aqueous/organic proportion, 4) the injecting solution, 5) the flow-rate and the separation temperature. Moreover, a stationary phase based in Fused-Core^{TM} particle technology bare silica column and a zwitterionic stationary phase were tested.

Among the two HILIC columns tested the zwitterionic stationary phase was found to be the best column for separate all analytes.

The optimized HILIC conditions were adapted to be coupled to a triple quadrupole (QqQ) tandem mass spectrometry (MS/MS) with electrospray ionization (ESI). Optimal LC-MS/MS conditions were performed using ESI in positive mode while acquisition data was acquired in multiple reaction monitoring (MRM) mode. An optimized SPE-HILIC-MS/MS method was applied for the determination of ICM in different complex environmental waters. The method presented good results and enabled the determination of ICM at low concentration levels in these types of samples.

[1] D. McCalley, J. Chromatogr. A 1217 (2010) 858.
[2] P. Jandera, Anal. Chim. Acta 692 (2011) 1.
[3] S. Pérez, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1235.
[4] W. Krause, P. W. Schneider, Top. Curr. Chem. 222 (2002) 107.

NDEO7

TREATMENT OF SAMPLES TO DETERMINE RADIONUCLIDES TROUGHT FLOW ANALYTICAL TECHNIQUES

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The preconcentration and the radiochemical separation of different radionuclides which can be present in environmental samples are usually needed steps since these radionuclides can be present in this kind of samples at very low concentration levels, and also the presence of other interfering compounds can be a problem. Moreover, the most used detection systems for these radionuclides present as a critical point a low selectivity which can be in some cases a serious problem, specially when interfering radionuclides are present in these samples. To overcome these problems, specific resins have been recently developed for the determination of several radionuclides such as radium, uranium or strontium among other isotopes[1,2]. UTEVA resins (dipentyl-pentylphosphonate group) that are used for actinide radionuclides separation or Sr-resin (18-crown-6 ether) for strontium, are some of the most used.

Commonly, the separation and preconcentration processes are carried out manually. Sample preparation is a part of the analytical process that accounts for over 70% of the total analysis time and the procedures involved in this step are the main contributors to the associated errors. Then automation is an attractive strategy since flow analytical techniques allow the developments of fully or semi automated methods in the pre-treatment sample achieving the minimization of sample handling, reagent volumes, time and cost per analysis and improvement of reproducibility [3]. The combination of multisrynge flow injection analysis (MSFIA) with Lab-on-valve (LOV) systems allow the automation of all the steps of resin extraction process: the loading of the sample, the cleaning of interferences and the elution of the target radionuclide. This combination also enables the integration of various analytical reagents in the selection valve with a great potential for miniaturization of the entire of instrumentation.

In this study, we used two different methods using a MSFIA-LOV [4,5] system combined with different resins: Uteva or Sr-Spec resin. The extraction conditions of each resin were optimized to extract uranium (U-238, U-234, U-235) and thorium (Th-232, Th-230) using UTEVA resin and Lead (Pb-210) and Strontium (Sr-90) using Sr- resin. The methods were applied to determine these radionuclides in different sludge and water samples collected in a potable water treatment plant.

[1] Triskem International, France: http://www.triskem–International.com/, (last visit: February 2012).
[2] O. Egorov, J.W. Grate, J. Ruzicka, Anal. Chem. 71 (1999) 345.
[3] Y. Fajardo, J. Avivar, L. Ferrer, E. Gómez, M. Casas, V. Cerdà, Trends Anal. Chem., 29 (2010) 1399.
[4] M. Manuel, V. Cerdà, J. M. Estela, Trends Anal. Chem., 21 (2002) 199.
[5] J. Wang, E.H. Hansen, Trends Anal. Chem., 22 (2003) 225.

OPTIMIZATION OF METABOLIC COMPOUNDS EXTRACTION IN YEAST SAMPLES

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Nowadays studies to analyze metabolic profile of yeast are having a special interest due to the rapidly profiling of a specific physiological condition and allow the complement features of trasncriptomic and proteomic analysis.

GC-MS was described as a robust quantification method for metabolomic studies in plant extract[2]. The main advantages of this technology are that it has long been used and therefore stable protocols, chromatogram evaluation and interpretation are available. Although not all protocols are suitable for yeast extraction and sample condition. For this reason, we proposed different changes in an extraction method witch have had very good results in metabolites plants extraction, described in Fiehn *et. al.*[1]. Therefore, our aim is to optimize the protocol for yeast samples to analyze metabolites such as carbohydrates, amino acids, organic acids and sugar alcohol.

The optimized method was divided in three steps: quenching, extraction and derivatization of samples. First, yeast culture has been exposed immediately to a quenching solution in order to make its metabolism stop. After this, to extract the intracellular metabolites, cell pellets were sonicated in presence of glass beads, MeOH:H_2O (1:1) and ribitol as a internal standard. To facilitate the separation between broken cells and solvent, and to avoid any non polar substances in the extract, a two phase system was created by adding chloroform. The upper layer was removed and taken to dryness using speed-vac evaporator. The dry residue was derivatized by two steps. Firstly, a methoxymation reaction was carried out to get reducing sugars and secondly, the use of sillylation with MSTFA reagents allows a better volatilization of substances to be analyzed by GC-MS[3].

This method has been optimized by changing: the quenching method (MeOH+NaOH solution *vs.* freezing), the process to break cells (sonication *vs.* boil), the absence of chloroform cleaning step, the dryness time, the derivatization reagents (MSTFA *vs.* BSTFA), and derivatization time.

An application of the optimized method is done focusing on dehydration process. We choose desiccation in yeast due to the importance of this process in the Active Dry Wine Yeast (ADWY) production industry to make more robust yeast strains. For this study we worked with laboratory strain to get an easier metabolic profile; also, this strains can be genetically modified. According to this we compare the metabolomic profiles between different conditions: desiccated and non-desiccated.

[1] O. Fiehn, J. Kopka, P. Dörmann, T. Altmann, R. Trethewey, L. Willmitzer, Nature Biotechnology 18, 1157.
[2] J. Lisec, N. Schauer, J. Kopka, L. Willmitzer, AR. Fernie, Nature Protocol 1: 387.
[3] U. Roessner, C. Wagner, J. Kopka, R. Trethewey, L. Willmitzer, The Plant Journal 23(1) (2000) 131.

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ACCURATE AND RELIABLE DETERMINATION OF CAROTENOIDS IN APPLE FRUITS AND JUICES BY HPLC-PDA

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Carotenoids are a group of phytochemicals that prevent the risk of lifestyle-related diseases, including cancer due to their antioxidant properties. In human diet, they are present in many foodstuffs of both animal and plant origin, but principally in fruits and vegetables[1,2]. A large amount of studies have lately been published describing different methods to quantify carotenoids in different fruits[3]. Apples are consumed worldwide and have been related as a source of carotenoids . This study, we has been focused on the development of an analytical methodology that allows the characterization of the carotenoid profile of different varieties of fresh apple fruits and commercial juices.

The method that has been established for the identification and quantification of carotenoids in apple uses liquid chromatography with an ultraviolet-diode array detector. Compounds were tentatively identified by congruent retention times and UV-vis spectra with those of standards. For compounds with no commercial standard the identification was carried out by comparison with the following parameters: maximum λ values, spectral fine structure and elution order according to similar chromatographic systems.

The main xanthophylls found in apples were violaxanthin, neoxanthin, luteoxanthin, lutein, zeaxanthin, antheraxanthin and β-cryptoxanthin and the hydrocarbon β-carotene. The presence of *cis-trans* isomeric forms worsens the chromatographic separation. In this work, we were able to resolve the geometrical isomers of violaxanthin and neoxanthin and to ensure their identification by mass spectrometry.

