SEQA 2015

XX REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA

Santiago de Compostela • 01-03 de Julio de 2015

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SEQA

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Santiago de Compostela, 01–03 de julio de 2015

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BIENVENIDA

Nos reunimos en Santiago de Compostela, en nuestra XX Reunión como Sociedad Española de Química Analítica (SEQA), tras un largo recorrido de casi cuatro décadas y en las que nuestra comunidad societaria ha experimentado profundos cambios, acordes con la evolución social, universitaria y científica vividas en la dinámica social y económica del país.

A nuestras espaldas quedan las reuniones iniciales que focalizaron su atención en la dimensión docente de la Química Analítica y su encaje en las reformas universitarias del momento. Desde aquel soporte inicial el tiempo transcurrido ha permitido, no sin esfuerzo, que esta comunidad científica crezca, avance en la diversidad de campos de investigación y profundice en los terrenos que aborda; toda esa riqueza se hace patente en el programa científico de estas jornadas. Contamos, en el apartado de conferencias invitadas, con la aportación de tres compañeros que proporcionarán una visión de la rica capacidad investigadora que atesoramos; igualmente nos acompañan cuatro científicos de otros países que son referentes mundiales en sus respectivos campos de investigación. Pero el grueso del programa científico lo nutren los numerosos asistentes a este encuentro con sus ideas y trabajos que serán presentados en las intervenciones orales o mediante póster. Estas contribuciones, desde muy diversos ángulos, confluyen en una mirada nueva que, sin duda, ayudará a hacer retroceder el inmenso ámbito de la incertidumbre y crecer el terreno de la certeza, en definitiva a impulsar la ciencia.

Esta atractiva realidad, plasmada en una densa agenda y a la que os damos la Bienvenida, se cimenta sobre el buen hacer de quienes nos precedieron; los fundadores de la SEQA no son ya tan jóvenes, algunos no están con nosotros, otros se han retirado o están a punto de hacerlo. Sobre la fortaleza de sus hombros la Sociedad ha sabido crecer y aglutinar a una comunidad joven que, a pesar de enfrentar un incierto futuro profesional, apuesta por estar activa y presente en estos encuentros. A esta juventud renovada dedicamos una, si se nos permite, más especial acogida. Esta presencia de jóvenes se beneficia de un amplio programa de becas otorgadas por la propia Sociedad y de un no menos generoso número de premios y contribuciones económicas de distintos Patrocinadores, a quienes agradecemos su esfuerzo y colaboración. Nuestra Sociedad está convencida de la necesidad imperiosa de favorecer el vínculo entre distintas generaciones de investigadores.

No soplan vientos favorables al incremento de recursos públicos para el impulso y desarrollo de la I+D y por el contrario, el escenario de la crisis económica y financiera se prolonga sin despejar el futuro; por ello es más decisiva la aportación privada, los patrocinios de entidades y organizaciones que prestan su colaboración para hacer posible la realización de estas reuniones científicas y que profundamente agradecemos.

Y damos una última bienvenida. Es la bienvenida a un futuro que será brillante si mantenemos la calidad y el rigor de nuestro trabajo, en el marco sólido de una Sociedad cohesionada, con la firme convicción de que unidos contribuimos al valor y riqueza de la Química, a la formación de grandes investigadores y docentes y, con todo ello, a la construcción de una sociedad más abierta, culta y capaz de enfrentarse a un mundo cada vez más exigente y competitivo.

ELENA DOMÍNGUEZ
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Conferencias Plenarias

PL1

SOLID PHASE MICROEXTRACTION – "GREEN" ALTERNATIVE SAMPLING/SAMPLE PREPARATION STRATEGY FOR CLINICAL INVESTIGATIONS

J. Pawliszyn[1]

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Recent trends in clinical sample preparation include shift towards automation, high-throughput, miniaturization, and extraction methodology with low or no solvent consumption ("green chemistry"). In the presentation the ways in which solid-phase microextraction (SPME) can complement currently used techniques will be discussed. This will involve the overview of SPME in many biological applications, including doping inspection, clinical control, therapeutic drug monitoring and metabolomics studies. The most promising application of SPME, which distinguishes this method from other extraction and sample preparation techniques, is its applicability to on-site and in vivo sampling with no need of sample withdrawal. A few examples of in vivo tissue analysis during transplantation as the alternative to standard approaches will be discussed. Finally, the importance of high throughput and automation in the view of clinical laboratory requirement as the large-scale drug screening will be shown.

In this presentation, an automated, high-throughput method based on thin-film solid phase microextraction and liquid chromatography mass spectrometry will be introduced for simultaneous quantitative analysis of 110 drugs chosen from ten classes and varying in physical and chemical properties. The developed SPME method was optimized in terms of sorbent selection, extraction pH, ionic strength of the sample, washing solution, extraction and desorption times for analysis of urine, blood and saliva samples. Chromatographic separation was obtained in reversed-phase mode and detection was utilized with full scan orbitrap or triple quadrupole mass spectrometer. The developed method was validated according to the Food and Drug Administration (FDA) criteria, taking into account Minimum Required Performance Level (MRPL) values provided by the World Anti-Doping Agency (WADA). The developed assay offers fast and reliable multi residue analysis as an attractive alternative to the standard methods that are currently used in anti-doping laboratories.

We will also introduce an in vivo solid phase microextraction method, which combines sample preparation, metabolism quenching and extraction as well as eliminates sample collection as an approach to monitor graft function at different stages of the medical procedure related to organ transplantation. To ensure the best analytical performance of the method, various aspects of the protocol were studied and optimized including selection of coating length, analyte coverage, transportation and storage conditions with particular attention paid to stability of the extracted compounds and convenience of the approach for clinical setup. The applicability of the developed method for determination of metabolic profile of the organs and for monitoring of drug metabolism was verified during lung and liver transplantation in pig models. A few examples of in vivo tissue analysis during transplantation as the alternative to standard approaches will be discussed.

The ability to deliver a rapid prognostic metric of clinical condition is certainly important in the critical-care setting (emergency and surgery units). Time in decision-making when choosing and implementing a therapeutic strategy could be the difference between life and death. For this reason, molecular diagnostic and prognostic instruments, which are able to provide doctors with fast and reliable results, are highly desired in such facilities for a personalized diagnosis and treatment of patients. Ideally, the assessment of such molecules (drugs, metabolites and biomarkers) before, during, and after surgery and/or emergency, should be performed in real or close to real time. Nevertheless, such metric has a number of challenges, which cannot be easily overcome by standard high-throughput assays; for example, compound selectivity, space resolution, and analysis of unstable/short-lived metabolites. Hence, a compromise between sample preparation and direct analysis is needed in order to provide high resolution, sensitivity and quantitation accuracy. Succinctly, SPME does not require any sample collection because

extraction takes place in situ by inserting a biocompatible microfiber directly into tissue, blood or other biological matrix for a short period of time. Alternatively, the same device can be used for ex vivo analysis using a small amount of the studied sample. Despite the multiple advantages of SPME, few efforts have been done regarding its direct coupling to MS towards the analysis of drugs and biomarkers in vivo and in complex matrices. This work presents multiple strategies recently developed for the direct coupling of SPME to MS. In order to have a broader range of applications, different SPME geometries such coated fibers and meshes, as well as ionization approaches such DART and ESI, were studied.

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PL2

RAMAN SPECTROSCOPY – POSSIBLE SOLUTION FOR UNMET MEDICAL NEEDS?

J. Popp[1,2]

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A comprehensive analysis to determine diagnostic, prognostic and predictive factors in a few steps or ideally in one-step requires the development of new, fast and reliable approaches that support and supplement routine medical diagnostics and therapy. In the past years, medical photonics has witnessed the development of optical / photonic approaches that are potentially in a position to meet these aforementioned challenges. In this context, spectroscopic approaches like e.g. Raman spectroscopy are especially noteworthy.

Raman spectroscopy is an especially efficient method since it probes molecular vibrations distinct for each type of molecule. A Raman spectrum can be seen as a characteristic "molecular fingerprint" of every sample. Since the end of the 20th and the beginning of the 21st century we observed an almost explosive increase in the application of Raman spectroscopy to address biomedical questions [1]. The ability to obtain specific chemical information labelfree makes Raman spectroscopy attractive for many clinical investigations of bodily fluids, pathogens, cells, and tissue biopsies. In this contribution, we will summarize our recent results in implementing various Raman-approaches for infectious diseases and cancer, as these types of diseases harbor unmet needs regarding diagnosis and therapy.

We will start with highlighting the potential of Raman microspectroscopy for an early diagnosis and therapy of infectious diseases with special focus on sepsis. In the field of sepsis, the fast identification of pathogens, their resistances and the specific host is crucial for choosing the appropriate initial antibiotic therapy to save lives in intensive care units. It will be shown that Raman hold great promise as point-of-care approaches to address these challenging tasks [2-5]. The application of Raman spectroscopy as point-of-care test requires chip-based sampling methods offering the opportunity to handle small sample volumes and to apply sample preparation steps. Here, we will present innovative chip-based bacterial isolation strategies out of complex sample matrices (e.g. blood or urine). In this context, we will e.g. introduce a Raman compatible silicon chip with aluminium squares suitable for the isolation of microorganism from complex media by using antibodies as capturing molecules [6]. Furthermore, we report about a dielectrophoresis Raman setup for the fast characterization of urinary tract pathogens by capturing bacteria directly from patients' urine samples in microstructured chip regions using spatial non-uniform electric fields [7].

The second part of this presentation focuses on Raman studies on eukaryotic cells for biomedical applications. Here, we will report about the great potential of Raman spectroscopy for a label-free discrimination between normal and (circulating) tumor cells and towards establishing a Raman spectroscopic hemogram [8-10]. In particular we will report about the recent progress we made towards Raman activated cell sorting (RACS) by coupling Raman spectroscopy with microfluidics and micromanipulation approaches [11,12].

Besides single cells, whole tissue sections like biopsy specimens can be characterized by means of Raman-microspectroscopy. The processing of the specific Raman-maps via mathematical approaches enables an objective evaluation of the tissue samples for an early diagnosis of cancer (= spectral histopathology) [13-15]. The potential to couple the Raman system via optical fibers to the point of measurements has enabled within the last years besides *ex-vivo* Raman studies on excised tissue also *in-vivo* Raman studies, i.e. Raman endospectroscopy. We will introduce novel Raman fiber probes for *in-vivo* tissue screening to reliably diagnose and screen cancer and other diseases like atherosclerosis in internal organs like e.g. colon, stomach or aorta [16].

The low Raman scattering cross section results in long acquisition times limiting the recording of Raman images of large tissue areas and thus, clinical applications. The acquisition times can be reduced by utilizing non-linear Raman approaches like CARS (coherent anti-Stokes Raman scattering) and allows recording Raman images of single characteristic Raman bands in real time. It will be shown, that the joint use of linear Raman microspectroscopy and CARS microscopy allows for complementary characterization of the type and chemical composition of the tissue samples [17,18]. In order to improve the diagnostic result CARS microscopy can be easily combined with second harmonic generation (SHG) and two-photon fluorescence (TPF) microscopy. SHG and TPF highlight morphological / structural features by displaying collagen structures (SHG) and the spatial distribution of autofluorophores like e.g. NAD(P)H, flavines, elastine etc. Overall, we will present the development of a compact CARS/SHG/TPF multimodal nonlinear microscope in combination with novel fiber laser sources for use in clinics [19]. The diagnostics potential of this compact multimodal microscope as compared to conventional histopathological images has been demonstrated for a broad variety of different cancer entities [20-22]. These examples show the great potential of multimodal imaging to complement established clinical pathological diagnostic tools.

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PL3

NANOSCALE TECHNOLOGY PROVIDING IMPROVEMENTS IN SEPARATION SCIENCE

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Nanomaterials continue to stretch the limits of capabilities perceived as well established. For example, while it is well known that decreased particle sizes increase chromatographic efficiency. However, a minimal particle size of 1-2 microns was perceived as the lower limit feasible for practical applications in chromatographic separations. New innovations in nanoparticle and nanofiber synthesis have broken this barrier. Today, particles and fibers in the 200- 500 nm diameter range have been shown to be viable materials for chromatographic supports in thin layer chromatography and HPLC

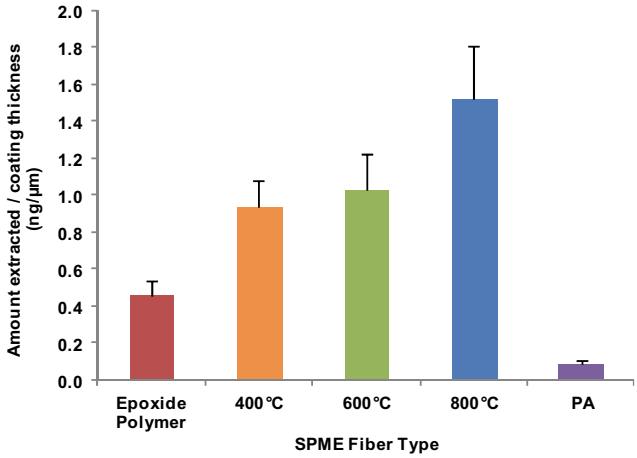

Figure 1. Extraction efficiency of metoprolol [1]

As an example, Figure 1 compares the extraction efficiency for trace levels of metoprolol using nanofibrous epoxide polymer solid phase microextraction device (SPME), carbon nanofiber SPME devices with the carbon processed to the listed temperatures to a commercially available polyacrylic acid based SPME device. Clearly the extraction efficiency using the nanofibrous devices show substantial improvements as well.

Innovations in chromatography, extraction capabilities, as well as new media for MALDI and SALDI desorption mass spectrometry will be described. As one example, Figure 2 (left) compares the SALDI mass spectrum for polyethylene glycol (PEG) with an average molecular weight of 3400 with an optimized MALDI mass spectrum. Clearly a substantial improvement in the signal/noise for PEG is obtained.

Illustrations of substantial improvements in speed of analysis, efficiency and lowered detection limits will be noted.Unique applications of this technology based on the ordered media will be highlighted.Finally, questions on improvements of these attributes will be posed based on detailed studies on flow dynamics and nano and molecular order in the media will be discussed.

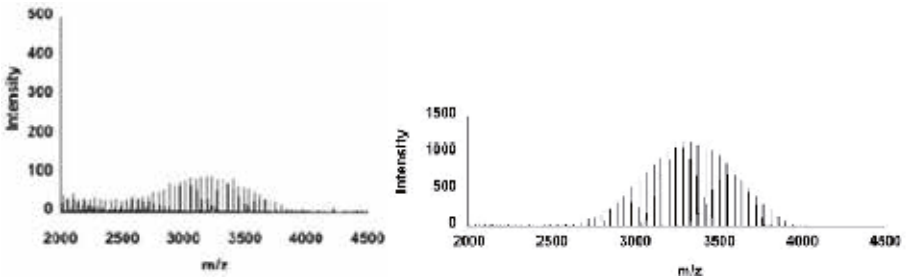

Figure 2. Mass spectra of polyethylene glycol with average molecular weight of PEG 3,400 [2]

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PL4

LA REVOLUCIÓN GENÓMICA: DE LA SECUENCIACIÓN SANGER A LA SECUENCIACIÓN DE NUEVA GENERACIÓN

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En más de un siglo de avances continuos de la Genética es muy difícil situar un punto de inflexión que marcase el comienzo de la Genómica y de hacerlo seguramente sería el descubrimiento de las técnicas de secuenciación de ADN en la década de los 70 y concretamente el método de Sanger, por el que este recibió su segundo Premio Nobel de Química. La técnica de secuenciación de Sanger es todavía, después de más de 30 años el *gold standard* en secuenciación y es usada por todos los laboratorios de genética del mundo y fue esencial para conocer la secuencia de los genomas.

El Proyecto Genoma Humano fue el otro punto de inflexión [1, 2]. Cuando comenzó en 1990 pocos podían imaginar que en el año 2003 se considerase ya finalizado, y esto fue posible gracias a una serie de desarrollos esenciales en muchas áreas diferentes entre los que destaca descubrimiento de la PCR por Kary Mullis (por lo que consiguió el Premio Nobel de Química en 1993), avances en la química y particularmente la tecnología de fluorocromos, en la física, como la electroforesis capilar y especialmente el gran desarrollo de la informática que en genómica dio lugar a una nueva disciplina en auge como es la bioinformática que ha pasado a tener una importancia clave en la investigación genómica.

Es difícil imaginar cuantos aspectos de la ciencia, de la cultura y de nuestras propias vidas están experimentando avances gracias a esta revolución genómica. Entre ellas la Medicina forense ya que gracias al estudio del ADN podemos identificar no solo un individuo a partir de muestras insignificantes sino conocer su origen geográfico, edad o algunas características físicas con bastante precisión. También la genética de poblaciones que se ha convertido en una herramienta auxiliar de la historia que complementa a otras clásicamente utilizadas como la arqueología o la lingüística e incluso a estas les da datos añadidos para entender muchos de sus hallazgos. Y, más aun, la secuenciación del genoma de otros homínidos como el hombre de Neanderthal [3] nos ha mostrado el dato sorprendente de no solo convivieron con el Homo Sapiens en Europa sino que se mezclaron en cierta proporción y que existen trazas del genoma Neanderthal en el Homo Sapiens moderno.

Pero los proyectos de secuenciación de genomas no se limitaron a la especie humana y cada vez más conocemos del genoma de otras especies, particularmente de las de mayor interés comercial, gracias a los cuales se está produciendo una revolución sin precedentes en mejora genética y prevención de enfermedades en agricultura y ganadería.

Pero la revolución más importante y la más esperada desde el lanzamiento del Proyecto Genoma Humano se está viviendo en la Medicina. No hay especialidad médica que no necesite actualmente análisis genéticos y el número de pruebas que se solicitan está creciendo a un ritmo superior al 20% anual. Los avances en genómica han tenido una repercusión más notable en algunas especialidades como la Microbiología, la Anatomía Patológica, la Hematología y por supuesto la Genética pero todas se han visto afectadas en menor o mayor medida.

En lo que se refiere a la genética clínica se ha producido simultáneamente, debido al avance tecnológico, un cambio en el espectro del componente genético de la enfermedad identificable y si antes lo mayoritario en el diagnóstico era el estudio de grandes defectos de los cromosomas, lo que denominamos cromosomopatías, ahora son notable mayoría las pruebas diagnósticas de enfermedades mendelianas y sobre todo complejas (esto es la que tienen un componente genético y ambiental más balanceado y que son las enfermedades más comunes) y entre las que destaca, a nivel de pruebas solicitadas, el cáncer ya que los análisis moleculares son esenciales para el diagnóstico, pronóstico y tratamiento de muchos de ellos.

El gran avance en el conocimiento de la enfermedad compleja vino en primer lugar de la mano de los avances en tecnología de microarrays que nos permitieron hacer análisis masivos de expresión del genoma y análisis masivo de la variación que hay entre individuos. En este último aspecto fue esencial el desarrollo del proyecto internacional HapMap.

En cuanto a la enfermedad mendeliana continuamente se encuentran nuevos loci responsables de enfermedades genéticas y las nuevas tecnologías y en particular las tecnologías de secuenciación de nueva generación están revolucionando no solo la investigación genómica sino el diagnóstico clínico.

La secuenciación Sanger genera una única lectura de un fragmento de ADN por reacción, mientras que en las tecnologías de secuenciación masiva o de nueva generación (NGS) el proceso de secuenciación se paraleliza, produciéndose millones de secuencias simultáneamente (generalmente más cortas que las obtenidas por el método Sanger). Las secuencias individuales son más propensas a contener errores, sin embargo, como cada región es secuenciada muchas veces, todas ellas en su conjunto permiten recomponer la secuencia de interés. Esto permite análisis de conjuntos de genes, exomas o genomas completos en un tiempo muy breve y a un coste muy bajo. Una tercera generación de secuenciadores que permiten secuenciar moléculas de ADN o ARN únicas abre nuevas posibilidades en campos como la Medicina forense o el cáncer.

La NGS ha permitido que se hayan completado proyectos internacionales como el Proyecto 1000 genomas, el Atlas del Genoma del Cáncer o el *International Cancer Genome Consortium* que han producido avances muy importantes en el conocimiento de la enfermedad y que se hayan lanzado recientemente proyectos como el 100,000 genomas o la *Precision Medicine Initiative* que pretende la secuenciación del genoma de un millón de individuos lo que va a conllevar a una nueva clasificación de las enfermedades basada en sus causas moleculares y una medicina personalizada que ya está comenzando a ser una realidad.

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Conferencias Invitadas

IN1

ADVANCED ELECTROCHEMICAL BIOPLATFORMS FOR DETECTION AND DIAGNOSIS OF CANCER BIOMARKERS

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Nowadays, it is widely accepted that the prompt diagnostic of a certain disease is a key factor in the patient survival, thus requiring efficient and reliable analytical methods for such purpose. Among the many diseases affecting mankind, breast cancer is of great relevance due to its high incidence, prevalence and mortality worldwide. During the last years, the demand of efficient, simple and disposable devices with short response times, easy-to-use, low-cost, and suitable for their mass production and to perform decentralized and routine analysis has increased in the medical diagnosis field.

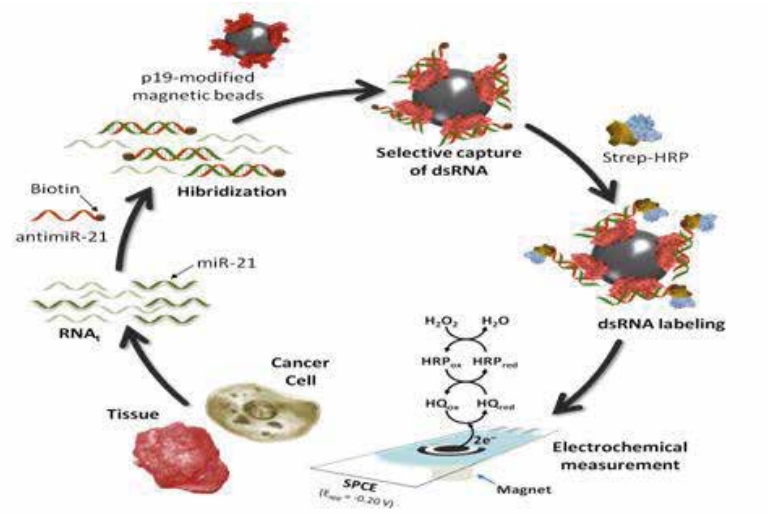
Within this context, different strategies implying the design and preparation of electrochemical biosensors for the sensitive, selective and rapid biosensing of cancer-related biomarkers will be summarized and discussed in this presentation.

The first approach involves the use of a novel reduced graphene oxide-carboxymethylcellulose hybrid nanomaterial as an efficient electrochemical scaffold for the detection of the TP53 gene with single nucleotide polymorphism [1]. The TP53 gene is the most commonly mutated gene in people who have cancer. Two different configurations involving amino-terminated hairpin specific capture probes of different length covalently immobilized by carbodiimide chemistry onto the −COOH rich rGO-CMC nanostructured platforms through their amino moiety were implemented and compared. Upon hybridization, a streptavidin- peroxidase (Strep-HRP) conjugate was employed as an electrochemical indicator. Hybridization was monitored by recording the amperometric responses measured upon the addition of 3,3′,5,5′-tetramethylbenzidine (TMB) as a redox mediator and H_2O_2 as an enzyme substrate. Limits of detection of ∼3 nM were obtained without any target or signal amplification and a complete distinction was observed between perfectly matched and the single-base mismatched DNA. The implemented methodology was applied to analyze the endogenous TP53 status in different human cell lines.

The second approach involves the determination of human epidermal growth factor receptor 2 (ErbB2) which is a breast cancer prognostic marker that plays an important role in breast cancer cell proliferation and malignant growth. We have developed an amperometric magnetoimmunosensor for ErbB2 determination in human serum, cell lysates and intact breast cancer cells, by using carboxyl modified magnetic beads as solid support and SPCEs as electrochemical transducers [2]. After covalent immobilization of antibodies against ErbB2 on the surface of modified MBs, target protein is sandwiched by the HRP detector antibody. Then amperometric measurements were carried out at -0.2 V by using the hydroquinone/hydrogen peroxide system. The LOD achieved with this methodology is 577 times lower than the cut off value for sErbB2, so then the developed magnetoimmunosensor can be applied as a useful and sensitive tool for the early diagnosis of breast cancer.

Another recent development in our group is the preparation of of magnetobiosensors involving RNA binding viral protein p19 as highly selective biorecognition element for miRs quantification [3]. miRs are a class of endogenous and small non-coding RNAs which play an important role in various cellular processes. Many studies have revealed that alterations in miR-expression are involved in the initiation and progression of human cancers suggesting that some miRs can function as tumor suppressor genes or oncogenes. The strategy we have developed combines the benefits of using modified MBs, the viral p19 protein which is a protein that functions as a dimer to bind and sequester only small double-stranded of 19–23 nt long with nanomolar affinity in a size-selective and relatively sequence independent manner, and SPCEs to perform the electrochemical detection. miR-21 was chosen as target analyte since it has been identified as

the only miR over-expressed in a wide variety of cancers and a proven oncogen. This Figure conceptually illustrates the fundamentals of the approach.



The p19-based magnetosensor was evaluated as an in situ testing system for RNA samples extracted from breast-cancer cells and tissues.

Recently, two miRs have been shown to be associated with breast cancer: miR-21 and miR-205. While miR-21 acts as a non-specific oncogene, miR-205 is a breast cancer specific tumor suppressor. Therefore, the simultaneous interrogation of miR-21 and miR-205 will allow the unequivocal identification of breast cancer groups which is not possible with individual detection of these miRs. We implemented the first electrochemical magnetosensor to simultaneously detect the expression of these two different miRs in one single experiment. The methodology relied on the same fundamental commented before for the individual detection of miR 21 but preparing two different batches of modified MBs, one for each target miR and performing the amperometric detection at dual SPCEs [4].

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IN2

LA QUÍMICA ANALÍTICA COMO HERRAMIENTA AL SERVICIO DE LA CONSERVACIÓN DE PATRIMONIO

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1. Antecedentes

La conservación del patrimonio cultural es una actividad necesaria, a la vez que gratificante, para la sociedad moderna. El patrimonio es fuente de inspiración y reflejo de nuestra cultura e historia y, por tanto, es un bien valioso que debe ser legado a las futuras generaciones. Aunque la significación de los bienes culturales deriva primordialmente de su propio mensaje histórico, cultural y figurativo, su perdurabilidad a lo largo del tiempo depende de sus materiales constitutivos, por lo que su conocimiento y caracterización resulta esencial para asegurara su salvaguardia. Es en ese ámbito, en el que la Química Analítica presta su mejor servicio, integrándose con otras ramas del saber, en la más amplia actividad pluridisciplinar de estudio y preservación del patrimonio.

El uso de las ciencias químicas y físicas para resolver cuestiones de arqueología, historia y conservación del patrimonio se remonta al siglo XVIII como resultado de la progresiva aplicación práctica de las ideas de Johann Wincklemann (1717-1768) sobre la metodología de estudio de objetos artísticos y arqueológicos [1]. Citaremos el estudio microscópico de un fragmento de roca de Stonehenge llevado a cabo por Halley en 1720, el estudio de Klaproth sobre la composición de monedas (1795), los estudios de J. F. Gmelin (1781) [1], J. Haslam (1800), J. Chaptal (1809) y Sir Humphry Davy (1815) sobre pigmentos [2] o el discurso de John W. Mallet (1882), séptimo presidente de la American Chemical Society, para la defensa de tesis doctoral que versaba sobre objetos celtas en la University of Gottingen, como algunas de las primeras investigaciones químico-analíticas registradas. En 1888 se funda el Königliche Laboratory en Berlin al cual suceden el Fine Arts Museum Laboratory de Boston en la década de los años 20 y los Laboratorios del Museo del Louvre y del British Museum en 1931. En España se crea el Instituto Español de Prehistoria en 1957 y el Instituto de Conservación y Restauración de Bienes Culturales (ICRBC), actualmente Instituto de Patrimonio Histórico Español (IPHE), en 1985 [3]. En la actualidad el panorama nacional se ha visto considerablemente ampliado con la incorporación de otras instituciones (institutos, universidades, fundaciones) que incorporan grupos de expertos en química analítica en sus equipos de trabajo[i].

2. Información proporcionada por las técnicas químico-analíticas

La Química Analítica desempeña un papel esencial en la caracterización de los materiales integrantes de un bien cultural. La identificación de la técnica de ejecución artística o de la tecnología de producción del objeto es decisiva para su adscripción a una determinada región geográfica o su datación. El análisis químico de un pigmento permite, en algunas ocasiones, la autentificación de una obra pictórica.

El análisis químico es, asimismo, fundamental para establecer el estado de conservación del objeto o monumento así como las causas de su deterioro. Una vez identificadas las causas y establecido el tratamiento de intervención, se hace indispensable el control analítico de este último para garantizar su correcta implementación sobre el objeto. El seguimiento analítico de parámetros medioambientales (temperatura, HR, concentración de contaminantes atmosféricos, etc.) forma parte primordial de los protocolos de conservación preventiva de bienes culturales. En paralelo a todas estas actividades ligadas a la intervención directa sobre el patrimonio, el análisis químico se hace indispensable en el proceso de desarrollo y validación de nuevos materiales y procedimientos de conservación y restauración.

[i] Un listado muy amplio de instituciones y grupos puede ser consultado en (http//www.investigacionenconservacion.es).

3. Requerimientos de las técnicas químico-analíticas

La aplicación de una determinada técnica químico analítica en el estudio de un bien cultural está severamente condicionada por su carácter único e irrepetible. De este modo, estrategias de muestreo aleatorio o mediante patrón regular, que habitualmente se utilizan en el análisis de materiales, son de aplicación muy limitada a los bienes culturales. La elección de la técnica analítica está, también, muy condicionada en este ámbito. El tipo de información que se desea obtener (morfológica, composicional, estructural,..) es un primer aspecto a tener en cuenta. El carácter invasivo/no invasivo o destructivo/no destructivo del método analítico así como su sensibilidad y selectividad son, asimismo, aspectos que van a determinar la elección del método de análisis finalmente escogido. En la actualidad, el desarrollo de técnicas instrumentales no invasivas o no destructivas, altamente sensibles, hacen que, progresivamente, se vaya imponiendo el uso de estrategias multi-técnica en las que el objeto, o una única muestra, es sucesivamente analizado mediante distintos instrumentos.

4. Revisión de técnicas químico-analíticas al servicio de la conservación de patrimonio

En sus comienzos, a finales del s. XVIII, los métodos de caracterización de bienes culturales se limitaron al examen microscópico y a los ensayos microquímicos [4].Durante el s. XIX y comienzos del s. XX se incorporaron algunas técnicas espectroscópicas. A mediados del s. XX (1930-70) tiene lugar el desarrollo de gran número de técnicas espectroscópicas, espectrométricas, cromatográficas y de datación. Peroes a partir de 1970, sin duda, cuando se produce el mayor auge en la aplicación del análisis químico al estudio y conservación del patrimonio gracias al control digital, a la estandarización de las plataformas informáticas que soportan la instrumentación analítica y al desarrollo de equipos portátiles [3, 5].

En acuerdo con el criterio de Lahanier [6], los métodos científicos actualmente aplicados al estudio de patrimonio cultural pueden clasificarse en tres grandes grupos: a) métodos de examen basados en el registro de imágenes obtenidas a partir de radiación electromagnética o electrones; b) métodos analíticos y c) métodos de datación. Los métodos de análisis químico, a su vez, pueden clasificarse en cuatro categorías atendiendo al tipo de información que proporcionan [7]: i) composición química del objeto; ii) estructura cristalina y molecular; iii) morfología y distribución estratigráfica y iv) textura superficial y microdominios.

5. Perspectivas futuras

En el momento presente las técnicas instrumentales desarrolladas a lo largo del s. XX se han incorporado a los laboratorios de museos y centros de investigación especializados en los cuales éstas se aplican de forma rutinaria en la caracterización de objetos y en el control de los tratamientos de conservación. Las tendencias actuales parecen ir dirigidas hacia la implementación de las técnicas quimiométricas y estadísticas que permitan una mejor discriminación y cuantificación de objetos y materiales. En el campo de la proteómica se están haciendo notables progresos en la aplicación de estas técnicas al análisis de aglutinantes orgánicos de tipo proteico. Se está progresando considerablemente en el desarrollo de equipos espectroscópicos, espectrométricos y electroquímicos portátiles no invasivos o no destructivos, con mayor sensibilidad y resolución. Por otra parte, se está ampliando las prestaciones de gran número de técnicas espectroscópicas y cromatográficas con instrumentación más robusta que permite el análisis de áreas de la muestra proporcionando mapas composicionales a escala micro y nanoscópica.

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IN3

ESPECTROMETRÍA DE MASAS E ISÓTOPOS ESTABLES ENRIQUECIDOS: UN VIAJE DESDE LO DESCONOCIDO A LO DESCONCERTANTE

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La Espectrometría de Masas se está imponiendo como técnica analítica de rutina en multitud de campos científicos, desde el análisis medioambiental hasta el control de calidad de los alimentos sin olvidar otros campos importantes como la metabolómica, la proteómica cuantitativa o el análisis antidopaje. Las fuentes de ionización más comunes: el plasma de acoplamiento inductivo, la ionización electrónica y el electrospray permiten la ionización de casi cualquier elemento o compuesto químico y, por tanto, su detección mediante Espectrometría de Masas. La emergencia de equipos en tándem de triple cuadrupolo ha permitido alcanzar límites de detección extremadamente bajos y su precio asequible ha contribuido a la expansión de la técnica en laboratorios de rutina. Además, la Espectrometría de Masas permite la confirmación de la presencia de un determinado compuesto en la muestra, uno de los requisitos de la legislación vigente, lo que justifica su preponderancia actual sobre otras técnicas analíticas.

Un aspecto menos conocido y mucho menos utilizado de la Espectrometría de Masas es su capacidad de proporcionar distribuciones isotópicas en átomos y moléculas. Esta capacidad abre el campo de aplicación de la Espectrometría de Masas al uso de isótopos estables enriquecidos y a la medida de modificaciones intencionadas de las abundancias isotópicas de átomos y moléculas, con multitud de aplicaciones en ciencia y tecnología. La aplicación más conocida en Química Analítica es el análisis por Dilución Isotópica (Isotope Dilution Mass Spectrometry, IDMS) donde compuestos marcados isotópicamente se utilizan como patrones internos para proporcionar resultados analíticos de alta calidad metrológica. Tanto es así que la IDMS está considerada como técnica de referencia trazable directamente al Sistema Internacional de unidades.

En los últimos años se ha desarrollado una herramienta de cálculo matemático, la Deconvolución de Perfiles Isotópicos (Isotope Pattern Deconvolution, IPD) basada en la regresión lineal múltiple, que permite un tratamiento de datos unificado sea cual sea la modalidad de uso de los isótopos estables enriquecidos (atómica o molecular) o su campo de aplicación. Esta herramienta permite calcular la fracción molar de todos y cada uno de los perfiles isotópicos presentes en una muestra o sistema biológico proporcionando información cuantitativa de alta calidad en sistemas complejos. La Figura 1 resume las distintas alternativas actuales al trabajo con isótopos estables enriquecidos o moléculas marcadas isotópicamente. Dependiendo del número de perfiles isotópicos distintos utilizados así como del momento en que esos perfiles isotópicos se añaden a la muestra o sistema considerado podemos encontrarnos con diversas aplicaciones siendo la IDMS sólo una pequeña parte del campo de aplicación de la metodología. Por ejemplo, en estudios de metabolismo mineral se pueden utilizar dos perfiles isotópicos distintos añadidos a distinto tiempo al sistema. El primer perfil isotópico proporciona la información metabólica mientras que el segundo perfil isotópico permite la cuantificación tanto del perfil isotópico natural, endógeno, como del primer perfil isotópico añadido. En estudios de marcaje de productos u organismos se añaden dos perfiles isotópicos distintos del mismo elemento, al mismo tiempo y con una relación molar fija entre ambos perfiles. Esta relación molar es constante e independiente de la concentración del elemento de perfil isotópico natural lo que confiere una gran estabilidad a la marca isotópica generada. En otro orden de cosas, los dos perfiles isotópicos añadidos pueden pertenecer a dos especies químicas distintas que se interconvierten durante el proceso analítico (por ejemplo creatina y creatinina). La medida de los perfiles isotópicos finales en los dos compuestos permite la corrección de las posibles reacciones de interconversión y, por tanto, una medida exacta de la concentración de ambos compuestos en la muestra.

El uso de dos isótopos enriquecidos de bario para el marcaje transgeneracional de truchas y salmones; el uso de tres isótopos enriquecidos de mercurio para la determinación de mercurio inorgánico, metilmercurio y etil mercurio en sangre, orina y pelo; la determinación de perfiles isotópicos de distintos metabolitos en el estudio del metabolismo de la glucosa en cultivos celulares; el estudio de la interconversión entre creatina y creatinina durante el proceso analítico; y el uso de glicina marcada isotópicamente para estudiar la síntesis de péptidos marcados serán algunos de los ejemplos utilizados en este viaje de lo desconocido a lo desconcertante.