Usually the extraction of carotenoids requires the use of environmentally hazardous organic solvents and long extraction times. We have used an extraction procedure that has been optimized and includes a saponification step in order to release the esterified xanthophylls. Thus, the developed method allowed recoveries of carotenoids around 90 %.

[1] P. Astorg, Trends in Food Science & Technology 8 (1997) 406.
[2] N.I. Krinsky, E.J. Johnson, Molecular Aspects of Medicine 26 (2005) 459.
[3] M.G. Dias, L. Oliveira, M.F.G.F.C. Camöes, B. Nunes, P. Versloot, P.J.M. Hulshof, J. Chromatogr. A 1217 (2010) 3494.

NDE10

CHARACTERIZATION OF TRIAMCINOLONE ACETONIDE METABOLITES IN HUMAN URINE FOR DOPING CONTROL PURPOSES

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Glucocorticosteroids are included in the list of banned substances in sports of the World Antidoping Agency. They are prohibited when administered by oral, intravenous, intramuscular or rectal routes. However, inhalation, intra-articular administration and topical preparations are allowed. Therefore, the distinction between different routes of administration through the analysis of urine samples is needed in antidoping control. For some corticosteroids (e.g. budesonide), different administration routes can be distinguished by setting thresholds for the concentrations of particular metabolites, proving that a good knowledge of the metabolism is needed to look for differences depending on the route of administration. The aim of this study was to investigate the metabolism of triamcinolone acetonide (TA).

TA was administrated to two healthy male volunteers by intramuscular injection (20 mg). Urine samples were collected periodically up to 6 days after administration. Samples were hydrolyzed with β-glucuronidase enzymes and subjected to liquid-liquid extraction with ethyl acetate in alkaline conditions. The extracts were analysed by liquid chromatography coupled to tandem mass spectrometry. Precursor ion scan methods (m/z 121, 147, 171) and neutral loss methods (20 and 38 Da) were applied for the open detection of TA metabolites. Two main compounds, TA and 6β-hydroxy-TA, and 6 minor metabolites were detected using those methods. Mass spectra of TA and those metabolites which are commercially available were studied in detail in order to find selective and specific and sensitive transitions for TA metabolites. Characteristic neutral losses were observed in positive (58 Da, 44 Da) and negative electrospray ionization (142 Da). Based on these experiments, multiple reaction monitoring methods were applied to urine extracts and three additional metabolites were detected. Some of the metabolites were characterized by comparison with standards of the compounds (TA, 6β-hydroxy-TA, and triamcinolone). Other metabolites were characterized using mass spectrometric data. Metabolites resulting from 6,7-dehydrogenation, hydroxylations, oxidation of the 11-hydroxyl group, and reduction of the 20-keto group were detected. Some of them have not been previously described.

DEVELOPMENT OF A CARBOHYDRATE SILYLATION METHOD IN ROOM TEMPERATURE IONIC LIQUIDS

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Room temperature ionic liquids (RTILs) are low melting point salts which exist in liquid phase at or below room temperature [1]. They possess unique and attractive properties such as low volatility, variable viscosity and miscibility, chemical and thermal stability, etc. In recent times, there is a high interest in the use of RTILs for different chemical reactions, including carbohydrate processing [2-3], being considered a possible recyclable alternative to traditional volatile organic solvents [4].

The analysis of carbohydrates dissolved in RTILs has commonly been carried out by HPLC-IR [4-5]. However, the low sensitivity and resolution of this technique make the development of new methods a required task for the analysis of complex mixtures of carbohydrates. GC could be an appropriate technique for this purpose; a previous derivatization step being mandatory. Silylation is the most common procedure used for the derivatization of carbohydrates [6]; however, the reaction efficiency needs to be evaluated in RTILs. In this work, a silylation method for different mono-, di- and trisaccharides in RTILs has been optimised.

1-Ethyl-3-methylimidazolium dicyanamide ([emim][dca]) was selected among different RTILs as it appears to be suitable to dissolve monosaccharides [7-8]. Different silylation reagents (TMSI, HMDS, HMDS+TMCS, BSTFA+1%TMCS, BSTFA, BSA) and operating conditions (time, temperature, stirring, use of ultrasounds (US), etc.) were evaluated. Phenyl-□-D-glucoside and octadecane were used as internal standards. Derivatised carbohydrates were recovered in heptane and analysed using a HP 7890 gas chromatograph coupled to a flame ionization detector, using nitrogen as carrier gas. A capillary column 35% dimethyl-65% diphenypolysiloxane (30 m x 0,25mm x 0,10µm*df*) was used.

An effective silylation of carbohydrates was obtained when TMSI, BSTFA+1%TMCS or HMDS+TMCS (3:1) were used as reagents. In all cases, 30 min at room temperature were enough to achieve the appropriate derivatization. No significant differences were observed when the reaction was carried out in static conditions, under vortex agitation or submitted to US, the three procedures being suitable for the silylation of mono-, di- and trisaccharides. The optimized method can be of general application for the derivatization of carbohydrates in RTILs before their GC analysis.

[1] T. Welton, Chem. Rev. 99 (1999) 2071.
[2] H. Zhao, S. Xia, P. Ma, J Chem Technol Biotechnol 80 (2005) 1089.
[3] V. Vičkačkaitė, A. Padarauskas. Cent. Eur. J. Chem. 10 (2012) 652.
[4] A.A. Rosatella, L.C. Branco, C.A.M. Afonso, Green Chem. 11 (2009) 1406.
[5] A.P. Carneiro, O. Rodríguez, E.A. Macedo, Fluid Phase Equilib. 314 (2012) 22.
[6] A.I. Ruiz-Matute, O. Hernández-Hernández, S. Rodríguez-Sánchez, M.L. Sanz, I. Martínez-Castro, J Chromatogr B, 879 (2011) 1226.
[7] Q. Liu, M.H.A. Janssen, F. van Rantwijk, R.A. Sheldon, Green. Chem 7 (2005) 39.
[8] M.E. Zakrweska, E. Bogel-Yukasik, R. Bogel-Yukasik, Energy Fuels 24 (2010) 737.

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COSMETICS SURVEY FOR ALLERGENS AND PRESERVATIVES USING PRESSURIZED SOLVENT EXTRACTION (PSE) AND MATRIX SOLID-PHASE DISPERSION (MSPD)

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The safety of ingredients is a top priority for the cosmetics industry. The free circulation of cosmetic products in the market and the safety of cosmetics placed on it have to be ensured and guaranteed by the respective governments. In the European Union context, a new Cosmetic Products Regulation [1] has been adopted in order to strengthen certain elements of the regulatory framework for cosmetics, such as in-market control, with a view to ensuring a high level of protection of human health. This Regulation is already into force, and it will apply from 11th July 2013. Among the new features, Article 19 from Ch. VI is dedicated specifically to "labeling" and directly affects the groups of ingredients selected in this study.

In this context, a survey of a variety of cosmetics for some of the target ingredients was conducted. Different leave-on and rinse-off cosmetics from national and international brands, covering a wide range of prices, were purchased from local shops, supermarkets, department stores and pharmacies. They included products intended for baby and child care. The analytical methods implemented for the cosmetic samples (based on PLE and MSPD) were previously optimized and validated for suspected allergens [2,4] and multiclass preservatives [3,5] in our research group. Twenty-five suspected fragrance allergens and 13 multiclass preservatives were considered in this study.