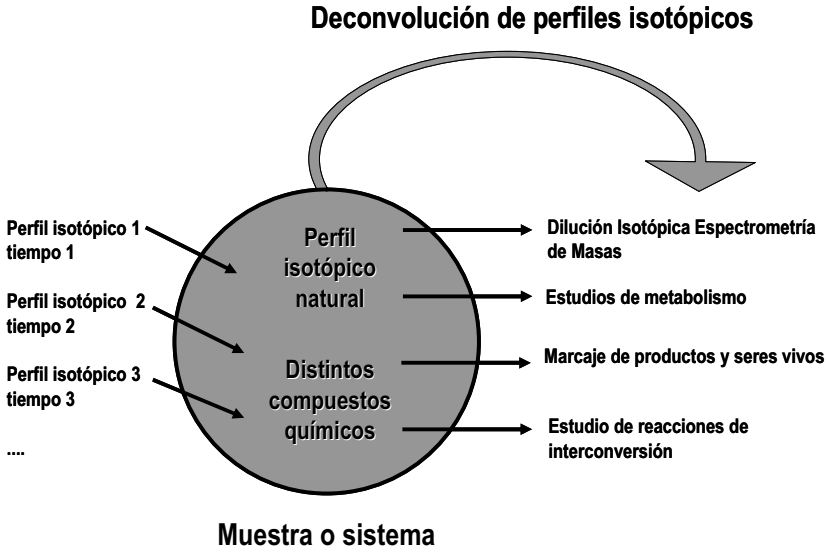

Figura 1. Distintas alternativas en el trabajo con isótopos estables enriquecidos y moléculas marcadas isotópicamente.

Comunicaciones Orales

O1

DISOLVENTES SUPRAMOLECULARES: LÍQUIDOS NANOESTRUCTURADOS SENSIBLES A ESTÍMULOS AMBIENTALES PARA LA EXTRACCIÓN DE COMPUESTOS ORGÁNICOS

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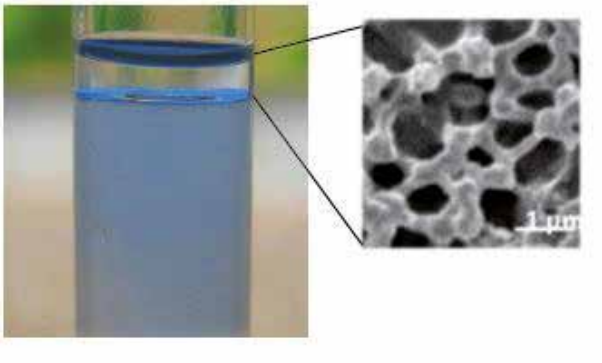
El desarrollo de disolventes alternativos a los disolventes orgánicos ha despertado un enorme interés en los ámbitos industrial y científico, sectores en los que se deben atender las exigencias de las políticas medioambientales y sociales. Los disolventes supramoleculares son líquidos nanoestructurados generados a partir de una disolución acuosa o hidro-orgánica de moléculas anfifílicas a través de un proceso espontáneo de autoensamblaje. Estas fases líquidas nanoestructuradas proporcionan una oportunidad única para el diseño y síntesis de disolventes funcionales con la eficiencia y selectividad requerida para el desarrollo de estrategias innovadores en procesos de extracción analítica e industrial.

En esta comunicación se presentarán algunos de los avances obtenidos en los últimos años en relación a la síntesis, caracterización y aplicación en procesos de extracción analítica de los disolventes supramoleculares. El desarrollo de la Química Supramolecular y, consecuentemente, la mejor comprensión de los mecanismos de autoensamblaje molecular, ha proporcionado las bases para el diseño de disolventes con propiedades programadas para cumplir funciones específicas. La caracterización de las nanoestructuras que los conforman sigue siendo un gran reto debido a la labilidad que se deriva del tipo de interacciones que conducen el proceso de autoensamblaje. El uso de técnicas de preparación de muestras como vitrificación está permitiendo la obtención de excelentes microfotografías mediante TEM Y SEM (ver figura). Las propiedades intrínsecas de los disolventes supramoleculares (ej. regiones de distinta polaridad en la nanoestructuras, diferentes tipos de interacciones, elevada concentración de compuestos anfifílicos) los convierten en ideales para procesos de extracción eficientes en análisis multiresiduo, el desarrollo de procesos generales de extracción, en estudios epidemiológicos y campañas de monitorización ambiental.



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O2

IMMUNOAFFINITY COLUMNS-ION MOBILITY SPECTROMETRY: COMBINING SELECTIVITY AND SENSITIVITY

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Ion mobility spectrometry (IMS), an analytical technique for the determination of volatile and semivolatile compounds based on the gas-phase separation of the resulting ions under a weak electric field at ambient pressure, reached its maturity between the late XX century and early XXI century [1]. Since then, IMS has been primarily used for the analysis of explosives, illicit drugs and chemical warfare agents with dedicated commercially available equipment. However, the analytical potential of IMS, particularly as regards operational speed and sensitivity, has extended its scope to the pharmaceutical, food and feed, clinical, polymer, petrochemical and environmental industries [2].

However, IMS measurement of complex samples, such as beverages, food, blood, urine or saliva, produces a mixture of reagent and analyte product ions [3], which could complicate the interpretation of IMS spectra of drug metabolites and interfere in analyte determination. Moreover, the measured number of theoretical plates of IMS rarely exceeded 5000, which compared to the typical number of theoretical plates obtained with other separation techniques (LC:25000, GC:120000 and CE:300000) [4], is easy to imagine that the lower separation efficiency has limited the applicability of the technique. Therefore, the incomplete resolution of peaks is a common situation in the analysis of complex samples by IMS, being necessary the application of a sample pretreatment step.

Over the last years, new materials have been studied to replace the standard solid phase extraction (SPE) supports, such as molecular imprinted polymers, polymer composites, carbon nanotubes and nanoparticles and immunoaffinity supports. Immunoaffinity chromatography (IAC) columns take advantage of the extreme affinity and specificity usually displayed by antibodies to provide a sort of SPE method that enables the selective extraction of the target analyte even from complex matrices.

The aim of this study is to highlight the advantages of IAC-IMS coupling in terms of sensibility, selectivity, accuracy and speediness of analysis using as example the determination of strobilurins, a fungicide family commonly used in high-value crops, including grapes, strawberries, peppers, tomatoes, and cereals, and anilinopyrimidines, a fungicide family commonly used in grapes, apples and cereals, from relevant matrices. The IAC columns have been evaluated in terms of immunosorbent binding capacity, optimum elution conditions, and reusability. The applicability of the methodology has been tested in the analysis of fruits, juices and wines.

Acknowledgements
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O3

COUPLING IONIC LIQUIDS AND GAS CHROMATOGRAPHY BY THERMAL DESORPTION-PROGRAMMED TEMPERATURE VAPORIZATION

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Ionic liquids (ILs) show valuable characteristics that have made them one of the most investigated topics in recent years. Due to their low vapor pressure, high thermal stability, and great capacity to dissolve a wide range of organic compounds, ILs have been proposed as an alternative to organic solvents in liquid-liquid microextraction (LLME). The direct injection of the IL employed as extractant is not compatible with common GC sample introduction techniques. Despite IL are thermally stable at high temperatures, they are negligible volatile, accumulating in the GC injector and column. However, the use of IL extracts on GC may be advantageous, since their low volatility avoids the interference of organic solvent peaks, and their high solubility allow the determination of a wide range of analytes. Usually, the GC analysis of compounds extracted by ILs requires a previous back extraction into a GC compatible organic solvent, after water dilution of the IL phase. This procedure is time consuming, and may lead to a reduction in the overall efficiency of the extraction process. The GC direct injection of small volumes of the IL extracts without further manipulation leads to IL contamination within the GC liner that requires a frequent cleaning and maintenance.

The difficult coupling of IL-LLME and GC can be accomplished using external interfaces that facilitate the transfer of the extracted analytes from the IL microdrop to the GC column, while prevent the entry and contamination of the GC system by IL. Aguilera-Herrador et al. [1] proposed a self-made interface, in which target analytes were volatilized by heating and transferred to the GC column by a helium gas flow. Canals and co-authors [2] proposed the employment of a commercially-available thermodesorption unit (TDU), in which a removable insert containing the IL drop was placed inside the desorption tube. These assemblies do not completely assure no dragging of the IL drop to GC column. In addition, only a small fraction of the resulting IL extraction phase can be effectively analyzed.

TDUs have been extensively used in SBSE-GC hyphenation, they are a versatile tool that allow the introduction of glass inserts capable of contain 150 µL of liquid sample into the thermal desorption tubes. The use of these glass microvials for the injection of large sample volumes is known as "direct microvial insert thermal desorption" [3]. This technique may be adapted to IL injection into GC systems, allowing the employment of larger volumes of the IL extract, while assuring no IL contamination in the GC system.

Within this approach, IL is placed in a glass microvial placed inside the TD tube, and submitted to thermal desorption. When the IL is heated, extracted compounds are thermally desorbed (vaporized), while less volatile IL remains. A carrier gas drags the analytes to a PTV injector where they are focused before entering into the chromatographic column. Once desorption step is over, PTV is heated, leading to the entrance of the retained compounds to the GC column. This assembly has two main advantages, on the one hand, the whole IL drop resulting from LLME (~20 µL) can be submitted to thermal desorption. On the other hand, the tube shape of the glass microvial assures no IL entry to the GC system, and even if some IL vapors are dragged, they would be retained in the disposable PTV liner. Some applications of this IL-GC-MS hyphenation have been developed, including the determination of parabens and bisphenols in aqueous samples, the analysis of organophosphorous pesticides in environmental samples and the study of phthalate esters migration from plastics to isooctane.

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O4

ENVIRONMENTAL -*OMICS* COMBINATION TO EVALUATE THE TERRESTRIAL AND AQUATIC ECOSYSTEMS AFFECTED BY METAL POLLUTION USING FREE-LIVING MOUSE *MUS SPRETUS* AND CRAYFISH *PROCAMBARUS CLARKII* AS BIOINDICATORS IN DOÑANA NATIONAL PARK (SPAIN).

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Anthropogenic and natural activities have increased contaminants levels in the terrestrial and aquatic ecosystems. In this sense, the importance of monitoring the exposure and studying the effects of heavy metals on living organisms has increased in the last years. Studies of small mammals for evaluate terrestrial ecosystems, mainly free-living mice (*Mus spretus*), and crayfishes (*Procambarus Clarkii*) for aquatic ecosystems have been used as bioindicators in numerous environmental studies because they can provide useful information for assessment of risk of metals to humans [1-5]. In the present work, we consider the use of mice and crayfishes to assess its biological response or mode of actions against contaminants in the relevant ecological area of Doñana National Park and surroundings (southwest Spain) in which many migrating birds land for breeding and feeding. In order to obtain a global vision of the metal toxicity mechanisms and of the responses that metals elicit in the organisms, new and more potent methodologies are needed.

Omics technologies (genomics, transcriptomics, proteomics/metallomics, metabolomics and ionomics) offer a valuable alternative in this field since they provide massive information about biomolecules in cells and organisms under the toxic metals effects. *Omics* differs from traditional hypothesis-driven research because it is a discovery-driven approach and they provide a more general appraisal of molecules altered under pollutant exposure. We demonstrated here the successful applications of heterologous microarrays, proteomics methodologies (2-DE, iTRAQ®), metallomics, ionomics or metabolomics in separates studies. Nevertheless, an overall evaluation of changes that contaminants induce in cells is only possible by integration of *–omics*, since the transcripts induced by pollutants (transcriptomics) encode proteins with altered expression profiles, which undergo post-transductional modifications (proteomics). In addition, many proteins related to environmental issues are bound to metals that make advisable the use of metal-tagged techniques (metallomics) and most are related to oxidative stress enzymes. Moreover, metabolomics and ionomics provide information about what is actually happening in the organisms, since metabolomic and ionomic profiles also reflect the influence of external factors (metal exposure, diet), providing easier understanding of complex biological systems under environmental issues.

The highest responses corresponded to bioindicators living areas placed between the Guadiamar stream and the Guadalquivir River, according to the extended and intensive use of agrochemicals in such areas. In addition, this area is suffered the input of metals transported by the Guadiamar river during the rupture of Aznalcollar mine tailing pond in 1998 [6].

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O5

ELECTROCHEMICAL GENOSENSOR FOR THE CONTROL OF GMO IN FOOD AND FEED USING HELICASE-DEPENDENT AMPLIFICATION

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The application of genetically modified organisms (GMOs) has revolutionized agronomic practices; however, high reluctance to food derived from GM crops has been shown by society due to its potential side effects on health and environment.

EU legislation states all products containing approved GM material must be labeled to ensure consumer choice, setting a threshold value of 0.9 % per individual ingredient, while unauthorized GM varieties are strictly forbidden. Therefore, the development of reliable detection analytical methods is of prime importance for the implementation of labeling rules.
DNA-based methods to determine the identity of food material at genetic level are officially accepted to quantitatively evaluate the presence of GMOs in food and feed, particularly, those based on the real-time polymerase chain reaction (RT-PCR); however, they require laboratories equipped with expensive instruments and staffed with highly qualified personnel to be carried out.

In our group, we have developed an accurate, sensitive and inexpensive detection system which combines helicase-dependent isothermal amplification (HDA) with subsequent analysis of unpurified amplicons by an enzyme-amplified electrochemical genosensor. This methodology provides unambiguous discrimination of 0.5 % GM cereals in less than 4 hours through the detection of the Cauliflower Mosaic Virus 35S promoter, the promoter of choice in biotech crops, thus resulting in a promising alternative for rapid primary on-site screening of GMO.

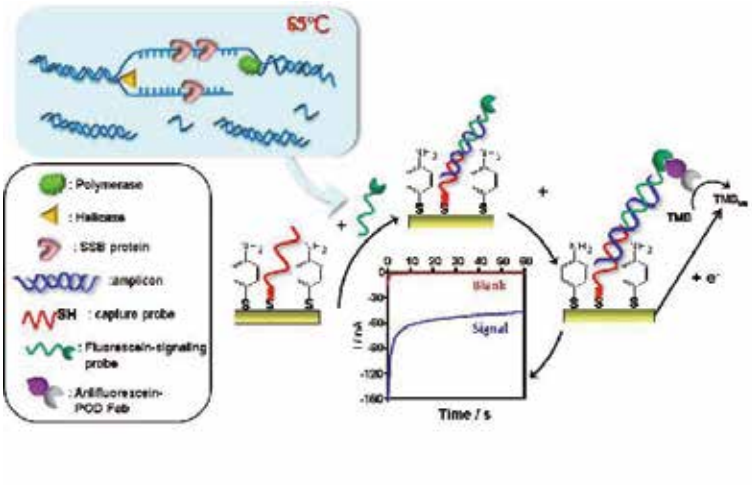

Figure1: Schematic representation of the methodology.

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O6

SISTEMAS ANALÍTICOS TOTALES EN SUPERFICIES TERMOCRÓMICAS

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El desarrollo de soluciones integradas sensibles, versátiles y baratas es uno de nuestros retos. En particular, esta comunicación presenta el desarrollo de sistemas analíticos totales basados en la tecnología de etiquetaje de discos compactos, empleando plataformas y lectores de disco convencionales como única instrumentación. El recubrimiento termocrómico de estos discos les confiere propiedades muy atractivas para el desarrollo de nuevos sistemas de análisis. Esta tecnología presenta además un conjunto de características superiores a las mostradas por los sistemas sensores basados en la tecnología CD, DVD o Blu-ray, admitiendo la funcionalización química y derivatización física de la superficie del disco. Además, permite irradiar selectivamente el área de la superficie de sensado que contiene el ensayo, acelerando así el proceso de lectura y reduciendo el volumen de los registros. Por otro lado, dada la elevada potencia del láser (40 mW, 780 nm), alrededor de 40 veces mayor que la de los lectores/grabadores de discos convencionales, permite incrementar la magnitud de las señales registradas. Otras de las prestaciones que hacen interesante a la tecnología desarrollada es la ausencia de limitaciones ópticas, superando ampliamente las prestaciones de los discos compactos estándar. De esta forma, las propiedades ópticas del resto de la plataforma no interfieren en el proceso de irradiación y lectura del disco. Este hecho amplía las posibilidades de efectuar nuevos diseños y aplicaciones analíticas que incluyen diversas etapas. Asimismo, a partir de discos de etiquetaje se obtienen plataformas de ensayo con un elevado grado de sofisticación mediante procedimientos muy sencillos. El uso de esta tecnología permite realizar ensayos en materiales con propiedades mejoradas para el sensado (silicio, PMMA, PDMS, poliestireno, nitrocelulosa, metales, geles), permitiendo desarrollar sistemas totales de análisis aplicados en el ámbito de las ciencias de la vida.

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O7

NOVEL AMPEROMETRIC MAGNETOIMMUNOSENSING PLATFORMS FOR FAST, SENSITIVE AND SELECTIVE DETERMINATION OF FOOD ALLERGENS

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Food allergies, defined as an immune response to food proteins, affect as many as 8% of young children and 2% of adults in westernized countries, and their prevalence appears to be rising like all allergic diseases, becoming one of the major health concerns nowadays. So far, there is no effective treatment for food allergens suffers and the only way to manage the health hazards resulting from allergens is to avoid the specific allergen containing food [1]. Milk and peanuts are two of the most common sources of food allergies. The prevalence of sensitization to these specific food allergens varies based on the age and characteristics of the studied population, but studies incorporating diagnostic food challenges currently estimate that the prevalence of cow's milk allergy in infants is 2.5%, and peanut allergy is estimated to be between 0.8 and 1.5% in young children in US and England [2]. Although there are available several analytical methods for the determination of the main allergens of these two food components, there is still an urgent need to develop alternative methods able to perform rapid determinations with high sensitivity and selectivity with low-cost instrumentation and adaptable to miniaturization, ideal to perform decentralized and routine analysis.

Within this context, different strategies implying the design and preparation of amperometric magnetoimmunosensors for the sensitive, selective and rapid biosensing of the main allergens associated with cow milk (β-lactoglobulin, α-lactalbumin) and peanuts (Ara h 1 and Ara h 2) will be summarized and discussed in this presentation. The developed methodologies, based on the appropriate use and coupling of a pair of selective antibodies, carboxylic acid modified magnetic beads and disposable screen-printed electrodes, allow the determination of the endogenous content of the target analytes in complex samples (milks, food extracts and saliva). All the implemented scaffolds, which compared advantageously with other commonly used methodologies, can be considered truthful and promising analytical screening tools in the development of user-friendly devices for *on-site* determination of the target allergens in relevant samples.