In summary, in the analyzed leave-on samples, 8 out of the 25 SAs were no detected, whereas about the 35% of the samples did not contain any of the analyzed suspected fragrance allergens. The rest of the leave-on samples contained an average of 5 fragrance allergens per sample. Seven out of 25 SAs were not detected in the rinse-off samples analyzed; only 2 samples did not contain any fragrance allergen, and only 2 samples included just one allergen each. The rest of the samples contained 2 to 7 SAs, with an average of 5 allergens per sample.

Regarding the multiclass preservatives, in the case of leave-on cosmetics, 80% of the samples contained at least one preservative and the third of the samples presented 5 of the target preservatives. Parabens were the most abundant preservatives and 5 preservatives were not detected in any sample. Only 4 out of 28 samples did not contain any of the targets. The presence of parabens in the rinse-off cosmetics is less frequent than in leave-on cosmetics; only 8 samples contained at least 1 paraben and among these samples the average content was 3 parabens per cosmetic product.

Both PLE and MSPD methods are adequate to verify that marketed personal care products comply with currently in force EU legislation, since the obtained LOQs are well below the limit set on it as regards labeling requirements. Finally, the degree of compliance with the regulation on the labeling has been also evaluated in all the leave-on and rinse-off samples.

[1] Official Journal of European Union, L342 (2009) 59
[2] J.P. Lamas, L. Sanchez-Prado, C. Garcia-Jares, M. Lores, M. Llompart, J Chromatogr A 1217 (2010) 8087.
[3] L. Sanchez-Prado, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Chem. 82 (2010) 9384.
[4] L. Sanchez-Prado, J.P. Lamas, G. Alvarez-Rivera, M. Lores, C. Garcia-Jares, M. Llompart, J. Chromatogr., A 1218 (2011) 5055.
[5] L. Sanchez-Prado, G. Alvarez-Rivera, J. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Bioanal. Chem. 401 (2011) 3293.

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ANALYTICAL STRATEGY BASED ON THE USE OF GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT AND HYBRID QUADRUPOLE TIME-OF-FLIGHT MS ANALYZERS TO INVESTIGATE POTENTIAL POLYMERIC MIGRANTS INTO FOOD SIMULANTS

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Packaging is one of the main ways to conserve foodstuff. However, migration of chemical substances from packaging to food may have a negative impact on the quality of products with some toxicological implications on human health. For this reason, the interaction between packaging and food has to be studied, assessed and controlled according to European Regulation No. 1935/2004 (EU, 2004,) which establishes the main principles about materials and articles intended to come in contact with food.

Recent European Regulation No. 10/2011 (EU, 2011) gives a list of monomers and additives that are permitted in the manufacture of plastic packaging. However, it is important to take also into account other substances not included in the target lists that may be produced by authorized initial reactants and additives: polymerization and degradation compounds, impurities and non-intentionally added substances (NIAS).

Until now, GC-MS and LC-MS with quadrupole and time-of-flight (TOF) analyzers have been used to determine particular families of migrants. Only few studies have reported multiresidue analysis covering several types of potential migrants [1]. Most of developed methods are focused on target analysis and only a limited list of potential contaminants is monitored. This work aims to investigate potential GC-amenable migrants from plastic packaging to food simulants using TOF and hybrid quadrupole time-of-flight (QTOF) mass analyzers. Using these high resolution techniques, accurate-mass full-spectrum acquisition data at high sensitivity are provided, highly useful for screening and identification/elucidation purposes. Two ionization techniques, the traditional electron ionization source (EI) and the atmospheric pressure chemical ionization source (APCI), recently developed for GC, have been complementary used for detection and reliable identification of potential detected migrants. In a first step, GC-(EI) TOF MS has been used for the identification of the compounds by library matching and accurate mass measurements. In a second step, the soft ionization promoted by GC-APCI-QTOF MS has been used for confirmation of the identity of the potential compounds, as the presence of the molecular ion and/or protonated molecule is very valuable for identification and confirmation [2]. Some reference standards have been subsequently acquired and injected for the final unequivocal identification and confirmation of the compounds detected.

The samples were supplied by a local provider and they are the usual material structure for ready-meals stored at ambient temperature. Migration tests were carried out in a specific conditions of time and temperature between the plastic material and the simulant food established by the European Committee for Standardization (CEN).[3].

This work shows that the combined use of GC-(EI)TOF MS and GC-(APCI)-QTOF MS is a powerful and promising approach for the identification and confirmation of NIAS in food packaging materials.

[1] Skjevrak I, Brede C. Food additives and Contaminants, 22 (2005) 1012.
[2] Portolés T, Pitarch E, López Francisco J, Hernández F, Niessen Wilfried M.A. Rapid Commun. Mass Spectrom. 25 (2011) 1589.
[3] BS EN 13130-1:2004; "Materials and articles in contact with foodstuffs. Plastic substances subject to limitation. Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants"

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POLYMERIC IMIDAZOLIUM IONIC LIQUIDS AS SORBENTS FOR SOLID PHASE MICROEXTRACTION

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The chemical synthesis of two monomers, namely 3-(but-3''-en-1''-yl)-1-[2'-hydroxycyclohexyl]-1*H*-imidazol-3-ium bis(trifluoromethanesulfonyl)imide and 1-(2'-hydroxycyclohexyl)-3-(4''-vinylbenzyl)-1*H*-imidazol-3-ium bis(trifluoromethylsulfonyl)imide, has been previously described [1]. Due to these two ionic liquids have appropriate alkene functionality, which allows their polymerization, were subjected to either a free radical polymerization withazobisisobutyronitrile (AIBN). So, twopolymeric ionic liquids have been synthesized and used as coatings for solid-phase microextraction (SPME).

A 2^3 two-level full factorial design (FFD) was performed to investigate the effects of extraction time, temperature and equilibration time, in order to obtain the maximum sensitivity. Low and high levels were selected based on preliminary tests. The order of the experiments was fully randomized to avoid possible memory effects of the analytical apparatus. This experimental design allows the evaluation of the effects of the main factors and their interactions. Sodium chloride (3 g) and stirring speed (900 rpm) were fixed. In all cases, data analysis was performed by means of the statistical package Statgraphics Centurion XV for Windows Version 15.2.06 manufactured by Statpoint Technologies (Warrenton, VA, USA).

These new fibers exhibit good film stability, high thermal stability and long lifetimes and were used for the extraction of volatile compounds in different samples.

[1] J. Gónzalez-Álvarez, D. Blanco-Gomis, P. Arias-Abrodo, D. Díaz-Llorente, E. Busto, N. Ríos-Lombardía, V. Gotor-Fernández, M. D. Gutierrez-Álvarez, Anal. Chim. Acta 721 (2012) 173.

A FAST AND SELECTIVE METHOD FOR THE DETECTION OF DIAZEPAM IN URINE BY MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION

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Benzodiazepines are a large class of drugs, widely prescribed as anxiolytic, hypnotic, anticonvulsant, myorelaxant and amnesic agents [1, 2]. They are included in the World Health Organization Model List of Essential Medicines (WHO EML) and are the medications most commonly used for the treatment of adult individuals with Generalised Anxiety Disorder (GAD) [3]. Treatment with benzodiazepines is recommended in the short-term only. Long term use may induce dependence and withdrawal symptoms. The use of benzodiazepines during the first trimester of pregnancy has been associated with congenital malformations in the infant [4]. Apart from their therapeutic applications, benzodiazepines are considered common abused drugs, and they are frequently involved in clinical and forensic cases.

In this research, several specific molecularly imprinted polymers (MIPs) for diazepam were synthesized using methacrylic acid (MAA) as functional monomer and different crosslinking agents and conditions of synthesis. Polymers I were synthesized using ethyleneglycol dimethacrylate (EGMA) as crosslinked and acetonitrile as solvent by photochemical (UV irradiation at 365 nm) or thermal (oven at 60ºC) initiation. In Polymers II, divinyl benzene was used as crosslinked and toluene-acetonitrile (3:1) mixture as solvent. Precipitation polymerization was carried out with agitation [5].

The interaction forces between the analyte and the polymer are driven by hydrogen bonding and the solvent plays an important role in the recognition step. To determine the effect of solvent on the diazepam MIP, the imprinted and non-imprinted polymers were conditioned, loaded and eluated with different solvents and volumes. Scanning Electron Microscope (SEM) images, Freundlich and Langmuir isotherms were used for the characterisation of polymers.
Synthesised MIPs have been used to extract diazepam from urine samples via molecularly imprinted solid-phase extraction (MISPE) protocol. Recent studies have demonstrated that the MISPE method has a higher selectivity than the SPE method, decreasing matrix interferences [6]. The quantification of diazepam was carried out by the mean of HPLC-DAD. The MISPE methodology is an attractive tool for extracted diazepam in urine and is applicable to the analysis of diazepam.

[1] P. P. Roy-Byrne, D. S. Cowley, “Benzodiazepines in Clinical Practice: Risks and Benefits”, American Psychiatric Press, Washington, DC., 1st edition, 1991.
[2] S. C. Sweetman, “Martindale: The Complete Drug Reference”, Pharmaceutical Press, London, 36th edition, 2009.
[3] World Health Organization, “Model List of Essential Medicines”, Geneva, Swiss, fifteenth edition, 2007, available from http://whqlibdoc.who.int/hq/2011/a95053_eng.pdf (last accessed on June 2012).
[4] World Health Organization, “17th Expert Committee on the Selection and Use of Essential Medicines Proposal”, Geneva, Swiss, available from http://www.who.int/entity/selection_medicines/committees/expert/17/application/Section24_GAD.pdf (last accessed on June 2012).
[5] R. Joso, E.H. Pan, M.H. Stenzel, T.P. Davis, C. Barner-Kowollik, L. Barner. J. Polym. Sci., Part A: Polym. Chem 45 2007 3482.
[6] R.A. Anderson, M.M. Ariffin, P.A.G. Cormack, E.I. Miller. Forensic Sci. Int. 174 (2008) 40.

PERFORMANCE OF LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF CYTOSTATIC COMPOUNDS IN WASTEWATER

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According to the high incidence of cancer worldwide, the amount of cytostatic drugs administered to patients has increased. These compounds are excreted to wastewaters, and therefore, become potential water contaminants. At this stage, very little is known on the presence and elimination of cytostatic compounds in Wastewater Treatment Plants (WWTP). The aim of this study was to develop a liquid chromatography-high resolution mass spectrometry (LC-Orbitrap-MS) method for the determination of the outmost used cytostatic compounds in wastewaters. Extraction and analytical conditions were optimized for a set of cytostatic compounds in wastewater. Both solid phase extraction (SPE) using Oasis 200 mg HLB cartridges and direct injection analysis were evaluated. Mass spectral characterization and fragmentation conditions were optimized at 50,000 resolving power (full width at half maximum, *m/z* 200) to obtain maximum sensitivity and identification performance. Quality parameters (recoveries, limits of detection, repetitivity) of the methods developed were determined and best performance was obtained with direct water analysis of the centrifuged wastewater. Finally, this method was applied to determine the presence of cytostatic compounds in wastewaters from a hospital and urban effluents and influents and effluents from 3 WWTP. The analysis of wastewaters using direct injection analysis revealed the presence of cyclophosphamide and epirubicin in WWTP influents and hospital and urban effluents at μg L^{-1} levels. However, no traces were observed in WWTP effluents. The results obtained in this study demonstrate the capability of LC-Orbitrap-MS for the accurate trace analysis of these very polar contaminants.

DETERMINATION OF COTININE IN URINE OF AS BIOLOGICAL MARKER OF TOBACCO USING SPE AND HPLC-DAD

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Smoking is considered one of the main risk factors for lung cancer and cardiovascular and respiratory diseases, and the World Health Organization has declared the tobacco smoke as the second leading cause of death worldwide [1]. In humans, about 70–80% of nicotine is converted to cotinine (COT) [2] by hepatic cytochrome P450 (CYP2A6). In order to measure exposure to environmental tobacco smoke, we analyze the levels of cotinine. Cotinine can be measured in urine, and it is considered the best indicator of exposure to environmental smoke.

A sensitive, simple and low-cost method based on solid phase extraction and HPLC-DAD detection was developed for the determination of cotinine in urine. The chromatographic separation was obtained with a column Synergi Polar Phenomenex at 25ºC, which allowed us to retain the cotinine without using an organic modifier, and elute it with enough retention time to separate it from the specific interferences of the urine. Previous to chromatographic analysis, the urine sample was submitted to purification by solid phase extraction (SPE). The sample was eluted with dichloromethane (DCM), this allowed to obtain a clean residue. Then it was dried under nitrogen gas. The dried product was reconstituted with 100 μL 15% acetonitrile-85% water and 25 μL aliquot was injected. To control the extraction process, 2-phenilimidazol was used as internal standard. The eluate was detected at a wavelength of 259 nm.

We obtained a detection limit of 12 μL mL^{-1} and a recovery of 97%. The linearity range used was from 25 to 4000 μL mL^{-1}.

The method was successfully used for the determination of cotinine in urine samples collected at different volunteers and allowed distinguish between smokers and non-smokers.

[1] WHO (2012) WHO global report: mortality attributable to tobacco. WHO.
[2] N.L. Benowitz, J.Hukkanen, P. Jacob Handb. Exp. Pharmacol. 192 (2009) 29.

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POSTERS

OMT

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OMTO1

UTILITY OF URINE SAMPLES EXPOSED TO SUBOPTIMAL CONDITIONS FOR METABOLOMIC STUDIES

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Sample collection and storage conditions are crucial factors for the stability of urinary metabolites. With the expansion of metabolomics to the clinical domain, however, sample collection conditions cannot be controlled as tight as in the laboratory setting. A typical example is sample collection outside of a hospital from patients with restricted mobility. Consequently, a question arises: should such samples be discarded completely or should we try to explore whether they still retain some physiologically relevant information. To this end, we analyzed 117 urine samples that were collected in two different conditions: at the clinical setting under optimal conditions or by patients at home followed by post delivery to the clinical setting. All samples were analyzed by liquid chromatography – mass spectrometry (LC-MS) and proton nuclear magnetic resonance (NMR) [1, 2].

As expected, multivariate modelling revealed clear differences between the two sampling condition for both LC-MS and NMR data sets. However, the sets of the differential metabolites appeared to be platform-specific, which clearly emphasizes the complementary nature of NMR and LC-MS [1]. NMR identified carboxylic acids related with bacterial metabolism. MS and MS2 data were matched against metabolite libraries leading to the identification of carboxylic acids, peptides and urea and nucleoside derivatives related with bacterial metabolism and time-dependent chemical transformations of urine. The further analysis of the samples that were exposed to suboptimal conditions revealed that age and body mass index remain as dominant traits of the metabolic profile, although their influence was stronger for LC-MS data.

In conclusion, we have shown that urine samples exposed to suboptimal conditions have different metabolic profile, but, they still retain useful physiological information. Thus, those samples can still be used for metabolomic studies with careful interpretation of the results.