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O8

(BIO)ANALYTICAL STRATEGIES FOR ASSESING NANOPARTICLES TOXICITY

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In the last years, nanoparticles (NPs) have received a great attention for their use and applicability in many new consumer products. A recent estimate suggests that more than 1000 NP-containing consumer products are currently on the market. In addition, their ability to advance science with novel analytical and medical tools also make them relevant to both physical and life sciences.

NPs are generally defined as spheres with a diameter between 1 and 100 nm. This small size gives them unique properties, especially because their specific surface area is larger and their reactivity is increased or different compared to bulk materials. These specific features are often linked with a potential toxicity. The products and applications of NPs include electronics, optics, textiles, medical applications, cosmetics, food packaging, water treatment technology, fuel cells, catalysts, biosensors and agents for environmental remediation. As a result of these applications, exposure of NPs to the environment and humans are becoming increasingly widespread. Consequently, different metals in the form of NPs have gained an increasing access to tissues, cells and biological molecules within the human body.

To date, the impact of NPs exposure to human health and the environment is not fully assessed. Research efforts to evaluate the toxic potential of NPs have presented some serious and far-reaching challenges and there remains an urgent need for well-designed studies that will generate data so that risk assessments for NPs can be conducted.

In this communication, we present different analytical and bioanalytical strategies to study the interaction of different NPs, including AgNPs, TiO_2NPs, SeNPs and quantum dots (Cd/Se), with cells and living organisms in order to assess their potential toxicity. To correlate any toxic reaction with a NP type, it is indispensable to investigate if NPs are attached to the cell surface or if they enter cells. If NPs are found in cells, their localization in different compartments such as endosomes, lysosomes, mitochondria, the nucleus or the cytosol, may also provide some answers regarding their potential toxicity. For these purposes, we have used ICP-MS and microscopic techniques. Our results show that the degree of internalization as well as the cellular localization is highly dependent on the type and size of the NP studied, rather than the type of cell.

We have also carried out bioanalytical assays to evaluate specific cellular pathways or cellular responses to NPs exposure including cell viability, cell proliferation and cell migration assays, and flow cytometry-based methods to determine the degree of apoptosis after exposure or changes in the cell cycle pattern. Additionally, we have used mass spectrometry-based high throughput discovery platforms for the identification of key proteins involved in the molecular mechanisms related to cell-NPs interaction.

Our results have shown that while some of the NPs induce a general stress response and result highly toxic at relatively low concentrations, other NPs exert their toxicity through specific pathways. In this case, such NPs could represent promising chemotheraputic agents when used under controlled conditions and modified with targeting ligands.

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O9

DETERMINATION OF PAHs IN FOOD USING SERS DETECTION

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Polycyclic aromatic hydrocarbons, PAHs, and their metabolic transformation products may be carcinogenic [1]. PAHs are originated from environmental sources (natural and anthropogenic), industrial food processing, packaging materials and certain cooking practices In fact; the main source of exposure to PAHs for non-smokers and non-occupationally exposed adults is food [2]. Discrepancy in PAHs results in food is mainly due to the type and fat content of the food, cooking process, temperature cooking, type of fuel used and proximity and direct contact with heat. PAHs are volatile and persistent. They are poorly soluble in water and soluble in lipophilic solvents.

Analysis of fatty matrices for PAHs determination requires the following: extraction, cleaning of the extracts and the use of high sensitive techniques due to the low concentrations of PAHs fixed as maximum levels permitted in current legislation.

Surface-enhanced Raman spectroscopy (SERS) is an extremely highly sensitive analytical technique based mainly on the relevant electromagnetic enhancement induced by nanostructured metal surfaces and associated to their localized plasmon resonance (LPR) [3].

The affinity of adsorbates toward metal nanoparticles surface is a key factor in the detection of pollutants due to the short-range effect of SERS effect. This can be increased by modifying the chemistry of the interface by a proper functionalization of the NPs. We have recently paid much attention to surface metal NPs modification by incorporation of molecules having both a high affinity to the metal and to the pollutant.

We have previously demonstrated that intense SERS spectra can be obtained from polycyclic aromatic hydrocarbons (PAHs), also in principle inactive in SERS, by changing the metal surface affinity with an appropriate functionalization with host molecules such as calixarenes or nanotubes [4]. However, up to now this new approach has not been tested for PAHs determination in real samples.

In this work we will present the potential capability to determine light PAHs in food samples by functionalization of Ag nanoparticles with viologens (lucigenin), a group of N-containing assemblers with a high electron acceptor character. The PAHs: naphthalene, acenaphthalene, acenaphthylene, fluorene and phenanthrene extracted in ACN have been determined, without the need to apply a separation technique, by the proposed developed method. Its analytical characteristics will be presented. The high sensitivity, simplicity in the sample treatment and selectivity achieved are very remarkable.

Acknowledgments
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O10

DETECCIÓN DE PARTÍCULAS INDIVIDUALES MEDIANTE ICPMS: BARRIDO ULTRARRÁPIDO VS. CONVENCIONAL

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La Espectrometría de Masas con Plasma de Acoplamiento Inductivo (ICPMS) es una técnica multielemental, utilizada de manera rutinaria para la determinación del contenido elemental total. Esta técnica puede utilizarse en el modo denominado de detección de partículas individuales (single particle ICPMS, SP-ICPMS) tanto para detectar, caracterizar en tamaño y cuantificar nanopartículas inorgánicas, como para detectar y cuantificar formas solubles, sin necesidad de separaciones previas, a niveles de concentración de ng L^{-1}.

En el modo de detección individual, cuando una nanopartícula es introducida en el ICP, sus átomos producen un paquete de iones gaseosos en el plasma, que se miden como un pulso individual en el detector. La intensidad de este pulso es proporcional a la cantidad de átomos del elemento en la nanopartícula y, por tanto, a la masa del elemento y al tamaño de nanopartícula, si su composición, forma y densidad son conocidas.
Para conseguir detectar nanopartículas individuales es necesario trabajar a concentraciones de partículas suficientemente bajas (del orden de 10^8 nanopartículas L^{-1} o menores) y a frecuencias de adquisición de datos suficientemente altas (del orden de 100 Hz o mayores). De esta manera, el número de pulsos es proporcional a la concentración en número de nanopartículas.

Los paquetes de iones generados por nanopartículas individuales en el plasma tienen una duración de aproximadamente 500 µs. Hasta muy recientemente, los instrumentos comerciales de ICPMS con analizadores de cuadrupolo sólo permitían trabajar con tiempos de lectura del orden de milisegundos (tiempos de lectura recomendados: 3-10 ms), de forma que los paquetes de iones se registran como pulsos individuales. Sin embargo, la última generación de ICPMS de cuadrupolo permite llevar a cabo barridos en modo ultrarrápido (frecuencias de adquisición de hasta 100 kHz), con tiempos de lectura del orden de microsegundos (10-100 µs). En estas condiciones, los paquetes de iones se registran como señales transitorias individuales, proporcionando información adicional sobre el perfil resultante. El uso de barridos ultrarrápidos supone unas ventajas añadidas a la hora de trabajar en SP-ICPMS, permitiendo la monitorización de más de un isótopo en una misma nanopartícula, reduciendo la capacidad de detección de especies disueltas, así como la incertidumbre de los resultados.

En este trabajo se lleva a cabo un estudio comparativo de la detección de partículas individuales mediante el uso de barridos convencionales (tiempos de lectura en el rango de los ms) y ultrarrápidos (tiempos de lectura en el rango de µs), evaluando las prestaciones de esta nueva forma de trabajo en SP-ICPMS. Del estudio realizado, se pone de manifiesto la importancia de seleccionar correctamente las concentraciones de nanopartículas en función del modo de barrido para conseguir resultados de la máxima calidad.

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O11

KINETIC-SPECTROSCOPY THREE-DIMENSIONAL CHEMILUMINISCENCE: A NEW APPROACH IN LUMINISCENCE.

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The time-resolved chemiluminescence elucidates the kinetic characteristics of the chemiluminescence emission. This technique is based on conventional flow-injection analysis and provides the analyst with the whole time profile for the chemiluminescence signal, thereby facilitating the resolution of complex mixtures of analytes. Using a back-thinned CCD spectrometer to obtain chemiluminescence spectra in this context has the advantage that it affords the acquisition of whole spectral signals in a few milliseconds. This solves the traditional limitation imposed by the fact that chemiluminescence emission usually lasts only a few seconds and is thus nearly impossible to measure with conventional means. However, modern spectrometers allow several spectra to be recorded while the chemiluminescence signal develops. The kinetic–spectroscopic 3D chemiluminescence technique acquires a certain number of 2D spectra during signal development that are subsequently assembled into a 3D spectrum with the software CLTotal[1].

We used two different types of software to obtain 3D kinetic–spectrometric profiles. BWSpec 3.26_41, which was run on the CCD spectrometer via a USB 2.0 interface, was used to acquire and store 2D spectra, as well as to export them as ASCII files. The software affords spectral acquisition at variable times and its timeline acquisition mode allows users to record and save (as ASCII files) a preset number of 2D spectra one by one.

Once 2D spectra were obtained, we used the custom-made software CLTotal, developed by our research group, to produce and process 3D spectra. CLTotal is based on software developed in the 1990s for a similar purpose using 3D fluorescence spectra. CLTotal allows the user to import previously acquired 2D chemiluminescence spectra and assemble them into a single 3D spectrum. The 3D spectrum can then be subjected to various graphical and mathematical treatments. However, the most interesting use of this new software is to obtain 2D spectra by following different trajectories in the 3D spectrum. For example, a horizontal cut allows an emission spectrum at a specific time to be obtained; similarly, a vertical cut provides a kinetic measurement at a specific wavelength. The user can also perform cuts along straight lines of variable slope or non-linear cuts according to a mathematical equation. The 2D spectra thus obtained are kinetic–spectroscopic spectra.

To demonstrate the potential of kinetic–spectroscopic measurements for the simultaneous determination of analytes with overlapped spectra, this methodology was applied to the simultaneous determination of benzo(a)pyrene and benzo(k)fluoroanthene. Polycyclic aromatic hydrocarbons (PAHs) are well-known pollutants resulting from incomplete combustion of hydrocarbons. These substances have aroused much interest on account of their potential deleterious effects on human health. PAHs usually reach water through discharges from industrial and wastewater treatment plants. This has raised the need for effective analytical methods to determine PAH concentrations in water with a view to monitoring water quality and industrial contamination. Chemiluminescence spectroscopy has proved a reliable analytical methodology for the determination of PAHs by effect of these compounds boosting the peroxyoxalate chemiluminescence emission upon reaction with hydrogen peroxide. This has promoted its use for determining PAHs.

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O12

MAGNETIC NANOPARTICLES-NYLON 6 COMPOSITE AS A NOVEL SORBENT FOR DISPERSIVE MICRO SOLID PHASE EXTRACTION

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The easy synthesis of magnetic nanoparticles-nylon 6 composite is here presented. The sorbent is synthesized in a quick and one-step method based on a solvent changeover, playing with the different solubility of the polymeric network in formic acid and water. The combination of magnetic nanoparticles (MNPs) with micrometric polymers is especially interesting in the microextraction context. In this sense, the composite presents a high extraction capabilities due to the polymeric network while maintains a magnetic behaviour that simplifies the isolation of the sorbent from the sample, since an external magnet is employed for this aim.

The new material has been characterized by different techniques including infrared spectroscopy (FT-IR), superconducting quantum interference device (SQUID) and transmission and scanning microscopy (TEM and SEM). The extraction performance of the composite under a dispersive micro solid phase extraction format was evaluated by determining two different analytical problems. Polycyclic aromatic hydrocarbons (PAHs) in water and bisphenol A (BPA) in milk samples were analysed, using liquid chromatography combined with an UV-Vis detector for their determination.

Firstly, four PAHs (benzo[*b*]fluoranthene, fluoranthene, indeno[*1,2,3-cd*]pyrene and phenanthrene) were determined in water samples. The developed methodology allows the determination of the analytes with limits of detection in the range from 0.05 μg L^{-1} (benzo[*b*]fluoranthene) to 0.58 μg L^{-1} (phenanthrene) and the repeatability of the method was better than 6.9 % at the limit of quantification level. The recovery study was performed in three different water samples obtaining relative recoveries from 80 to 111%, which demonstrated the applicability of the hybrid sorbent for the selected analytical problem.

After that, the synthesized composite was also employed for the dispersive -SPE of BPA in milk samples. Before the extraction, an adapted milk precipitation process was developed bearing in mind that the analyte has a high tendency to interact with the fatty compounds of the milk. As the common precipitation agent (EDTA-McIlvaine buffer) only allows the transfer of 30% of the analyte to the supernatant, acetonitrile was added to the medium to permit the transference of all the analyte. The developed methodology provides a limit of detection of 3.05 μg L^{-1}, which is in agreement with the specific migration limit (SML) established by the European Union. The repeatability, in terms of relative standard deviation, was better than 9.1% (at 100 μg L^{-1}). The performance of the method was evaluated through the analysis of milk samples including whole, defatted and skim. Since no sample contained the analyte in a measurable concentration, samples were spiked at 100 μg L^{-1}. The values were in the range from 86 to 99% so the applicability of the method for the selected analytical problem was demonstrated in spite of the complexity of the matrix.

O13

DESARROLLO DE UN MÉTODO DE *SCREENING* MULTI-CLASE EN PELO POR DISPERSIÓN DE MATRIZ EN FASE SÓLIDA Y CROMATOGRAFÍA LÍQUIDA DE ALTA RESOLUCIÓN –ESPECTROMETRÍA DE MASAS EN TÁNDEM

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El objetivo de este trabajo es el desarrollo de un método multi-clase para la detección de drogas de abuso en pelo (principales metabolitos de la cocaína, anfetaminas, opiáceos y drogas de diseño). En comparación con el análisis de orina y sangre, el uso del pelo como matriz biológica alternativa presenta una serie de ventajas tales como una menor invasividad, no viola la privacidad de la persona, es más difícil una posible contaminación o adulteración. Además, en pelo se puede detectar drogas hasta 1 ó 2 años. Con este método se analiza una gran variedad de drogas de abuso en muy poco tiempo, con un tratamiento sencillo y un coste mínimo.

El pretratamiento de la muestra optimizado se basa en la abrasión de la muestra (50 mg de pelo) con 180 mg de alúmina (dispersante) en presencia de 20 µL de dithiothreitol, (DTT) durante 5 minutos (previa a la etapa de abrasión se añaden los patrones internos, 500 µg L^{-1}). La muestra dispersada se trasvasa a un tubo eppendorf, y la extracción de los compuestos de interés se lleva a cabo con 1,4 mL de una mezcla MeOH / NH_4Ac 2mM bajo irradiación con ultrasonidos (37 kHz) durante 40 min. Los compuestos extraídos (cocaína, benzoilecgonina, ecgonina metil éster, cocaetileno, morfina, 6-monoacetilmorfina, codeína, anfetamina, metanfetamina, buprenorfina, norefedrina, etc.) se determinan por cromatografía líquida de alta resolución – espectrometría de masas en tándem (HPLC-MS/MS) empleando una columna Kinetex 5µ C18 100 Å (fase reversa).

La metodología previamente descrita ha sido validada acorde a las directrices propuestas por la *Food and Drug Administration* (FDA), y ha sido aplica con éxito al *screening* de drogas de abuso en muestras de pelo.

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O14

METHODOLOGICAL DEVELOPMENT FOR THE DETERMINATION OF COXIBs IN WASTEWATERS USING OFF-LINE SOLID PHASE EXTRACTION (SPE) COUPLED TO LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-Q-TOF)

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The development and performance evaluation of a method for the simultaneous determination of six pharmaceuticals belonging to the class of non-steroidal anti-inflammatory drugs (NSAIDs) which present high selectivity for the cyclooxygenase (COX)-2 isoform of COX in environmental waters are presented. The method involves an off-line mixed mode (reversed-phase and strong anionic exchange) solid phase extraction (SPE) for the selective concentration of Coxibs in combination with liquid chromatography (LC) quadrupole time-of-fligth (QTOF) mass spectrometry (MS). The use of a strong anionic exchange sorbent (Oasis MAX) led to a significant reduction of matrix effects, during electrospray ionization (ESI), in comparison with results reported for mixed mode weak anionic exchange sorbent (Oasis Wax) and polymeric reversed phase sorbents (Oasis HLB and Strata X).

Quantification limits were established between 0.031 and 3.1 ng L^{-1} for influent wastewater and for effluent wastewater the limits were ranged between 0.017 and 1.48 ng L^{-1}.

Finally, the method was applied to determinate the analytes in wastewaters. Among the pharmaceuticals investigated, two of them (celecoxib and etoricoxib) were detected in the treated and raw wastewaters at low levels (ppt) and two metabolites were found, the carboxylatedcelecoxib and the hydroxylatedetoricoxib.

O15

CARACTERIZACIÓN DIRECTA DE COMPUESTOS VOLÁTILES MEDIANTE SEPARACIÓN BIDIMENSIONAL POR TAMAÑOS Y COMPOSICIÓN HACIENDO USO DE UN SISTEMA HÍBRIDO DE MOVILIDAD IÓNICA DIFERENCIAL Y ESPECTROMETRÍA DE MASAS

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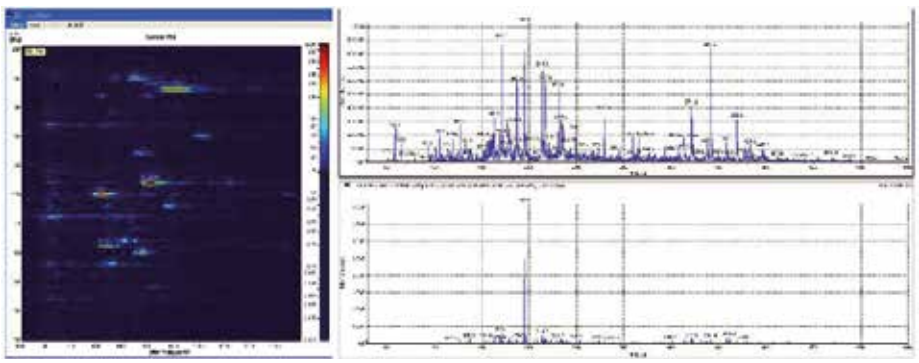
La clasificación directa de compuestos volátiles puede llevarse a cabo mediante técnicas de movilidad diferencial (DMA, differential mobility analyzers). Cuando tales dispositivos se acoplan con espectrómetros de masas de operación a presión atmosférica, el instrumento resultante (DMA-MS) se convierte en una poderosa herramienta analítica capaz de proporcionar información sobre tamaño y composición de los compuestos analizados. Esta información dual puede ser aprovechada con éxito en aplicaciones relacionadas con los estudios de compuestos con actividad asociada al grado de empaquetamiento, o como un elemento adicional de eliminación de interferencias y de aumento de la sensibilidad.