[1] E. Nevedomskaya, O.A. Mayboroda, A.M. Deelder, Mol Biosyst, 7 (2011) 3214-3222.
[2] T. Pacchiarotta, P.J. Hensbergen, M. Wuhrer, C. van Nieuwkoop, E. Nevedomskaya, R.J. Derks, B. Schoenmaker, C.A. Koeleman, J. van Dissel, A.M. Deelder, O.A. Mayboroda, J Proteomics, 75 (2012) 1067-1073.

OMT02

EFFECT OF DIETARY POLYPHENOLS ON K562 LEUKEMIA CELLS: A FOODOMICS APPROACH

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In this work, a global Foodomics strategy has been applied to study the anti-proliferative effect of dietary polyphenols from rosemary on two human leukemia lines, one showing a drug-sensitive phenotype (K562), and another exhibiting a drug-resistant phenotype (K562/R). To this aim, whole-transcriptome microarray together with a MS-based non-targeted analytical approach (via capillary electrophoresis-time of flight mass spectrometry, CE-TOF MS, and ultrahigh performance liquid chromatography-time of flight mass spectrometry, UPLC-TOF MS) have been employed to carry out Transcriptomics and Metabolomics analyses, respectively. Functional enrichment analysis was done using Ingenuity Pathway Analysis (IPA) software as a previous step for a reliable interpretation of transcriptomic and metabolomic profiles. Rosemary polyphenols altered the expression of ~1% of the genes covered by the whole-transcriptome microarray in both leukemia cell lines. Overall, differences in the transcriptional induction of a number of genes encoding phase II detoxifying and antioxidant genes, as well as differences in the metabolic profiles observed in the two leukemia cell lines suggest that rosemary polyphenols may exert a differential chemopreventive effect in leukemia cells with different phenotypes. IPA predictions on transcription factor analysis highlighted inhibition of Myc transcription factor function by rosemary polyphenols, which may explain the observed antiproliferative effect of rosemary extract in the leukemia cells. Metabolomics analysis suggested that rosemary polyphenols affected differently the intracellular levels of some metabolites in the two leukemia cell sublines. Integration of data obtained from Transcriptomics and Metabolomics platforms was attempted by overlaying datasets on canonical (defined) metabolic pathways using IPA software. This strategy enabled the identification of several differentially expressed genes in the metabolic pathways modulated by rosemary polyphenols providing more evidences on the effect of these compounds.

OMTO3

DEVELOPMENT OF A NEW METHOD FOR LIPID PROFILING OF YEAST CELLS USING GC-MS

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Recent rapid growth of lipidomics is mainly attributed to technological advances in mass spectrometry. Development of soft ionization techniques, in combination with computational tools, has spurred subsequent development of various methods for lipid analysis [1]. However, none of these existing approaches can cover major cellular lipids even in vegetable, animal or human cells.

The description of lipid profile of yeasts have an special interest for wine industry, even so, yeast is better known to be a powerful experimental system to study biochemical, cell biological and molecular aspects of lipid synthesis because most bioshynthetic routes of lipids in yeast are similar to those in higher eukaryotes[2].

Although, a few methods exist to profile yeast lipid content, generally the devices for this analyze, such as ESI-MS, MALDI-MS, APCI-MS and so on, are inaccessible to be used in normal laboratories as a routine research for their cost and complexity.

A gas chromatography coupled with mass spectrometry (GC-MS) new protocol was created. The method was based in previous reports [3], in order to analyze yeast samples. Here, also, we demonstrate that there is the possibility of combine different techniques in order to get the hole lipidomic approach. For instance, HTLC/TLC has been used for ages to analyze lipidomic profile. However, it used to be tedious and high time-consuming. In the present work these techniques have been optimized and combined with our new CG-MS method to have a single chromatogram for each group of lipid compounds.

The aim of this work was to optimize a method for GC-MS to cover majour yeast lipids in a single run; the protocol is very simple; briefly from a lipid extract obtained by the classical methodology of biphasic system and lower sample layer (non-polar) was directly injected into CG-MS. This method permits us to detect free fatty acids, squalene, intermediary of biosynthesis pathway of sterols and molecular species of diacylglycerides and triacylglycerides in one chromatogram.

To validate the method; yeast growth in two different conditions (in presence o absence of oxygen during the culture) were evaluated. The oxygen is need to synthesize yeast lipids, therefore, some different were expected to be changed in a quantitative and even qualitative form. Analyze confirm those differences and show an example of application of this new protocol.

[1] G. Shui, X. Li Guan, C. Pei Low, G.Huey Chua, J.Sze Yuin Goh, H. Yang, M.R. Wenk, Mol. BioSyst. 6 (2010) 1008.
[2] G. Daum, N.D. Less, M. Brad, R. Dickson, Yeast 14 (1998) 1471.
[3] M.A. Quail and S.L. Kell,. The extraction and analysis of sterols from yeast. Yeasts protocols: Methods in molecular biology 1996 vol. 53-chapter 14.

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OMTO4

CONTROLLED EXPERIMENT FOR EVALUATION OF STATISTICAL MODELS FOR DATA ANALYSIS IN METABOLOMICS

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A typical metabolomics experiment generates large data sets, due to the complexity of biological samples and the high sensitivity of modern analytical techniques such as mass detectors coupled to separation systems, which can generate several hundreds/thousands of variables per sample. The manipulation of these data sets is one of the challenging steps of a study in metabolomics, and it determines which metabolites present in an organism are candidates as potential biomarkers. Therefore, data processing step acts directly on the results generated in a study.

Works in the area of chemometrics have contributed to the interpretation of experiments that generate a lot of data for analysis [1], especially multivariate statistics [2] which includes tools such as principal component analysis (PCA), and discriminate analysis with partial least squares regression (PLS-DA), that are used extensively in studies of metabolomics. Other works point to important observations about the pre-processing of data in multivariate analysis in metabolomics [3, 4]. The present work discusses some changes in the selection of variables as potential biomarkers caused by changes in the stage of data processing.

To evaluate these modifications a controlled experiment was designed. Samples (n=30) contained equal amounts of a pool of urine and 20 standards of metabolites in 5 different concentrations, 13 of them changed randomly keeping the average in the two groups equal, while 7 increased or decreased classifying the samples into two groups . After that, samples were analyzed by capillary electrophoresis with time of flight mass detector (CE-TOF), under conditions previously described [5]. The profiles of the 30 electropherograms were aligned in MassProfiler Professional software (Agilent Technologies), and subjected to different processes of transformation, scaling of data, and treatment of missing values in PCA and PLS-DA models, analyzed in SIMCA-P+ software (Umetrics) and the R platform (open-source).

Data analysis showed significant differences when working with raw data or log transformed data, and scaling type unit variance (UV) or variance type Pareto (Par), modifying the estimates of covariance and errors associated with the variables of the PLSDA models. In addition, the treatment of missing values directly influenced the quality of the PCA models and metabolite concentrations between the groups of individuals prepared in the experimental design.

[1] R. Madsen, T. Lundstedt, J. Trygg, Anal. Chim. Acta 659 (2010) 23.
[2] K.H. Liland, TrAC Trends Anal. Chem., 30 (2011) 827.
[3] M. Katajamaa, M. Oresic, J. Chromatogr. A 1158 (2007) 318.
[4] M.M.W.B. Hendriks, F.A. Eeuwijk, R.H. Jellema, J.A. Westerhuis, T.H. Reijmers, H.C.J. Hoefsloot, A.K. Smilde, TrAC Trends Anal. Chem., 30 (2011) 1685.
[5] J.Godzien, D. Gracía-Martínez, P. Martinez-Alcazar, F.J. Ruperez, C. Barbas, Metabolomics (2011).