El instrumento descrito en la presente comunicación se basa en una arquitectura de movilidad iónica de tipo planas que incluye un elemento ionizador a presión atmosférica por electropulverización. Los compuestos ionizados son separados por tamaños basados en su movilidad eléctrica (U) y guiados al espectrómetro de masas dónde experimentan su separación en base a la relación masa/carga (m/z). En este modo híbrido de operación, se pueden obtener espectros de masas específicos a valores de movilidad fijos, o representaciones 3D correspondientes a la obtención de espectros de masas completos a los distintos valores de movilidad. El equipo permite diferentes modos de operación incluyendo el muestreo directo mediante succión capilar, o la colección de material mediante el uso de filtros captadores.

La comunicación que se presenta quedará ilustrada con los diferentes modos de operación en matrices diversas alimentarias, medioambientales o de interés en seguridad. Como ejemplo del potencial del equipo se muestra en la figura adjunta una ampliación de la zona del espectro de masas entre 150 y 200 Th para una bebida energética. La señal de cafeína es perfectamente visible en el espectro de masas no resuelto en movilidad, aunque queda rodeado de una multiplicidad de señales correspondiente a las diversas señales adyacentes. Mediante la aplicación del filtro de movilidad es posible obtener el espectro de modo selectivo, aumentando la relación señal-ruido en el espectro resultante.



O16

ABSOLUTE PROTEIN QUANTIFICATION AND PHOSPHORYLATION DEGREE DETERMINATION USING capLC-ICP-QQQ

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The observed associations of variations in protein levels to important alterations of biological processes, are placing quantitative proteomics as a top priority in current research. Electrophoretic techniques first, and more recently mass spectrometry (MS) have been used for quantification of proteins to a relative level (by comparison to a reference biological state). The use in the last few years of elemental MS techniques with an ICP (inductively coupled plasma) ionization source, has opened a new door to absolute protein quantification without the need for specific standards (a great advantage in comparison to molecular MS-based techniques in absolute quantification of proteins [1]). Protein phosphorylation is one of the most important post-translational modifications (PTMs) as it is involved in many significant cellular processes. Traditionally, most of the studies have focused on phosphorylation sites determination rather than quantification or dynamic studies. Unfortunately, previous efforts in determination of the phosphorylation degree by ICP-MS were limited by the need of a previous knowledge of the protein concentration [2].

Sulfur and phosphorus are elements extremely important in biomolecules. Sulfur in cysteine and methionine amino acid residues, and phosphorus (phosphate) as the most common PTM in proteins. The ICP-MS detection of both elements has been traditionally hampered due their high ionization potential and numerous polyatomic interferences. In this context, ICP-QQQ has been recently proposed for removing interferences with high efficiency providing very low detection limits of such elements [3]. Thus, a methodology for absolute protein quantification based on ICP-QQQ has been here evaluated, by coupling the ICPMS detector to capillary reversed phase liquid chromatography (capLC) via a total consumption nebulizer as a sample interface.

Results obtained using species-unspecific Isotope Dilution Analysis (IDA) for the determination of different standard proteins were validated. This implies a significant development in protein quantitative studies, because of its simplicity and because specific standards are no longer needed (provided that stoichiometries are known). Of course, further optimization could make this methodology appropriate to simple protein mixtures, opening a wide range of possibilities in targeted proteomics.

Moreover, the simultaneous determination of P/S ratios by ICP-QQQ on transient signals in phosphoproteins and phosphopeptides has also been conducted. Bovine milk β-casein and Ovoalbumin were chosen as model proteins. The analysis of the intact protein by capLC-ICP-MS allowed to obtain the global protein phosphorylation degree, phosphate and methionine as unspecific calibrants. In addition, the tryptic digestion has been also investigated to evaluate the phosphorylation degree for individual phosphopeptides.

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O17

PRINTING INTERNAL STANDARDS FOR LA-ICP-MS BIOIMAGING STANDARDISATION. COMPARISON OF THE DIFFERENT NEPHROTOXIC BEHAVIOUR OF CISPLATIN, CARBOPLATIN AND OXALIPLATIN

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Cisplatin, carboplatin and oxaliplatin are currently used in cancer chemotherapy. However, these drugs differ in their efficacy and adverse effects, such as nephrotoxicity. The study of the different distributions of the aforementioned drugs along the kidney may help to understand their different nephrotoxic behaviour. Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has shown high potential to improve the understanding of the distribution of trace elements in complex biological processes at tissue an cellular level. In fact, previous Pt-bioimaging studies performed by LA-ICP-MS on the whole 3 μm sagital kidney slices from Wistar rats treated with cisplatin, point out that nephrotoxicity may be related with the accumulation of cisplatin and its derivatives in the kidney cortex [1]. Unfortunately, results obtained by LA-ICP-MS are subjected to several variations concerning the sample matrix and instrumental drifts. Therefore, in order to compare bioimages obtained at different measuring sessions and/or for different tissues a standardisation method has to be developed. Recently, several internal standardisation methodologies have been proposed by different authors for standardisation in LA-ICP-MS [2, 3]. In this work, an internal standardisation method based on printing an iridium-spiked ink onto the surface of the sample has been employed to evaluate the different distributions of cisplatin, carboplatin and oxaliplatin along the kidney. The deposition of the internal standard was performed by printing the Ir-spiked ink onto the top of fresh kidney tissue slices of 4 μm with a conventional office printer. Moreover, this methodology was also applied to study variations on Cu and Zn distributions as a result of Pt-based drugs treatment. Results shown that the different distributions observed for the three drugs may be related with their different nephrotoxic behaviours, since the bioimaging maps obtained revealed that oxaliplatin presented a different distribution than those obtained for cisplatin and carboplatin.

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O18

REAL-TIME HIGH-RESOLUTION TANDEM MASS SPECTROMETRY IDENTIFIES FURAN DERIVATIVES IN EXHALED BREATH

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The identification of molecules in exhaled human breath is of interest in the search for new biomakers of diseases. Around 900 compounds have been detected in breath so far, comprising a huge range of chemical functionalities and volatilities. However, proper identification of these compounds is still difficult, and efforts need to be made to develop techniques with better identification capabilities. Furan derivatives are a set of compounds whose presence in breath has been suggested but not yet clearly understood. Even though some of them have been related to smoking or fungal infection, their source is still unclear since they are not part of the known human metabolome. Proper identification of the whole family of furan derivatives in the exhalome would be useful for a better understanding of the origin and function these compounds, their source (the main hypothesis being that they are related to the interplay between gut microflora and human metabolism), and their possible usefulness as biomarkers of diseases.

Even though GC-MS is the preferred technique for breath analysis, GC-MS is slow and other techniques for rapid, real-time analysis of breath have been developed, such as proton-transfer-reaction mass spectrometry (PTR-MS) and selected-ion flow-tube mass spectrometry (SIFT-MS). In a recent publication [1], our group has shown that secondary electrospray ionization (SESI) interfaced to high-resolution mass spectrometry (HRMS) allows the real-time analysis of several oxidative stress biomarkers in breath. SESI has proven to be able to detect compounds with significantly higher m/z values that those detected by PTR-MS and SIFT-MS, and has also shown promising identification capabilities.

Here, we couple SESI with tandem HRMS for identifying biomarkers in exhaled breath. Additionally, its combination with UHPLC for resolving isomeric forms is also explored. Ten healthy people were asked to repeatedly breathe in a LTQ Orbitrap mass spectrometer whose inlet was replaced with a low flow secondary electrospray ionization source (LF-SESI) that allowed the admission of breath samples through a heated tube. Up to 16 features were found to correspond with the expected $[M+H]^+$ ions of alkylfurans, furfural, furfurol, and acetyl- and acetone-furan. In the case of alkylfurans, SESI-HRMS allowed the detection of the whole homologous series, filling the existing gaps in the literature, and it expanded the series up to the C12-furan, a compound whose m/z value of 237 u is beyond the mass range of other real-time techniques such as PTR-MS and SIFT-MS. To further improve the identification process, real-time SESI-HRMS/MS experiments were run in a tripleTOF instrument for the furan derivatives previously detected. These HRMS/MS spectra were compared with those obtained from standards and were used for the generation of fragmentation pathways. These comparisons showed a clear match between the standards and the compounds detected in exhaled breath. To improve the identification of structural isobaric isomers, we added a UHPLC separation step before HRMS(/MS). Chromatographic peaks corresponding to at least two or three structural isomers were found for C5 to C12 alkylfurans. These chromatograms were compared with those obtained from standards showing perfect matches regarding retention times. These matches allowed us to identify twelve members from the homologous series of unbranched alkylfurans.

In conclusion, we showed that real-time HRMS/MS is a powerful analytical technique not only for the analysis of exhaled breath in real time, but also for the proper identification of biomarkers found in the exhalome, as demonstrated by applying this technique to the identification of furan derivatives. In addition, the combination of HRMS/MS with UHPLC, even though it cannot be applied in real time, allowed the identification of different isobaric structural isomers.

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O19

EVALUATION OF THE EFFECT OF HYBRID PALM OIL SUPPLEMENTATIONS ON PHOSPHOLIPID COMPOSITION OF HUMAN ERYTHROCYTES BY UPLC-(+)ESI-MS/MS ANALISYS

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Hybrid palm oil (HPO) is the crude oil obtained from the fruits of the interspecific hybrid palm *Elaeisoleifera* x *Elaeisguineensis*. HPO differ from the common palm oil for its fatty acid composition. HPO contains indeed higher amounts of unsaturated fatty acid and lower percentages of saturated fatty acids [1].It is obtained by exclusively mechanical processes and doesn't need refining procedures. Moreover, HPO is rich in antioxidant compounds such as β-carotene, tocopherols and significant amounts of tocotrienols [2].Considering composition and its content in bioactive substances, HPO could represent a tropical equivalent of the extra-virgin olive oil (EVOO), which is at the base of the Mediterranean diet, and the positive effect of which are nowadays well known and demonstrated. Despite its potential, only few studies about health effects of HPO on population have been performed.

The aim of this work is to investigate and compare the effects of HPO and EVOO supplementations on the human erythrocytes phospholipid composition. For this purpose, erythrocytes phospholipid molecular species profiles were studied involuntary and suitable patients, during a 3 months clinical trial.160 patients were admitted to the study and randomly assigned to two different groups. A group was submitted to an HPO daily supplementation, the other group, serving as control group, was treated with EVOO. A daily amount of 25 ml of the assigned oil was assumed from each patient. Analysis of target parameters was performed at baseline, and after the first, the second and the third month of treatment.

The phospholipid molecular species of erythrocytes have been fully characterized by ultra high performance liquid chromatography coupled online with positive electrospray ionization tandem mass spectrometry (UPLC-(+)ESI-MS/MS). A fast analytic method based on HILIC column and ESI source was developed to achieve the separation of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SPH) within 6 minutes. The molecular species of all the phospholipids were detected as their protonated molecular peak [M + H]+. The positive ionization was preferred to the negative ion mode because the molecular species of PC and PE are better identified: the fragments originating from the loss of the fatty acid moieties are formed. The identification of the phospholipids molecular species was confirmed by using standard PL solutions and comparing the mass spectra with those reported in the literature. The relative abundance of individual molecular species within a phospholipid class was calculated from the single ion current responses, since the peak intensity with ESI-MS among phospholipids species of the same class is considered similar.

The PC profile significantly changes during EVO and HPO treatments. Especially, the EVO and HPO supplementations for 1 month implied the enhancement of PC species containing saturated fatty acids. Differently, the PC profile from samples obtained after 3 months of supplementation is similar to that of the baseline samples. Unlike to PC, the Sph molecular species profile remain unaltered during the treatments and the PE profile was weakly affected.

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Comunicaciones Pósters

Nuevos Avances en Tratamiento de Muestra (TM), Alimentos (ALI), Nanotecnología (NT), Técnicas Ómicas (TO), Docencia (D)

P1

DETERMINACIÓN DE PARABENOS EN AGUAS MEDIANTE MICROEXTRACCIÓN CON ELECTROMEMBRANA (EME) Y DETERMINACIÓN CROMATOGRÁFICA

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Se conoce comúnmente con el nombre de parabenos a la serie homóloga de los ésteres del ácido *p*-hidroxibenzóico (o ácido 4-hidroxibenzóico). Son sólidos cristalinos, incoloros, poco volátiles y lipofílicos, aunque relativamente solubles en agua en un amplio rango de pH [1], disminuyendo su solubilidad en agua al incrementarse el tamaño de la cadena carbonada [2, 3]. Los parabenos son ampliamente usados como conservantes en productos cosméticos y para el cuidado de la piel. Debido a su amplia utilización actualmente son considerados como contaminantes emergentes, ya que se liberan en gran cantidad, y de forma contínua, en el medio ambiente a través de las aguas residuales.

Sus posibles efectos sobre el sistema endocrino han despertado preocupación por la seguridad en su uso y eliminación, lo que ha llevado a la regulación sobre la presencia de parabenos en los productos comerciales por parte de organizaciones nacionales y transnacionales. Este hecho ha dado lugar a un creciente interés en el desarrollo de métodos sensibles y fiables para su determinación en muestras ambientales, cosméticos y productos para el cuidado de la piel.

La búsqueda de nuevos métodos de extracción que disminuyan el consumo de extractante (con la consiguiente reducción en los costes y en el impacto ambiental) y el tiempo de extracción con respecto a los métodos bien establecidos (extracción líquido-líquido o extracción en fase solida), ha llevado al desarrollo de una serie de métodos de microextracción caracterizados por el uso de volúmenes de extractante del orden de microlitro, que permiten altos niveles de preconcentración. En los últimos años se han descrito varios procedimientos para la determinación de parabenos en distintas matrices utilizando microextracción en fase líquida usando fibras huecas (HF-LPME) [4], pero, hasta la fecha, no se ha propuesto la extracción de estos compuestos mediante electromembranas (EME).

En este trabajo se propone, por primera vez, un método de extracción de parabenos (EtilParabeno, PropilParabeno, EtilParabeno, isoButilParabeno, ButilParabeno) mediante electromembranas. La extracción se lleva a cabo a pH 4 en la fase donadora y pH 12 en la fase aceptora, la extracción se realiza, en estas condiciones, en 40 minutos a 30 Voltios. Los extractos fueron analizados mediante HPLC. Los límites de detección oscilan entre 0,72 y 1,43 $\mu g\ L^{-1}$.

La metodología propuesta no requiere una etapa de *clean-up* previa para muestras de aguas superficiales, obteniéndose factores de enriquecimiento tres veces superiores a aquellos descritos usando HF-LPME. El método propuesto ha sido aplicado a diversas muestras de aguas superficiales, obteniéndose resultados satisfactorios.

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P2

DETERMINATION OF ALDEHYDES IN CORK BASED PRODUCTS USING GAS-DIFFUSION MICROEXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

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Emission of volatile organic compounds (VOC), particularly aldehydes such as formaldehyde and acetaldehyde from cork and wood based products, is a well-known problem [1] that can affect the air quality of indoor environments and human health. Thermal treatments, the use of resins and other industrial processes may lead to the appearance of volatile compounds. Cork products have been vastly used as building materials due to its singular characteristics, such as thermal and sound insulation and for being a renewable and environmental friendly resource.

Gas-diffusion microextraction (GDME) was initially developed for the extraction of volatile compounds from liquid samples, particularly beverages [2], however, more recently was successfully applied to solid samples [3]. The GDME device consists in a small dimension Teflon module, with a microporous hydrophobic membrane at its bottom that allows the diffusion of volatile compounds from the sample to an acceptor solution. The acceptor solution is, in many cases, the derivatization reagent, which helps improve the extraction efficiency and allows the detection of the analyte by high performance liquid chromatography with UV detection.

In this work, the main objective was to determine the amount of aldehydes, such as formaldehyde, in cork based agglomerates using the GDME methodology. Two different derivatization reagents were used as acceptor solution in this study: 2,4-Dinitrophenylhydrazine, which reacts with both aldehydes and ketones, and the acetylacetone reagent (Hantzsch reaction), which is selective for the determination of formaldehyde. This method is based on the cyclization of formaldehyde with a β-diketone, such as acetylacetone, in the presence of ammonium acetate [4].

Using the GDME approach we were able to identify numerous aldehydes, including formaldehyde, benzaldehyde, hexanal and other longer chain aldehydes. The results showed significant differences between the samples studied, and a possible correlation between the results and cork industrial production could be made. Similar formaldehyde concentrations were obtained using the two different derivatization methods, and levels lower than 9.0 mg kg^{-1} were found in the analysed products.

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P3

METODOLOGÍA ANALÍTICA AVANZADA PARA CONTROL DE CONTAMINANTES DE ALIMENTOS PROCESADOS EN PLASMA HUMANO

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Durante el procesado de alimentos se pueden generar compuestos potencialmente tóxicos y marcadores de calidad que ponen en riesgo la salud de los consumidores. Entre ellos cabe destacar el 3-monocloropropano-1,2-diol (3-MCPD), el 1,3-dicloro-2-propanol (1,3-DCP) y el 2,3-dicloro-2-propanol (2,3-DCP). Estos compuestos presentan actividad genotóxica y mutagénica [1].

Debido a la escasez de información sobre la presencia de estos compuestos en matrices biológicas tales como plasma humano, se ha desarrollado un método de análisis eficiente y rápido para la extracción y la determinación de tres cloropropanoles en plasma humano. La preparación de muestra se basa en la microextracción líquido-líquido dispersiva asistida por ultrasonidos (UA-DLLME) [2]. Las condiciones óptimas fueron: 1 mL de muestra; 1,5 mL de acetonitrilo, como disolvente de dispersión; 90 µL de cloroformo, como disolvente de extracción; 50 µL de HFBI (agente derivatizante); 1,7 min de agitación en ultrasonidos a 30 ºC, y 5 min de centrifugación a 3500 rpm.

El método de cuantificación necesita una reacción de derivatización (integrada en el proceso de extracción) seguido de la separación con cromatografía de gases y detección con espectrometría de masas en tándem (GC-MS/MS). Las moléculas se ionizan con una fuente de impacto electrónico (EI) con una energía de ionización de 70 eV, en modo de ion positivo, a una temperatura de 250 °C. El análisis se llevó a cabo en modo MRM (*multi reaction monitoring*). El método fue validado en términos de linealidad, límites de detección (LOD) y cuantificación (LOQ), precisión y exactitud [3]. Se obtuvo un rango lineal entre 5 - 200 ng mL^{-1} para 1,3-DCP, 10 - 200 ng mL^{-1} para 2,3-DCP y 10-400 ng mL^{-1} para 3-MCPD, con coeficientes de determinación de 0.9997. Los límites de detección se encuentran en el intervalo de 0.3-3.2 ng mL^{-1}. La precisión (RSD) varió entre 1.9-10 % (n=9) y las recuperaciones se encuentran entre 91% y 101%.

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P4

OPTIMIZATION OF THE MICROEXTRACTION BY PACKED SORBENT FOR DETERMINATION OF SIX ANTIDEPRESSANTS IN HUMAN URINE

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Depression is a chronic psychiatric illness that can eventually lead to suicidal behavior. Antidepressants use has increased in recent years resulting in a higher consumption. As a result, a many patients are involved in numerous cases of poisoning.

A method of microextraction by packed sorbent (MEPS) followed by high performance liquid chromatography with diode array detector (HPLC-PDA) was developed for the simultaneous determination of six antidepressants in human urine samples.The chromatographic method was performed using aXBrigde^{TM}Shield RP 18 column(250 x 4.6 mm, 5 µm particle size) and a mobile phase composed of acetonitrile and phosphate buffer 1mM (pH 3) at a flow of 0.7 mL min^{-1} in the gradient elution mode, obtaining a total analysis time of 10 minutes.

During the method optimization, the extraction parameters as the type of sorbent material, type and volume of elution solution, number of extraction cycles, volume and pH of sample, type and volume of washing solution were studied.

Anasymmetric screening design ($2^2 3^3 4^1$//16) was used as a screening method in order to select the variables that have influence on the MEPS efficiency procedure, providing the following conditions: 300 µL of sample volume, 50 µL of MeOH as elution solvent, 0.5 min of drying time and a C8-SCX as sorbent phase. The parameters, such as pH of sample and number of strokes, were optimized using aCCD (α=1.414) response surface design in further combination with desirability providing the following conditions: pH 7 and 10 strokes.

The analytical method was validated by determining the linearity, selectivity, limits of detection and quantification, precision and accuracy.

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P5

DETERMINATION OF UV FILTERS IN BATHING WATERS BY COUPLING STIR BAR SORPTIVE-DISPERSIVE MICROEXTRACCIÓN TO THERMAL DESORPTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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UV filters are organic compounds present in a wide variety of cosmetics, taking advantage of their ability to absorb or reflect solar ultraviolet (UV) radiation both in the UVA and UVB ranges [1]. In order to guarantee consumers' health, the compounds that can be used as UV filters in cosmetics and their maximum allowed concentrations are regulated in each country [1]. However, the large use of cosmetics containing these compounds has led to their release into the aquatic environment either by direct (e.g. swimming) or indirect (e.g. wastewater) sources [2]. Furthermore, different studies have demonstrated that some UV filters, even at trace levels, may have a negative impact in the flora and fauna of the aquatic ecosystem [3, 4]. Hence, from an environmental point of view, it is becoming increasingly important to develop sensitive and selective analytical methods to monitor their concentration at trace levels. Moreover, in order to improve the method sensitivity and/or eliminate some potentially interfering compounds, extraction techniques are usually needed.

Recently, the authors of this work have presented for the first time a new microextraction technique termed stir bar sorptive-dispersive microextraction (SBSDμE) [5] that combines the principles of SBSE and DSPE into a single method. It is mediated by the use of a stir bar coated with surface modified magnetic nanoparticles (MNPs) as sorbent material. At low stirring rate, it acts as SBSE, whereas at higher rates MNPs are dispersed as in DSPE. This approach affords a series of advantages to both SBSE (lower extraction time and nanosorbents with various coatings can be employed depending on the analytes) and DSPE (easier extraction and post-extraction treatment, reducing the manual intervention).

The aim of this work was to expand the analytical utility of SBSDμE to the determination of trace amounts of UV filters in water samples by coupling it, for the first time, to thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS), using a simple, effective and solvent-free desorption step. Under optimized conditions, a MNPs-coated stir bar was immersed in 25 mL aqueous standard (or sample) solution adjusted to pH 4 and 5 % NaCl (w/v), and highly stirred for 30 min at room temperature. Then, stirring was stopped and nanoparticles collected onto the stir bars, which were removed and placed into a glass tube to accomplish TD directly coupled to GC-MS.

The method was successfully validated showing good linearity, limits of detection and quantification in the low ng L^{-1} range and good intra- and inter-day repeatability (RSD < 12 %). The developed approach was applied to two environmental waters (river and sea) and swimming pool water with satisfactory recovery values (80 – 116 %), indicating that the approach enables to determine these organic compounds at (ultra)trace level.

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P6

MESOPOROUS SILICA BASED MCM-41 AS SOLID-PHASE EXTRACTION SORBENT COMBINED WITH MICRO-LIQUID CHROMATOGRAPHY-QUADRUPOLE-MASS SPECTROMETRY FOR THE ANALYSIS OF PHARMACEUTICALS IN WATERS

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Even though the important development of instrumentation in Analytical Chemistry, sample preparation is a crucial step to achieve enough sensitivity and/or specificity when determining multiple analytes at trace levels in complex samples.

Silica and carbon based mesoporous materials, as well as surface modified mesoporoussilicas, have been used to remove organic contaminants from water [1] and for sample preparation for extraction of metal ions, adsorption of organic compounds, size selective enrichment of peptides/proteins, specific capture of post-translational peptides/proteins and enzymatic reactor for protein digestion [2].

This work reports the first application of the silica based mesoporous material MCM-41 as sorbent in solid phase extraction (SPE), to pre-concentrate pharmaceuticals of very different polarity (atenolol, nadolol, pindolol, timolol, bisoprolol, metoprolol, betaxolol, ketoprofen, naproxen, ibuprofen, diclofenac, tolfenamicacid, flufenamicacid andmeclofenamicacid) in surface waters. The analytes were extracted from 100 mL water samples at pH 2.0 (containing 10^{-3} mol L^{-1} of sodium chloride) by passing the solution through a column filled with 100 mg of MCM-41.

Following elution, the pharmaceuticals were determined by micro-liquid chromatography and triple quadrupole-mass spectrometry. Two selected reaction monitoring transitions were monitored per compound, the most intense one being used for quantification and the second one for confirmation. Matrix effect was found in real waters for most analytes and it was overcome using the standard addition method, which compared favourably with the widely used matrix matched calibration method. The detection limits in solvent (acetonitrile:water 10:90, v/v) ranged from 0.01 to 1.48 $\mu g\ L^{-1}$ and in real water extracts from 0.10 to 3.85 $\mu g\ L^{-1}$. The quantitation limits in solvent were in the range 0.02 - 4.93 $\mu g\ L^{-1}$, whereas in real water extracts were between 0.45 and 10.00 $\mu g\ L^{-1}$. When ultrapure water samples were spiked at two concentration levels of each pharmaceutical (0.1 and 0.2 $\mu g\ L^{-1}$) and quantified using solvent based calibration graphs, recoveries near 100% were obtained except for tolfenamic, flufenamic and meclofenamic acids. However, recoveries for all the pharmaceuticals were comparable or better than de described above, when river water samples (spiked at the same concentration levels) were quantified by the standard addition method and slightly worse using the matrix matched calibration method.

Five real samples (two river, one dam and two fountain water samples) were analyzed by the developed method, atenolol, timolol, betaxolol, nadolol and diclofenac being found in some of them, at levels higher than theirs quantitation limits.

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P7

EFFECT OF PHYSICAL PROPERTIES OF HOLLOW FIBERS USED IN LIQUID MICRO-EXTRACTION OF METALS FOR ENVIRONMENTAL ANALYSIS: SILVER TRANSPORT AS A MODEL SYSTEM

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Popularity of hollow fiber liquid micro-extraction (HFLPME) for metals analysis has grown during the last decade, and the number of publications regarding HFLPME increases every year. HFLPME has been applied in environmental samples for analysis of metals at trace level as well as for speciation studies. In this regard polypropylene fibers Accurel PP 50/280, Accurel PP Q3/2 and Accurel PP S6/ have been the most widely used.
Most of the systems applied for metals transport using hollow fiber extraction consist in a three phase configuration. In this configuration the metal is transported from the sample to the acceptor, inside the fiber, through an organic solution containing a ligand that facilitates metal transport from the sample to the acceptor solution.

Up to the date, optimization of pre-concentration systems is based on the study of the effects of chemical conditions, but criteria about fiber selection are not reflected in the literature. Inspite of fibers differ in pore size, porosity, wall thickness, etc., which can affect efficiency and/or selectivity, the influence of these factors is not normally taken into account.
In this work Ag^+ transport using tri-isobutylphosphine sulfide (TIBPS) has been used as a model to evaluate differences in metal transport due to the properties of the different fibers. Silver transport using TIBPS takes place by coupled co-transport. In particular, NO_3^- is required in the sample to facilitate Ag complexation by TIBPS and $S_2O_3^{2-}$ has been used as acceptor agent inside the fiber lumen. In this work the chemical and hydrodynamic conditions have been optimized for the three fiber types, and the results have been compared. Additionally, the effect of different fiber type on silver transport has been evaluated. Moreover, the effect of physical properties of fibers on speciation capability, of fibers, in the presence of salinity and organic matter has been studied.

Fibers Accurel PP 50/280 that present a higher effective surface showed the highest efficiency for metal transport, Accurel PP Q3/2 present intermediate efficiency but easier handling; finally, Accurel S6/2 offer poorer efficiency but the highest stability and in some cases they present more capability for speciation due to a higher wall thickness. For these reasons, it is important to clarify the criteria for fiber selection depending on the objective they are used for.

P8

ELECTROMEMBRANES AS A TIME EFFICIENT METHOD TO PRE-CONCENTRATE CADMIUM IN NATURAL WATERS

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Cadmium is considered to be one of the most toxic metals in the environment, being its presence in natural waters from both natural and anthropogenic sources, with deleterious effects on living organisms even at very low concentrations. To measure this metal concentration, some of the most frequently available analytical techniques, such as spectrometry, are not sensitive enough and, so, a sample pre-treatment is usually needed. One of the drawbacks of this step of a chemical analysis is related with the high solvent and chemicals consumption.

In this work a HF-LPME process by electromembranes for the pre-concentration of Cd in natural waters has been optimised using a multivariate methodology. The hollow fiber employed was Accurel PP S6/2 with the pores filled by octanol, as the organic solvent, and the lumen filled by nitric acid. The hollow fibers were immersed in the sample solutions containing 0.1 μg L^{-1} Cd(II). The response variable was the enrichment factor (EF), defined as follows:

$$EF = \frac{[Cd]_{Strip}}{[Cd]_{Feed}}$$

with $[Cd]_{strip}$ and $[Cd]_{feed}$ the Cd concentration in the receiving solution and initial Cd concentration in the sample, respectively.

First of all, a factorial design was performed to study those variables which affected EF the most, namely, extraction time, voltage applied, stirring rate, HNO_3 concentration, pH of the sample and fiber length. Secondly, a response surface methodology was applied to study extraction time, voltage applied, stirring rate, HNO_3 concentration and fiber length further.

By these experimental design, a 10.5 min agitation time, 73 V, 350 rpm, 0.49 M HNO_3 and pH 4.5 were chosen as the most adequate.

Under these optimum conditions, the proposed methodology was successfully applied to the determination of cadmium in different natural waters.

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P9

AN INNOVATIVE ARRANGEMENT FOR IN-VIAL MEMBRANE ASSISTED LIQUID-LIQUID MICROEXTRACTION. APPLICATION TO THE DETERMINATION OF ESTERS OF PHTHALIC ACID IN ALCOHOLIC BEVERAGES

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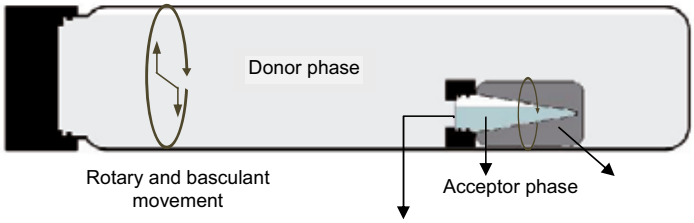
In membrane assisted extraction, the kinetics of the extraction strongly depends on the area of the membrane and agitation conditions. These two variables very significantly affect the characteristics of the different devices for membrane assisted extraction. Useful arrangements based on either tubular or flat sheet membranes have been described.

In this communication, an innovative setup that allowed effective agitation of both donor (aqueous) and acceptor (organic) phase is presented (Figure). It complements other known arrangements based on flat sheet membranes, as in-vial membrane liquid-liquid extraction [1] and stir membrane liquid-liquid microextraction (SM-LLME) [2].



Operational advantages of the presented arrangement are: a minimal handling and a low risk of accidental contamination or losses of analytes and sample carryover. The extraction scheme does not need any dedicated equipment than a simple roller mixer. Moreover, the reduced membrane surface wasted for extraction reduces the cost of the determination. It requires a long extraction time, but, a reasonable extraction time per sample is achieved running several extractions in parallel.

Using toluene as acceptor phase, phthalic acid esters (PAE) were extracted from alcoholic beverages (red wine, white wine, beer, low alcohol beer and sangria) and determined by gas chromatography-mass spectrometry. Matrix effects were detected, and consequently, the method of standard additions was used. As extensively reported, most of analysed alcoholic beverages were PAEs contaminated, being di-*n*-butyl phthalate a fairly frequent contaminant, as already reported for wine samples [3].

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P10

COMPARISON OF TWO IL-DLLME APPROACHES FOR THE DETERMINATION OF BENZOYLUREA INSECTICIDES IN WASTEWATER USING LIQUID CHROMATOGRAPHY-QUADRUPOLE-LINEAR ION TRAP-MASS SPECTROMETRY: EVALUATION OF GREEN PARAMETERS

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Dispersive liquid-liquid microextraction (DLLME) has been successfully applied to the extraction and concentration of a wide variety of pesticides from water samples [1, 2]. It was developed in 2006 by Rezaee and co-workers [3] and is based on the complete dispersion of the extractant solvent into the sample in order to enlarge the contact area between the extractant and the solution so that the equilibrium state is achieved in a short time. The advantages of DLLME method are simplicity of operation, rapidity, low cost, high recovery, high enrichment factor and decreasing waste generation [4].

Two dispersive liquid-liquid microextraction (DLLME) approaches including temperature-controlled ionic liquid dispersive liquid-liquid microextraction (TCIL-DLLME) and ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction (US-IL-DLLME) were compared for the extraction of six benzoylurea insecticides (diflubenzuron, triflumuron, hexaflumuron, teflubenzuron, lufenuron and flufenoxuron) from wastewater samples prior to their determination by high-performance liquid chromatography with a hybrid triple quadrupole-linear ion trap-mass spectrometer (LC-QqLIT-MS/MS). Influential parameters affecting extraction efficiency were systematically studied and optimized and the most significant green parameters were quantified and compared. The best results were obtained using the US-IL-DLLME procedure, which employed the IL 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) and methanol (MeOH) as extraction and disperser solvent, respectively. US-IL-DLLME procedure was fast, easy, low environmental toxicity, as well as it was able to successfully extract all selected benzoylureas. This method was extensively validated with satisfactory results: limits of detection and quantification were in the range 0.5-1.0 ng L^{-1} and 1.5-3.5 ng L^{-1}, respectively, whereas recovery rates ranged from 89 to 103 % and the relative standard deviations were lower than 13.4 %. The applicability of the method was assessed with the analysis of effluent wastewater samples from a wastewater treatment plant located in an agricultural zone of Almería (Spain) and the results indicated the presence of teflubenzuron at mean concentration levels of 11.3 ng L^{-1}. US-IL-DLLME sample treatment in combination with LC-QqLIT-MS/MS has demonstrated to be a sensitive, selective and efficient method to determine benzoylurea insecticides in wastewaters at ultra-trace levels.

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P11

ANALYSIS OF PESTICIDES WITH DIFFERENT POLARITY IN ENVIRONMENTAL WATERS USING SOLID PHASE EXTRACTION WITH CARBON NANOTUBES FOLLOWED BY LIQUID CHROMATOGRAPHY-QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETRY

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The most popular technique for preconcentration of contaminants in water samples is solid-phase extraction (SPE) using hydrophobic non-selective sorbents such as C18 and styrene-divinylbenzenepolimers [1]. However, nowadays, novel sorbent materials are being used in SPE, carbon nanotubes (CNTs) among them. These sorbents interact strongly with organic molecules via non-covalent forces such as hydrophobic, electrostatic and van der Waals interactions, hydrogen bonding and π stacking and these interactions, along with theirs hollow and layered structures, which involve large surface-to-volume ratios, makes them good candidates for use as sorbents, specially multi walled carbon nanotubes (MWCNTs) [2].

As for analytical techniques, liquid chromatography and electrospray ionization hybrid quadrupole linear ion trap (LC-QqLIT) mass spectrometry is an hybrid technique offering numerous advantages [3]. The QqLIT is a triple quadrupole in which the third quadrupole can be operated as either a quadrupole or an ion trap. In this way, the same analyzer can be run in two different modes, retaining the classical QqQ scan functions, such as selected reaction monitoring (SRM), while providing access to sensitive IT experiments.

In this work, a SPE method using packed MWCNTs as sorbent has been developed for the pre-concentration of eight pesticides in environmental water samples. After optimization of the main factors affecting the enrichment efficiency into the solid phase, 100 mL of water sample modified with 1% methanol, adjusted at pH 5.0 and containing 0.005 mol L^{-1} KCl were passed through 20 mg of MWCNTs at a flow rate of 1 mL min^{-1} and then eluted with 3 mL of acetone/*n*-hexane, 50:50 v/v. In order to fulfill the EU guidelines, two selected reaction monitoring transitions were monitored per compound, the most intense one being used for quantification and the second one for confirmation [3]. In addition, an information dependent acquisition (IDA) experiment was performed for unequivocal confirmation of positive findings.