OMTO5

METABOLOMIC APPROACH TO EVALUATE LIVER GROWTH FACTOR (LGF) EFFECT IN MICE WITH EMPHYSEMA CAUSED BY TOBACCO SMOKE EXPOSURE

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Tobacco smoke exposure is the principal cause of lung tissue destruction, which in turn results in emphysema that leads into shortness of breath. This injury and chronic bronchitis are both included into the term COPD (chronic obstructive pulmonary disease) which is a major reason of mortality and chronic morbidity in the world [1].

Liver growth factor (LGF) is an albumin-bilirubin complex with antifibrotic and antioxidant properties [2] that could be useful to promote lung tissue regenerating capacity in damaged lungs.

The current study has examined differences in metabolite profiles (*fingerprints*) of plasma from mice (strain C57I/6J, susceptible to emphysema) exposed to tobacco smoke during six months, part of them received a treatment with liver growth factor (group T + LGF), whereas the other group did not receive the treatment (group T). Age and sex-matched mice that were not exposed to smoke were maintained with or without treatment (groups C, C + LGF).

Metabolic fingerprints (untargeted analysis) of plasma after protein precipitation were obtained by RP-HPLC coupled to QTOF MS detector [3]. The signals (raw data) were processed with MassHunter Qualitative (Agilent Technologies) in order to obtain a list of entities (features) that were aligned, filtered, and further processed by MassProfiler Professional. Multivariate data analysis with SIMCA-P+ (Umetrics) using orthogonal partial least squares discriminant analysis (OPLS-DA) of the most significant metabolites provided models that highlighted the differences between control and smoke exposed mice in both conditions (C *vs* T and C + LGF *vs* T + LGF).

Accurate masses of features representing significant differences were searched through online public databases. Among the metabolites putatively identified as markers of the different conditions, it has been possible to find changes in molecules related to oxidative stress, oxidation metabolism and the adequate structure of the surface of the lung, which are strongly related to tobacco diseases [1,4].

[1] GOLD - Global Initiative for Chronic Obstructive Lung Disease, 2011. www.goldcopd.org
[2] L. Condezo-Hoyos, F. Abderrahim, M.V. Conde, C. Susín, J.J. Díaz-Gil, M.C. González, S.M. Arribas, Free Radical Biology and Medicine 46 (2009) 656.
[3] M. Ciborowski, J. Teul, J.L. Martin-Ventura, J. Egido, C. Barbas, PLoS One 7 (2012) e31982.
[4] G.D. Boutten A, Boczkowski J, Bonay M, Med Pregl, 2005 Sep-Oct.

OMTO6

A FINGERPRINTING BASED METABOLOMIC STUDY COMBINING GC-MS AND LC-MS TO INVESTIGATE SLEEP APNEA AND HYPOPNEA SYNDROME

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Sleep apnea and hypopnea syndrome (SAHS) refers to momentary, often cyclical cessations in breath rhythm (apneas) or momentary or sustained reductions in breath amplitude (hypopnea), with or without complete or partial obstructions of the upper airway [1]. This syndrome is the second disease in order of frequency among respiratory disorders, surpassed only by asthma and is quickly becoming a major cause of morbidity and the most common medical cause of daytime sleepiness. Despite the increasing number of scientific contributions in these areas, more efforts are necessary to improve the knowledge about multicomponent aspects of SAHS. Metabolomics by fingerprinting represents a good approach for monitoring, in a non-target manner, the main changes [2] connected to the pathophysiological mechanism of SAHS and also a valuable strategy to identify useful markers in disease diagnosis. Due to the wide structural diversity of those metabolites, different analytical platforms are necessary to capture the entire metabolome of a tissue [3]. Mass spectrometry used in combination with liquid (LC-MS) and gas chromatography (GC-MS) is an excellent tool to fulfill this purpose [4]. Hence, these techniques were applied to study the relative plasma metabolic fingerprints of early-mild to severe SAHS patients and healthy subjects as controls. Proper and precise sample pre-treatment are required to extract metabolites and achieve reproducible data, thus samples were prepared according to strict protocols in our lab as previously described [5, 6]. After deproteinization 36 patients' and 16 controls' samples were analyzed using LC-MS with a quadrupole-time of flight detector. Molecular feature extraction was performed by Mass Hunter Qualitative Analysis software (MH, Agilent) and the list of features was filtered and aligned by Mass Profiler Professional (MPP, Agilent) software. Accurate masses of statistically significant features were searched against databases and isotopic pattern distribution was also studied to assess putative identification. GC-MS sample treatment, consisting of deproteinization, methoximation and silylation, permits to decrease compound polarity and increase volatility and the thermal stability of polar metabolites. The relative plasma fingerprints of 45 patients and 13 controls, obtained using GC-MS with quadrupole detector, were deconvoluted using AMDIS open source software and identified by comparing their mass fragmentation patterns with the information held in the NIST and Fiehn's libraries. For data modeling SAHS patients were divided in four groups, according to the degree of severity. Both multivariate (MVDA) and univariate statistical analyses were performed to screen potential biomarkers. The high separation and prediction capability of MVDA models permitted to achieve a discriminant classification of the samples. Considerable differences in individual fingerprints of SAHS patients were highlighted by changes in the plasma level of phospholipids, endocanabinoids, branched-chain amino acids and carnitines. In addition, other identified metabolites could represent potential biomarkers of SAHS. These findings give an important contribution to the knowledge of SAHS, improving the capability to clarify its multicomponent aspects and permitting the identification of novel biomarkers of the disease.

[1] J.A. Dempsey, S.C. Veasey, B.J. Morgan, C.P. O'Donnell, Physiol. Rev. 90 (2010) 47.
[2] L.K. Schnackenberg, R.D. Beger, Pharmacogenomics 7 (2006) 1077.
[3] T. Kind, G. Wohlgemuth, d.Y. Lee, Y. Lu, M. Palazoglu, S. Shahbaz, O. Fiehn, Anal. Chem. 81 (2009) 10038.
[4] V. Shulaev, Brief Bioinform 7 (2006) 128.
[5] M. Ciborowski, J. Teul, J.L. Martin-Ventura, J. Egido, C. Barbas, PLoS One 7 (2012) e31982.
[6] G. Antonia, B. Coral, Gas chromatography-mass spectrometry (GC-MS)-based metabolomics, 2011.

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OMTO7

ANALYSIS OF RECOMBINANT HUMAN ERYTHROPOIETIN GLYCOPEPTIDES BY CAPILLARY ELECTROPHORESIS ELECTROSPRAY-TIME OF FLIGHT-MASS SPECTROMETRY

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Erythropoietin (EPO) is a hormone produced mainly by the kidney involved in the physiological feedback mechanism that maintains red blood cell number and tissue oxygen supply at adequate levels [1]. Human EPO (hEPO) is an approximately 30 kDa glycosylated protein [1,2] that shows three complex type *N*-glycans (in Asn24, Asn38 and Asn83) and one *O*-glycan (in Ser126) attached to the polypeptide backbone. Recombinant human EPO (rhEPO) has been extensively used to treat certain forms of anaemia [2]. However, it has become especially popular due to its misuse in endurance sports disciplines, being forbidden, together with its analogues, by sport authorities since 1989 [3,4].

Since 2003, the official method approved by the World Anti-Doping Agency (WADA) for the detection of rhEPO and its analogues is the U*rine EPO Test* [3]. Nevertheless, this method is long, tedious and does not permit confirmation by mass spectrometry (MS). Therefore, new methods for a simple and rapid screening and confirmation of these agents in human urine and plasma are necessary not only to control their abuse in sports, but also to verify the quality of these biopharmaceutical products.