Matrix effect was not found in real waters and, therefore, the quantitation was carried out with calibration graphs built with solvent based standards. With the exception of cymoxanil, detection and quatitation limits in surface waters were in the range 0.3-9.5 ng L^{-1} and 1.6-45.2 ng L^{-1}, respectively. Recoveries in ultrapure water were about 100%, except for the most polar pesticides (methomyl and cymoxanil) and the same behaviour was kept in all real water samples, in addition to a decrease in recoveries for the less polar pesticide phosalone in three real samples. The behaviour of phosalone can be explained because it interacted with the organic matter present in the water samples, reducing its adsorption onto the MWCNTs. Finally, the precision study yielded RSD (%) lower than 10% in all cases.

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P12

CARBON NANOTUBES FUNCTIONALIZED POLYDIMETHYLSILOXANE BASED SORBENT PHASES FOR IN-TUBE SOLID PHASE MICROEXTRACTION COUPLED TO CAPILLARY LIQUID CHROMATOGRAPHY.

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Carbon nanotubes (CNTs) opened a new investigation area in the development of alternative sorbent materials for in-tube solid phase microextraction (IT-SPME). Owing to their special characteristics, c-CNTs can establish strong interactions with organic molecules, via no-covalent forces, such as hydrogen bonding, π-π stacking, electrostatic forces and hydrophobic interactions [1]. In addition, CNTs surface can be chemically modified, resulting in functionalized sorbents in which different interactions with organic molecules are considered [2, 3].

The objective of the present work was to evaluate the performance of CNTs-PDMS coatings as extractive phase for IT-SPME coupled to Cap-LC. Carboxylic SWNTs (c-SWNTs) and carboxylic MWNTs (c-MWNTs) have been immobilized on a PDMS capillary column and their adsorption capacity has been compared with the adsorption capacity of commercial PDMS capillary columns. In addition, the percentage of diphenyl groups (5% and 35 %) on the PDMS sorbents has also been considered. The different capillary coatings have been tested for target analytes such as triazines, PAHs, organophosphorus and pyrethroids. Parameters such as extraction efficiency, precision and stability have been compared in order to elucidate the potential advantage of using CNTs-PDMS as extractive phase for IT-SPME as function of the structure of the target analytesand the different interactions that can take place between the analytes and the c-CNTs present in the sorbent material. The results demonstrated that a significant enhancement of the adsorption capability of PAHs was achieved with the c-SWNTs-$PDMS_{TRB-5}$ capillary column, mainly owing to π-π interactions. This sorbent phase also showed improved results compared with unmodified $PDMS_{TRB-5}$ for trade triazines. Pyriproxyfen and chlorpyrifos are better extracted by c-MWNTs-$PDMS_{TRB-35}$ and c-MWNTs-$PDMS_{TRB-5}$, respectively. Thus, it can be concluded that the adsorption capability of the target compounds towards CNTs-PDMS capillary columns depends not only on the polarity (log Kow), but also on the interactions that the analytes can establish with the CNTs. Therefore, the structure of the analytes plays a key role for practical applications of c-CNTs based PDMS sorbents for IT-SPME coupled to a chromatographic system. However, if all tested compounds want to be screened the best capillary option is the sorbent c-SWNTs-$PDMS_{TRB-5}$ achieving a good compromise bearing in mind its extraction efficiency.

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P13

NQS DOPED PDMS COMPOSITE AS AN ALTERNATIVE ENVIRONMENTAL OBSERVATION TECHNOLOGY: APPLICATION TO ESTIMATE NH_3 EMISSIONS FROM ANIMAL PRODUCTION INDUSTRIES

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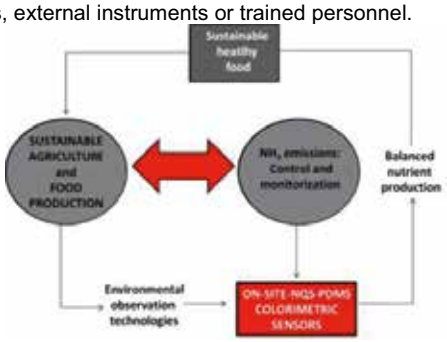
Ammonia and ammonium determination, control and monitorization in sustainable food production and agriculture is mandatory. As an example, the use of metabolic-by-products as nutrient to plants grown give rise to sustainably healthy food. Their viability and sustainability depends on the balance of N-production, and mainly on the NH_3/NH_4^+ content. In particular, NH_3 emissions from animal food production industries on sensitive ecosystems are an important issue, as conservation agencies seek to reliable methodologies for protecting the environment and human health [1]. Herein, the objective of the present work was the development of a green, simple and rapid colorimetric passive sensor to determine NH_3 in atmosphere from animal production industries. The proposed colorimetric device is based on the immobilization of the chemoresponsives dye1,2-Naphtoquinone-4-sulfonate (NQS) into Polydimethylsiloxane-Tetraethylortosilicate-SiO_2 nanoparticles composite [2-4]. When the resulting sensor is exposed to an atmosphere in presence of NH_3, this analyte is visually detected due to the change of colour from yellow to brown. The intensity of the colour is related to the concentration of NH_3 in air, and so quantitative analysis was carried out by measuring diffuse reflectance of the sensing membrane after the exposure to ammonia. Different sensor compositions and exposure times have been optimized. Under the optimum conditions, analytical parameters such as sensitivity, reproducibility and selectivity have been established. Satisfactory LOD was achieved (1 ppmv, exposure time= 72 h) taking into account the expected NH_3 concentrations (5-15 ppmv). Reproducibility was also tested, providing adequate results (RSD<13%). Finally, NH_3 concentration in a poultry farm atmosphere has been estimated with satisfactory results. The main advantages of the proposed device are the simplicity of the whole procedure, since reagent preparation is completely reduced and its portability. It is also a cost-effective and energy efficient device for its use in NH_3 monitoring campaign and routine analysis since it does not need power supplies, external instruments or trained personnel.



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P14

MAE *vs* MSPD FOR THE EXTRACTION OF PAHs IN MOSS

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In the last decades, the mosses are used as an alternative way of monitoring air quality, and nowdays an European Standard proposes the sampling protocol and the preparation of mosses to monitor the atmospheric contaminants (EN 16414-2014) [1]. Due to their morphological and physiological characteristics, mosses can accumulate pollutants from air making them excellent tools for biomonitoring of pollutants, such as Polycyclic Aromatic Hydrocarbons- PAHs [2]. These pollutants, generated by the incomplete combustion of organic materials, have carcinogenic and teratogenic properties [3], posing a great concern for human health.

Two different analytical procedures for the extraction of 16EPA PAHs in a complex matrix as moss samples were compared in this work: microwave assisted extraction (MAE) and a matrix solid-phase dispersion (MSPD). The determination of PAHs was performed in both cases by programmed temperature vaporization-gas chromatography-tandem mass spectrometry (PTV-GC–MS/MS). The comparison between the two proposed methods was performed taking into account not only the analytical parameters, but also the green analytical chemistry criteria.
The methods were validated for one moss species used as moss monitor in ambient air, obtaining similar high recoveries for both methods, between 83–116%, and good intermediate precision, lower than 11% for both.

Both methods were also compared with other procedures reported in literature for the analysis of PAHs in moss samples. MAE and MSPD, shown similar analytical method performance characteristics than the methods in literature. Nevertheless, when the green analytical chemistry principles are considered, the proposed techniques present many advantages in comparison with the classical procedures. Specially, the MSPD achieves a considerable reduction of solvent volume and total analysis time, using a very low amount of sample, and achieving very good quantitation limits. The low sample amount and time consumption permit to analyse a high number of sample replicates and minimize the variability intrinsic to biological matrices.

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P15

DETERMINATION OF DISSOLVED REACTIVE PHOSPHORUS USING DIRECTLY SUSPENDED DROPLET MICROEXTRACTION FOLLOWED BY MICROVOLUME UV-VIS SPECTROPHOTOMETRY

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Dissolved reactive phosphorus (DRP) is considered the fraction of phosphorus that contributes the most to the eutrophication of surface waters [1]. DRP refers to the fraction of phosphorus that responds to chemical reactions without previous hydrolysis or oxidation after filtration through 0.45 μm membrane filters. An analytical methodology for the determination of DRP in water samples is presented herein. The proposed method is based on the formation of 12-molybdophosphate malachite green ion pair, extraction of the ion pair by a microvolume of methyl isobutyl ketone, and determination with no dilution using a microvolume spectrophotometer [2, 3]. Under optimal conditions, the enrichment factor, defined as the ratio between the analyte concentration in the final extract and its initial concentration in the sample, was found to be 275 in 7.5 min. The limits of detection and quantification were determined as 6.1 and 20.5 nM, respectively. The repeatability, expressed as relative standard deviation, was 2.7% (n = 6). The proposed method was successfully applied to the determination of DRP in aqueous matrices, showing recovery values in the range of 95-104%.

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PROGRESS ON SAMPLE PREPARATION METHODS: ELECTROMEMBRANES FOR NICKEL PRECONCENTRATION IN NATURAL WATERS

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Analysis of trace metals in natural waters is a field of interest because of their potential toxicity and bioaccumulability. Some of them (Cd, Pb, etc.) are considered toxic even when they are present at very low concentration levels. On the other hand, metals such as Cu, Ni, etc., are essentials for normal cell growth at low concentrations but can be toxic when they are present at higher concentrations. In this sense, although Ni can be normally found at trace level in natural waters, it accumulates in organisms causing bioaccumulation through the trophic chain. As a consequence, monitoring its concentration in natural waters is of main concern. However, due to the very low detection limits required to determine natural concentrations of this metal, a preconcentration step previously to the instrumental analysis is still necessary.

There have been developed a great variety of methods to perform metals preconcentration from natural waters, with hollow fiber liquid phase microextraction (HF-LPME) as a clear alternative owing to several advantages such as simplicity, higher enrichment factors and lower waste generation. From the different configurations available for these systems, the three phase mode appears as the most suitable to metal analysis. In this configuration, the analyte is transported from the sample to a receiving solution inside the fiber (only a few microliters) through an organic phase (liquid membrane) inside the pores of the hollow fiber walls. Besides the advantages of these systems, they still present some drawbacks mainly related with relatively long preconcentration times. In order to increase the extraction speed, the application of an electrical potential difference as the driving force has been introduced, giving raise to the electromembrane extraction.

In this work, a HFLPME method based on electromembranes for Ni preconcentration from natural waters was optimized. Ni is transported through the pores of an Accurel PPS/2 hollow fiber impregnated with di-2-ethylhexyl phosphoric acid (dEHPA) dissolved in kerosene and into its lumen (containing a few microliters of a nitric acid solution). In order to get the maximum enrichment factor, the effect of several parameters such as extractant concentration in the organic phase, nitric acid concentration in the receiving solution, stirring rate, extraction time, applied voltage and fiber lengthwas studied.Under optimized conditions, an enrichment factor (EF) of 180 was obtained after 15 minutes of extraction.

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FABRIC PHASE SORPTIVE EXTRACTION FOR THE DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN WATER SAMPLES

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Since the inception of solid-phase microextraction (SPME) in 1989, techniques based on the sorption over a solid support have been progressing constantly in order to solve the limitations over the extraction efficiency. In this sense, stir-bar sorptive extraction (SBSE) and later, extraction with low-cost polymers was proposed. Here, the use of fabric phase sorptive extraction (FPSE) is proposed for the efficient extraction of four of the most consumed non-steroidal anti-inflammatory drugs (NSAIDs) in water, particularly ibuprofen (IBU), naproxen (NAP), ketoprofen (KET) and diclofenac (DIC). FPSE has been recently developed [1] and is based on the "sol-gel" technology over a polymeric fabric support.

For the extraction of IBU, NAP, KET and DIC, several parameters relevant to extraction efficiency were studied by means of a mixed level factorial design ($3^1 \times 2^2$) where the sorbent chemistry, matrix pH and ionic strength were evaluated simultaneously as well as their possible interactions. Three sorbents of different polarities sol-gel poly(dimethyldiphenylsiloxane) (sol-gel PDMPS), sol-gel poly(tetrahydrofuran) (sol-gel PTHF) and sol-gel poly(ethylene glycol) (sol-gel PEG)), sample pH in the range 2-12 and ionic strength (by addition of NaCl) from 0 to 30% made the domain of the experimental design. Also, three central-points were included in a total of fifteen experiences. Under the optimized conditions the most polar material (PEG) was selected, the pH fixed at 2 (in concordance with the acidic character of the analytes) and no salt addition. For the sample volume, 15, 30 and 70 mL were studied and although the chromatographic response is doubled when the volume increases from 15 to 30 mL, with the use of 70 mL the response remains practically at the same level. So, the intermediate value (30 mL of sample) was selected for further experiments. One of the most relevant premises of FPSE is to achieve the equilibrium in a very short time, due to its open geometry and thin-film of inherently porous sol-gel sorbent. In fact, this was confirmed by performing the extraction kinetics in the mentioned conditions. Thus, the equilibrium was reached in two hours for KET whereas for the rest of analytes values ranged from 45 to 90 min. In order to decrease the variability of the method, 2 h was the time selected to carry out the extraction. The next step was the desorption of the extracted analytes on FPSE media into a solvent, in this case ethyl acetate was selected according to the good compatibility with the later derivatization reaction and the subsequent determination by gas-chromatography-mass spectrometry (GC-MS). The desorption took place in 1 mL of ethyl acetate for 15 min. The extract was further evaporated to 40 µL and derivatization was performed with 10 µL of N-methyl-N-*tert*-butyldimethylsililtrifuoroacetaminde (MTBSTFA) for a period of 60 min at 60 ^{o}C

The method was validated in ultrapure water and the limits of quantification were in the low ng L^{-1} (2-15 ng L^{-1}), the linearity was excellent in the range tested (LOQ-20 µg L^{-1}) and the enrichment factor oscillated from 162 to 418 times, that could be translated in an extraction efficiency of 27-70%. The method was applied to influent, effluent and river water samples and no matrix effects were observed with relative recoveries from 92 to 116% and RSDs lower than 15%.

Finally, the concentrations detected in the influent showed an increase in the concentration of IBU (up to 10-15 µg L^{-1}) from previous studies. Further, KET and DIC seems to be more recalcitrant with levels between 90-450 ng L^{-1} in the effluent. All the NSAIDs were also detected in surface water at concentrations from 26 to 130 ng L^{-1}.

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GAS-DIFFUSION MICROEXTRACTION AND SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION FOR THE DETERMINATION OF BIOGENIC AMINES IN SOLID SAMPLES

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Biogenic amines (BA) are nitrogenous organic compounds of low molecular weight widely found in foods and beverages. They are mainly formed by decarboxylation of amino acids and can be generally divided into three main groups according to their chemical structure: aliphatic, aromatic and heterocyclic [1]. Traditionally, a liquid-liquid extraction or a solid-phase extraction process is used for the extraction of BA from foods, followed by gas or liquid chromatography. In solids an extraction in acid medium before a purification step is usually performed [2].

In this work, two innovative extraction procedures are proposed and compared for BA screening in cheese: gas-diffusion microextraction (GDME) [3] and salting-out assisted liquid-liquid extraction (SALLE) [4]. The extraction with GDME is especially directed towards volatile or semi-volatile amines, while SALLE can be used for both volatile and non-volatile amines. Both extraction methodologies present clear advantages over traditional techniques due to their simplicity, quickness and minimum extraction steps involved in the process, while being cost-efficient.

The GDME approach was developed to be a quick and simple microextraction methodology for the analysis of volatile amines. In this procedure BA are extracted with GDME and a pre-column derivatization of the extracted analytes is performed prior to HPLC-UV analysis. Several extraction conditions were evaluated, such as the time of extraction, pH and temperature of extraction. Different types of cheese were analysed and concentrations of methylamine, dimethylamine and ethylamine ranging from 2.3 ± 0.3 to 17.5 ± 0.9 mg kg^{-1} were found.

The SALLE procedure uses the salting-out effect as the basis for a liquid–liquid extraction. The addition of salt to a mixture of water and water-miscible organic solvent promotes a biphasic system and the partition of solutes between the two liquid phases. Several extraction parameters were optimized, such as pH, derivatization conditions of the analytes and organic phase volume in order to achieve better extraction of BA to the organic phase. This procedure, which allows to simultaneously perform the extraction and derivatization was successfully applied for the determination of several BA in different types of cheese.

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NEW APPLICATIONS OF GAS-DIFFUSION MICROEXTRACTION (GDME)

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Membrane-based extraction can be used to develop simple, high throughput, low cost and low solvent consumption sample preparation procedures. Besides extracting the analytes from the sample matrix to avoid the interference of other sample components in the analysis, it is possible to obtain a clean extract that will preserve the analytical instrument. A recently patented technique named gas-diffusion microextraction (GDME) has been developed in our research group [1, 2]. The extraction process is based on the compounds transfer from the sample (donor phase) through a gas-permeable membrane into an acceptor phase, usually liquid. The membrane embodies a thin air space inside its pores, and the mass transfer occurs by diffusion of the analytes in the gas form across the gas layer separating the two phases. That very small air gap accelerates the analytes extraction, especially those with low volatility that are not extracted by headspace techniques such as solid-phase microextraction (SPME). Since microextraction is used, the extraction is not exhaustive allowing monitoring the concentration of analytes along time without significantly altering the studied sample. GDME can be combined with labelling (or derivatisation) to improve separation and/or detection of the analytes. The use of a labelling reagent in the acceptor phase enhances the extraction selectivity and the enrichment factors. GDME has been used for the analysis of volatile compounds in beverages, namely important quality markers like diketones, aldehydes and biogenic amines [1, 3-4]. However, the versatility of GDME allows its use in a wide range of applications [5].

In this work, the application of the GDME technique to solid samples is presented. Different types of solid samples (popcorn, corn silage) and analytes (diketones, ammonia, amines) were studied. The influence of membrane nature, temperature of extraction, extraction solvent, mass of sample used for extraction and calibration procedure were some of the studied parameters.

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USE OF MAGNETIC MOLECULAR IMPRINTED POLYMERS (MAG-MIPS) FOR THE SIMULTANEOUS EXTRACTION OF 16 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN WATER SAMPLES

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Polycyclic aromatic hydrocarbons (PAHs) are a group of about 10000 compounds consisted of fused aromatic rings, not containing heteroatoms or carrying substituents. Most of the PAHs in the environment derive from incomplete burning of carbon-containing materials, such oil, wood, coal or garbage. These pollutants have a high persistence in the environment, low biodegradability and high lipophility, some of them being highly toxic [1]. Some decades ago, the US Environmental Protection Agency (EPA) included PAHs in the list of the 129 priority pollutants (U.S. EPA 1982). Official methods for analysis of PAHs include sample pre-treatments which involve some kind of extractions (liquid-liquid, solid-liquid) with the use of large amounts of organic solvents like hexane, methylene chloride or isooctane. (ISO 17993, 2004; EPA, 1989; EPA, 1990).