In this work, capillary electrophoresis electrospray-mass spectrometry has been used to detect and characterize the great variety of *O*- and *N*-glycopeptide glycoforms of recombinant human erythropoietin (rhEPO) using an orthogonal accelerating time-of-flight mass spectrometer to obtain their exact molecular masses (CE-TOF-MS) [5]. rhEPO was digested with trypsin and Glu-C and analyzed by CE-TOF-MS to detect O_{126}, N_{83}, N_{24}-N_{38} and N_{24} and N_{38} glycopeptide glycoforms, respectively. Neuraminidase was first used to enhance the detection of the glycopeptides and detect all possible glycoforms contained in each glycosylation site. O_{126} and N_{83} glycopeptides were extensively characterized. Twelve sialoforms corresponding to 5 different glycoforms were detected in N_{83}, and for the first time, a sulfated sialoform of this glycopeptide was also detected. In the case of O_{126}, different sialoforms with different types of sialic acids (Neu5Gc and Neu5Ac) were detected and a estimation of the relative percentage of Neu5Gc versus Neu5Ac was also carried out for this glycopeptide. N_{24} and N_{38} glycosylation sites were also characterized by CE-TOF-MS after Glu-C digestion and these results permitted to rule out some glycan combinations for N_{24}-N_{38} glycopeptide glycoforms. This study provided a reliable glycopeptide map of rhEPO and may be regarded as an excellent starting point to analyze rhEPO glycopeptides in biological fluids and detect the use of this hormone in sports.

[1] S.B. Krantz, Blood 77 (1991) 419.
[2] W. Fisher, Exp. Biol. Med. 228 (2003)1.
[3] F. Lasne, J. Ceaurriz, Nature 405 (2000) 635.
[4] J. Segura, J.A. Pascual, R. Gutiérrez-Gallego, Anal. Bioanal. Chem. 388 (2007) 1521.
[5] E. Giménez, R. Ramos-Hernan, F. Benavente, J. Barbosa, V. Sanz-Nebot, Rapid Commun. Mass Spectrom. 25 (2011) 2307.

OMTO8

APPLICATION OF HIGH RESOLUTION MASS SPECTROMETRY FOR THE CHARACTERISATION OF THERMAL TRANSFORMATION PRODUCTS OF QUINOLONES IN COW MILK

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Quinolones are antibiotics widely used in human and veterinary medicine. Its main use in veterinary is the treatment of infections but also can be their prevention (prophylactic use) or as a growth promoter although this last use, is forbidden for the European Union.

If a cow medicated is milked too soon, before all antibiotic and its metabolites are eliminated from the body, residues of the administered antibiotic and their metabolites can be found in the milk. On the other hand, milk is treated thermally before human consumption to eliminate pathogens microorganisms. These processes can affect the residues of antibiotics and their metabolites modifying their structures and given several transformation products (TPs).

In the last years, a lot of methods to the determination of residues of antibiotics have been developed. The presence of these residues is a potential risk on consumers because may have direct toxic effects as allergenic reactions or they may cause problems through induction of resistant strains of bacteria. In the case of metabolites and transformation products, there is unknown about how they affect to consumer health because these new compounds can be more persistent and harmful than the administrated drug.

In our work, some thermal processes were simulated and their effect was studied about four quinolones (enrofloxacin (ENR), ciprofloxacin (CIP), difloxacin (DIF) and sarafloxacin (SAR)) in cow milk. Using LC-ToF of high resolution and comparing the mass spectrums of spiked and blank milk, ions corresponded to different TPs were found. The structure assignation was obtained through the use of mass analyser LTQ-Orbitrap of high resolution, which allowed us make MS^n of the TPs and elucidate their structures.

[1] A. Junza, R. Amatya, D. Barrón, J. Barbosa. J. Chromatogr. B 879 (2011) 2601.
[2] M.P. Hermo, D. Barrón, J. Barbosa. J. Chromatogr.A, 1104 (2006) 132.
[3] P. Calza, C. Medana, F. Carbone, V. Giancotti, C. Baiocchi. Rapid Commun. Mass Spectrom. 22 (2008) 1533.
[4] B. Prasad, A. Garg, H. Takwani, S. Singh. Trends Anal Chem. 30 (2011) 360.
[5] A. Anadón, M.R. Martínez-Larrañaga, J. Iturbe, M.A. Martínez, M.J. Díaz, M.T. Frejo, M. Martínez. Res. Vet. Sci. 71 (2001) 101.

OMT09

SEARCH FOR MARKERS OF BLADDER CANCER WITH A METABOLOMIC APPROACH

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Metabolomics seeks to quantify small molecules that serve as physiological indicators within circulating biological fluids or tissues [1]. Measurement of limited biomarker compounds to diagnose a disease or monitor the efficacy of therapies is basically unsound and therefore more comprehensive snapshot of multiple metabolites must be taken. Such approach is commonly referred to as metabol(n)omics and allows to obtain a 'fingerprint' in a pathological condition and compare to a healthy model. After fingerprinting, specific markers of the diseases will be identified aiming both a biochemical interpretation and the finding of more accurate and specific sets of markers. Final aims are improving prognosis, diagnosis, and therapy monitoring tools. Analytical techniques are mainly separation techniques coupled to accurate mass spectrometry with chemical elucidation capabilities. In addition, dedicated informatic platforms are used for multivariate data analysis and metabolite identification leading to the discovery of specific disease markers.

Bladder cancer is more frequent in men than in women. Spain shows the highest bladder cancer incidence rates in men among European countries. The current use of cystoscopy for screening and detecting bladder cancer is invasive and expansive. Various urine based biomarkers have been used for this purpose with limited success [2]. Clinical studies addressed to determine appropriate markers that would allow establishing and interpreting the corresponding risk factors in a non-targeted approach through metabolic fingerprinting are necessary. This work aims looking for diagnostic markers of bladder cancer in urine with LC-QTOF-MS and CE-TOF-MS with previously developed methods according to well established developed methods in our group. As both techniques are complementary the varieties of metabolites to be detected become much higher.

Forty eight samples of urine from patients with bladder cancer were evaluated by LC-MS [3] and CE-MS [4]. Due to the high complexity of the disease, the samples were divided into four groups: patients with stable and high risk (SH); stable and low risk (SL); recurrent and high risk (HR); recurrent and low risk (HL). The diluted samples were diluted with water analyzed by LC-MS and CE-MS. Data were collected in positive ionization in full scan mode. Profiles were deconvoluted by Mass Hunter software respectively. Alignment and statistical treatment was performed with Mass Profiler Professional P7+ and SIMCA softwares. It was performed statistical analysis using SIMCA software.

Relevant metabolites were significantly different in the studied subgroups; their identification was performed with databases and confirmed by LC-MS/MS or by standards for those found with CE-MS. The models obtained with PLS-DA show a good separation between the groups, especially when it was compared patients with recurrent cancer of high and low risk. The biological interpretation of these findings showed some altered metabolic pathways.

[1] A.O. Caglayan, Int. CardioVasc. Thor. Surg.11 (2010) 779.
[2] H. J Issaq, *et al*., J. Urology 179. 6 (2008), 2422.
[3] J. Godzien, et al., J. of Proteome Res. 10 (2011) 837.
[4] E.P. Moraes, *et al*., Electrophoresis 32 (2011) 2055.

OMT10

METABOLOMIC STUDY OF STABLE vs UNSTABLE ATHEROSCLEROSIS PATIENTS SEARCHING FOR PROGNOSTIC MARKERS OF NEW CARDIOVASCULAR EVENTS

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Introduction: Many diseases with totally different clinical presentations are basically atherosclerosis related, and among them, myocardial infarction and stroke determine to large extent the mortality in Western style populations. Most of the acute manifestations of atherosclerosis share a common pathogenetic feature: rupture of atherosclerotic plaque [1]. Coronary arteries plaque disruption has been studied most extensively, and a number of correlations have emerged between the morphology of the culprit plaques, the degree of thrombus formation and types of ensuing ischemic coronary syndromes of patients. These observations have led to a concept of unstable atherosclerotic plaques: plaques with an unstable morphology giving rise to the onset of unstable coronary artery disease [2]. Finding differences in plasma among patients with atherosclerosis is the aim of this metabolomic study, searching for prognostic metabolites helpful for screening purposes.

Materials and methods: 38 plasma samples from patients with atherosclerosis were analyzed, 19 corresponded to stable atherosclerosis and 19 to unstable atherosclerosis that after the first event still had one or more cardiovascular events i.e., are recurrent patients. All of them formed a very homogeneous group in ages, genders, treatment, etc. Samples were analyzed by two analytical tools GC-Q-MS [3] and LC-QTOF-MS [4] with previously developed methods for obtaining as much signals as possible. Profiles were deconvoluted by AMDIS and Mass Hunter software respectively. Alignment and statistical treatment was performed with Mass Profiler Professional P7+ and SIMCA softwares.

Results and discussions: Initially by GC-MS 120 compounds were obtained, aligned and identified using the Fiehn library of metabolites, after statistical treatment 13 compounds were found that are significantly different in the two sample groups, these compounds can be divided into two classes: fatty acids and carbohydrates. As for LC-MS 1200 features were found 57 out of them were considered statistically different in the groups of samples, and they were submitted to different metabolite databases (Human Data Base, Metlin and MassTrix) where 38 of them could be identified. The compounds found by LC-MS can be divided into three main classes: carbohydrates, amino acids and some fatty acids and organic acids. These masses were also analyzed by MSxMS to confirm the identity. With the biological interpretation of these findings the altered metabolic pathways will be shown.

[1] E. Falk, Am. J. Cardiol. 63 (1989) 114E.
[2] M. J. Davies, Circulation 82 -suppl II (1990) II38
[3] A. Garcia et al., Methods Mol. Biol. 708 (2011), 191.
[4] M. Ciborowski et al., PLoS ONE 7, (2) (2012) e31982.

OMT12

METABOLOMIC APPROACH IN TYPE 1 DIABETES BY CE-MS

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Although Type 1 Diabetes Mellitus (T1D) is known since the ancient years, it is still a matter of research, in order to achieve deeper knowledge about the metabolic alterations resulting from the absence of self-produced insulin. Moreover, the chronic alterations common to diabetic patients (cardiovascular disease, inflammation) could be related to metabolic changes present since the early stages, and these changes could be a target for specific treatments, addressed to improve the life quality of long-term diabetic patients. Metabolomics is suited to help in the quest for such biomarkers; by means of non-targeted approaches a large number of small molecules in biological samples (metabolites) can be screened. With multivariate analysis tools significant variables can be detected, and further identified. Capillary electrophoresis offers broad possibilities in the analysis of a group of metabolites, challenging with other techniques such as reverse-phase liquid chromatography, and in T1D studies it has provided the opportunity to asses monitoring of the changes in metabolites, together with the contribution to the discussion of disease progression [1]. Hyphenation of MS may provide information for structural elucidation, as previously demonstrated [2,3].

For the present study children between 6-11 year old were enrolled, T1D and age-matched controls. Urine fingerprints were obtained with CE (Mod.7100) coupled to ESI-TOF (Mod 6224) from Agilent Technologies. Raw data were processed with MassHunter to obtain the molecular features in each electropherogram. MassProfiler Profesional was used to remove background noise and unrelated ions, to proceed with the multialignment and filtering of the results. In total 230 features out of 5367 entities where selected for further data treatment. Differences among profiles obtained in the different groups of study were evaluated for individual metabolites by using a paired-*t* test ($p \leq 0.05$) and the standard error of the coefficient was determined by jack knife, also used for multivariate statistical calculations and plotting. The metabolites responsible for the classification were putatively identified by their molecular mass in different databases (HMDB, METLIN) and confirmed with standards. Different metabolites related to amino acid metabolism were responsible for the classification, even though the T1D children were tightly controlled with insulin. The results highlight the capabilities of the technology to show up differences between groups and to shed light on some of the metabolic alterations present. The methodology and the biochemical interpretation of the changes will be presented in detail.

[1] C. Balderas, A. Villaseñor, A. García, F.J. Rupérez, E. Ibañez, J. Señorans, J. Guerrero-Fernández, I. González-Casado, R. Gracia-Bouthelier, C. Barbas, J Pharm Biomed Anal 53 (2010) 1298.

[2] E. P. Moraes, F.J. Rupérez, M. Plaza, M.Herrero, C. Barbas. Electrophoresis 32 (2011) 2055.

[3] J. Godzien, D. García-Martínez, P. Martinez-Alcazar, F. Ruperez, C. Barbas, Metabolomics (2011) DOI 10.1007/s11306-011-0351-y.

CAPILLARY GEL ELECTROPHORESIS-LASER INDUCED FLUORESCENCE OF DOUBLE-STRANDED DNA FRAGMENTS USING GELGREEN, A NEW FLUORESCENT DYE

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Nowadays, approaches focused on the replacement of reagents hazardous to human health are highly demanded in Green Analytical Chemistry. Numerous fluorescent DNA-staining dyes such as intercalating agents (ethidium bromide, YOPRO-1, etc.) are potent mutagens. GelGreen is a new non-hazardous DNA staining reagent successfully used in slab gel electrophoresis protocols. In the present work, GelGreen has been assayed to the analysis of double-stranded (ds) DNA by capillary gel electrophoresis (CGE) with laser induced fluorescence (LIF) detection. A CGE-LIF method developed in our laboratory was selected to study the performance of this novel dye. The DNA separation method is based on a dynamic coating with a cellulose derived polymer, that also acts as sieving matrix, and a washing routine with 0.1 N HCl and water. The effect of GelGreen concentration on resolution, signal/noise ratio and migration time of a wide concentration range of a standard dsDNA mixture was evaluated. The optimum concentration of GelGreen in the sieving buffer was established based on signal/noise ratio and resolution. Under optimum GelGreen concentration in the sieving buffer efficient and sensitive separations of DNA fragments with sizes from 100-500 base pairs (bp) were obtained. Also, a comparison in terms of resolution, time of analysis, LOD, LOQ, dynamic range, reproducibility, sizing performance and cost of analysis was established between the two optimized CGE-LIF protocols for DNA detection, based on the dye YOPRO-1 (typically used for CGE-LIF of DNA fragments) and the new GelGreen using different standard DNA mixtures. Analyses using YOPRO-1 are faster than those using GelGreen (31.1 min vs 32.4 min for the analysis of 100-500 bp DNA fragments). On the other side, sensitivity using GelGreen was 3-fold higher than that using YOPRO-1. The cost of analysis was significantly cheaper (25-fold) using GelGreen than with YOPRO-1. The resolution values and sizing performance in terms of linearity of the calibration curve and relative error were not significantly different between the two dyes (e.g., resolution length for the 100 and 200 bp DNA fragments was 2.2 for both dyes). The usefulness of the separation method using GelGreen is demonstrated by the characterization of polymerase chain reaction amplicons obtained during optimization of different DNA amplification systems. Our data demonstrated that GelGreen is well suited for the CGE-LIF analysis of DNA.

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