In the last years, processes like liquid-phase microextraction (LPE), solid-phase extraction (SPE) and solid phase microextraction (SPME) have been included in sample treatments, prior the analysis, for clean-up and preconcentration of PAHs in different kind of samples [2,3]. On the other hand, it is well known that PAHs are a class of highly lipophilic compounds without any pronounces functional groups in their molecules, non-covalent interactions like hydrogen bond, dipolar or ionic interactions that could be formed between PAHs and functional monomer. Taking into account this fact and in order to improve the selectivity of the micro-extractions, molecular imprinted polymers (MIPs) have been used as stationary phase, in SPE as well as in SPME, taking advantage of their unique imprinted and recognition properties. MIPs-based SPE has been demonstrated to be an efficient pre-treatment sample in a number of proof concept studies as well as for pre-concentration of environmental samples [4,5].

A new, rapid, simple and low cost method based on magnetic molecular imprinted polymers, is proposed for the simultaneous extraction of 16 PAHs. The PAH-magMIP used in this work is composed by crosslinked vinylic polymer; magnetite (Y-Fe_3O_4) content 5% w/w, 3 µm diameter, being the saturation magnetization 1.52 emu g^{-1}, supllied by NanoMyp®. Extraction was carried out in less than 30 minutes. LODs range between 1,3 ng L^{-1} for AN and 24,3 ng L^{-1} for NA. LOQs range between 4,33 ng L^{-1} for AN and 80,92 ng L^{-1} for NA. When DAD was used (for ACL), LOD and LOQ were 969 ng L^{-1} and 3226.77 ng L^{-1}, respectively. Compared to other mag-MIP-based pre-treatments reported in the literature which only some of the PAHs were extracted, this protocol is able to extract the 16 PAHs from water samples, is simple, low organic solvent consuming and recycled, being environmentally friendly compared to official methods for the extraction of PAHs.

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MÉTODO AUTOMATIZADO PARA LA DETERMINACIÓN DE POLIFENOLES EN JALEA REAL MEDIANTE CROMATOGRAFÍA DE FLUJO TURBULENTO-UHPLC-ORBITRAP-MS

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Aunque mucho menos conocida que la miel, la jalea real posee un gran potencial debido a las propiedades beneficiosas que se le atribuyen. En este sentido, es importante conocer la composición de los diferentes compuestos que posee, con el fin de ser capaces de atribuir correctamente sus propiedades, así como garantizar la calidad del producto para los consumidores. Entre sus componentes, aunque muy poco investigados en este tipo de matriz, se encuentran los polifenoles.

En el presente trabajo, se muestra el desarrollo de una metodología analítica que permite un análisis rápido y automatizado de los polifenoles presentes en la jalea real mediante la aplicación de cromatografía de flujo turbulento (Turboflow^{TM}). De este modo, y a pesar de la gran cantidad de dificultades que puede presentar el análisis de este tipo de matriz, por ser una sustancia gelatinosa con gran cantidad de carbohidratos, proteínas y lípidos [1], la aplicación de este procedimiento de limpieza permite simplificar la etapa de tratamiento de la muestra, que por parte del operador solo implica una simple dilución de la misma. A través de la columna de Turboflow, se consiguen eliminar todas aquellas posibles interferencias que de alguna manera podrían interferir en la ionización de los compuestos objeto de estudio, así como en la separación cromatográfica. La columna de Turboflow se acopla a un cromatógrafo de líquidos de ultra alta eficacia, empleando como sistema de detección un espectrómetro de masas de alta resolución tipo Orbitrap (UHPLC-Orbitrap-MS). Este acoplamiento, permite una rápida limpieza de la muestra, separación de los compuestos y una fiable determinación de los mismos. De este modo, sin la necesidad de llevar a cabo un método de extracción previo, se realiza el análisis en un tiempo total de 18 minutos, incluido el tratamiento de la muestra mediante la columna de Turboflow. Este sistema de limpieza requiere de tres fases móviles, compuestas por una solución acuosa de ácido fórmico al 0.5 %, metanol, y una tercera fase de limpieza de la columna de Turboflow, compuesta por la mezcla de acetonitrilo, acetona y 2-propanol, en la proporción 4:3:3 (v/v/v).

Para una correcta identificación de los compuestos, se desarrolló una base de datos con 52 polifenoles, obteniendo tanto su tiempo de retención, modo de ionización, ión característico y fragmentos necesarios para su correcta confirmación
El procedimiento analítico desarrollado fue validado para todos los polifenoles de la base de datos, obteniendo adecuados límites de cuantificación que van desde 10 hasta 150 µg/kg. La linealidad fue hasta 2000 µg/L, con unos coeficientes de determinación (R^2) superiores a 0.994 en todos los compuestos validados. La metodología desarrollada se aplicó al análisis de 9 muestras de jalea real, confirmando la presencia de 15 polifenoles diferentes en las muestras analizadas. En relación a los compuestos determinados, los ácidos fenólicos son los compuestos detectados a mayor concentración, representando un porcentaje superior al 68 % de la cantidad total de polifenoles en todas las muestras. Otros compuestos detectados pertenecen a la familia de las flavanonas, flavonas, flavonoles e isoflavonas en porcentajes muy inferiores (< 24 %).

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DISOLVENTES SUPRAMOLECULARES CONSTITUIDOS POR NANOESTRUCTURAS HEXAGONALES VOLÁTILES PARA LA SIMPLIFICACIÓN DEL TRATAMIENTO DE MUESTRAS BIOLÓGICAS

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El Bisfenol A (BPA), uno de los compuestos químicos con mayor producción mundial, se ha considerado tradicionalmente un estrógeno débil. Sin embargo, numerosos estudios in vitro realizados recientemente han demostrado que el BPA puede alterar la función normal del sistema endocrino y producir perturbaciones en las funciones celulares a concentraciones tan bajas como 0,23 ng L^{-1}. El consumo de alimentos embotellados y enlatados constituye la principal vía de exposición humana al BPA, ya que este compuesto se usa en la fabricación de plásticos policarbonatados y resinas epoxi utilizados en contenedores alimentarios y en recubrimientos internos de latas de conservas y bebidas. El Bisfenol A migra desde estos contenedores a las bebidas y a los alimentos, donde se pueden encontrar a concentraciones en los intervalos 0,1-3,4 ng mL^{-1} y 0,3-458 ng g^{-1}, respectivamente. Estudios recientes han puesto de manifiesto que el BPA está presente en un 95% de las muestras de orina humana analizadas (concentración media=0,72 ng mL^{-1}) [1], por lo tanto, es fundamental disponer de métodos sencillos, rápidos, económicos y respetuosos con el medio ambiente que permitan determinar bajas concentraciones de BPA en orina para evaluar la exposición humana a este contaminante.

Hasta la fecha, los métodos desarrollados para la determinación cuantitativa de BPA en orina requieren tratamientos de muestra largos y complejos, que generalmente incluyen extracciones repetitivas con grandes volúmenes de disolventes orgánicos o SPE, seguidas de una etapa de purificación. La cuantificación se lleva a cabo mediante cromatografía líquida con detección fluorimétrica o acoplada a espectrometría de masas.

En este trabajo se propone el uso de un nuevo disolvente supramolecular constituido por agregados inversos de hexanol para la extracción del BPA presente en muestras de orina humana con el objetivo de simplificar la etapa de tratamiento de muestra. El procedimiento implica la adición de 82,5 µL de hexanol y 150 µL de tetrahidrofurano a 1,267 µL de orina previamente hidrolizada. A continuación se agita la mezcla durante 7 min para favorecer la incorporación del analito al disolvente supramolecular generado *in situ* y después, se centrifuga para acelerar la separación de fases. Para eliminar interferencias de la matriz de la muestra, 75 µL del extracto obtenido (123 µL) se llevan a sequedad con nitrógeno y posteriormente, el analito se disuelve en 300 µL de una mezcla metanol:agua (50:50). El extracto purificado se analiza usando cromatografía líquida-espectrometría de masas. El límite de cuantificación del método es 0,022 ng mL^{-1} y la precisión, expresada como desviación estándar relativa, del 4,5%. La aplicabilidad del método se estudió analizando 20 muestras de orina. El BPA se encontró en el 100% de las muestras a concentraciones en el intervalo 0,14-0,70 ng mL^{-1}. Las recuperaciones obtenidas para muestras adicionadas con 1 ng mL^{-1} de BPA oscilaron entre el 96 y el 110%.

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THREE-PHASE SOLVENT BAR MICROEXTRACTION AS A SIMPLE ALTERNATIVE TO ANALYSE CADMIUM IN WATER SAMPLES

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Cadmium is considered to be one of the most toxic metals in the environment, with deleterious effects on living organisms even at very low concentrations. For this reason, it was included in the list of priority hazardous substances under the Water Frame Directive and the determination of its concentration in natural waters is a field of interest. Nevertheless, it is not an easy issue due to very low detection limits are required to determine natural concentrations of this metal. A preconcentration step previously to the instrumental analysis is still necessary.

Among the variety of methods available to perform metals preconcentration from natural waters, hollow fiber liquid phase microextraction (HF-LPME) presents several advantages as simplicity, high enrichment factors and short extraction times. These systems can adopt different configurations, being the three phase mode the most suitable to metal analysis. In this configuration a piece of a microporous polimeric support, the hollow fiber, is immersed in the sample with its lumen filled with a few microliters of an aqueous solution. The extraction is performed from the sample to the receiving aqueous solution through an organic phase placed in the pores of hollow fiber. In addition, it can be talked of a three-phase solvent bar microextraction system when the ends of the fiber are sealed and it is tumbled freely in the sample solution.

In this work, an available method for cadmium preconcentration from seawater using a bulk liquid membrane was optimized in a three-phase solvent extraction system [1]. In order to get the maximum enrichment factor, the effect of several parameters was studied such as the pH of the sample, the extractant concentration in the organic phase, the nitric acid concentration in the receiving solution, the stirring rate and the extraction time. Under optimized conditions, an enrichment factor (EF) of 262 was obtained after 180 minutes of extraction.

In addition, the effect of sample's salinity on the enrichment factor was also tested. A decrease in the EF was observed when sample´s salinity increase, so the use of citrate as mask agent was also tested.

Finally, applicability of the system to the analysis of cadmium in saline and no-saline waters was tested.

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NOVEL FABRIC PHASE SORPTIVE EXTRACTION METHOD FOLLOWED BY UHPLC-MS/MS FOR THE ANALYSIS OF BENZOTRIAZOLE UV STABILISERS IN SEWAGE SAMPLES

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Fabric phase sorptive extraction (FPSE) is a novel sample preparation technique developed by Kabir and Furton [1], which is a highly sensitive, fast, efficient and solvent minimized sample preparation approach [2]. FPSE integrates the advantages of sol–gel derived hybrid inorganic-organic sorbents and the flexible, permeable and rich surface chemistry of cellulose fabric, containing approximately 400 times higher sorbent loading than SPME fibers and providing better extraction efficiencies. Moreover it is a cheap device that can be reused and do not suffer from coating damage, unlike to SPME fibers or stir bars.

We have developed a procedure based on FPSE followed by ultra-high performance liquid chromatography with mass spectrometry detection for the determination of seven benzotriazole UV stabilizers (BUVSs) compounds in sewage samples from different wastewater treatment plants of Gran Canaria island. BUVSs are a group of compounds added in sunscreens and other personal care products, which can present negative effects over aquatic systems. They are mutagenic in bacterial systems and toxic in plants and can exert adverse effects on the fecundity and reproduction of fish [3].

The proposed method was optimized evaluating all the parameters involved in both extraction (sorbent, extraction time and ionic strength) and back extraction process (desorption time, solvent and volume). Under the optimized conditions, this methodology allows enrichment factors of 10 times with detection limits ranged from 0.07 to 0.50 $ng \cdot mL^{-1}$ and intra and inter-day RSDs lower than 11 and 30 % for all compounds, respectively.

The sample preparation procedure was then applied to determine the presence of target analytes in liquid samples from different wastewater treatment plants of Gran Canaria Island (Spain). Two compounds were measured in ranges of 17.0-60.5 $ng \cdot mL^{-1}$ (UV 328) and 73.3-99.3 $ng \cdot mL^{-1}$ (UV 360) in the three sewage water samples analysed.

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APPLICATION OF SALTING-OUT LIQUID-LIQUID EXTRACTION (SALLE) TO THE EXTRACTION OF POLYPHENOLS FROM BLUEBERRIES

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Salt-assisted liquid-liquid extraction (SALLE) is a homogeneous liquid-liquid extraction technique that has been receiving considerable attention for sample preparation of various types of samples. The procedure consists in adding a water-miscible organic solvent to an aqueous sample followed by the addition of a salt (or a mixture of salts) to induce the phase separation between water and the organic solvent, due to the salting-out effect. This process is very interesting for sample preparation since extraction of compounds to the organic solvent can occur, being the analysis performed over the organic phase [1].

Studies over the formation of two liquid phases in acetone-water-inorganic salts mixtures go back to the early 20th century. Since then, the interest in this type of liquid-liquid extraction procedure has been increasing, particularly for the analysis of biological samples [2]. Main advantages of SALLE are its low cost, simplicity and low solvent consumption. Besides, the extracts are readily compatible with various instrumental techniques such as gas and liquid chromatography [1, 3-4].

In this work the application of SALLE for the determination of polyphenols in blueberries is presented. Samples were extracted with acetonitrile-water-ammonium sulphate by decoction at room temperature, followed by centrifugation to improve liquid phases' separation. Finally, the organic extract was collected and analysed by high performance liquid chromatography with UV detection. The extracts' composition was characterized by liquid chromatography with mass spectrometry detection for compounds identification and confirmation.

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SALTING-OUT LIQUID-LIQUID EXTRACTION FOR THE DETERMINATION OF POLYPHENOLS AND AROMA COMPOUNDS IN PLANTS

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The salting-out effect has an interesting application for the creation of liquid biphasic systems in mixtures composed by water-miscible organic solvents and water. Adding salts, two distinguishable liquid phases are formed in which the upper phase is mainly composed by the organic solvent. Simultaneously, solutes can be extracted to the organic layer, resulting in a salting-out assisted liquid-liquid extraction (SALLE) [1]. The interest for this type of extraction procedure for analytical purposes has been increasing and it is the basis of the QuEChERS methodology [2, 3].

The application of SALLE to plants is well documented but mainly aims the analysis of contaminant compounds (pesticides, toxins). However, the potentialities of SALLE are vast and prone to be further explored. In this work, the use of SALLE for the chemical profiling of fennel (*Foeniculum vulgare*) seeds extracts is presented. It is a novel approach for SALLE, and a simple and environmentally friendly sample preparation tool for phytochemical characterization. Samples were extracted by decoction with an acetonitrile-water mixture (50:50, v/v) using ammonium sulphate to promote phase separation and the solutes partition to the organic layer. The extracts were analyzed by liquid chromatography with UV and mass spectrometry detection (HPLC-UV-MS/MS) and by gas chromatography with mass spectrometry detection (GC-MS). For GC-MS analysis, a dispersive solid-phase extraction (*d*-SPE) clean-up of the extracts was needed to remove residual water and other interferences.

The organic extracts analysis by HPLC and GC showed that it is possible to separate a wide range of compounds from the samples, allowing a very complete phytochemical characterization of the fennel seeds. By HPLC-UV-MS/MS analysis of the organic extract it was possible to identify various phenolic compounds, including quercetin derivatives, caffeic acid, coumaric acid and chlorogenic acid. The main compounds identified by GC-MS were estragole, fenchone, anysaldehyde, anethole, benzaldehyde, camphor and apiol.

The proposed methodology enables, in only one step, the separation of a broad range of compounds with distinct properties.

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ESTUDIO METABOLÓMICO DE LOS EFECTOS DEL ESTRÉS AMBIENTAL SOBRE COMPUESTOS BIOACTIVOS EN FRESAS MEDIANTE UHPLC-ESI-MS/MS

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En España la fresa es uno de los cultivos más importantes, especialmente en el suroeste de la península, en la provincia de Huelva gracias a las condiciones climáticas y a la calidad del agua que presenta esta zona. La importancia económica de este cultivo y su gran competitividad en el mercado, obliga a los productores a desarrollar nuevos métodos de cultivo, tales como el cultivo sin suelo [1] y el uso de variedades tempranas. Estas variedades entran en el mercado en la temporada de invierno con una mayor calidad del fruto, mayor resistencia al estrés (biótico y abiótico) así como con una mayor productividad.

La composición química de la fresa se ve afectada por una serie de factores pre y poscosecha. Entre los factores precosecha, la variedad es considerada la principal fuente de variación en la composición. Por otra parte, la calidad nutricional y nutracéutica también se encuentra influenciada por las condiciones de cultivo (climatología y técnicas de cultivo), el tiempo de muestreo y el grado de madurez, entre otros factores [2-5].

La metabolómica es una disciplina emergente dentro de la química analítica enfocada en la identificación de moléculas de bajo peso molecular (metabolitos). Inicialmente, sus principales aplicaciones se desarrollaron dentro del campo farmacéutico, pero hoy día, ha llegado a ser una poderosa herramienta en el campo de la agricultura y de la ciencia de los alimentos, siendo utilizada para caracterizar los cambios metabólicos en plantas después de ser sometidas a un estrés biótico o abiótico.

El objetivo del presente estudio se centró en los cambios en la composición de metabolitos secundarios (30 compuestos fenólicos) en respuesta a distintas condiciones de cultivo. Para ello se analizaron 54 muestras de fresas pertenecientes a tres variedades con distinta sensibilidad a las condiciones ambientales (Camarosa, Festival, Palomar) que crecieron en cultivo sin suelo con diferentes condiciones agronómicas (conductividad eléctrica, sustrato y cobertura).

La determinación de los compuestos bioactivos se realizó mediante UHPLC-ESI-MS/MS lo que permitió un incremento significativo en la sensibilidad del método y una reducción del tiempo de análisis respecto a los estudios realizados con anterioridad en nuestro grupo [6].

El análisis multivariante de los datos obtenidos, puso de manifiesto que los ácidos benzóicos, cinámicos, los flavan-3-oles, y la actividad antioxidante se ven influenciados de forma significativa ($p < 0.001$) por la cobertura y la variedad. Además, se observó que el sustrato tiene un efecto significativo sobre los flavonoles, mientras que la variedad afecta al contenido en ácido elágico y sus derivados así como en antocianos.

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ISOLATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM INFUSION AND COFFFEE BY SOLID-PHASE EXTRACTION AND DETERMINATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Polycyclic aromatic hydrocarbons (PAHs) are hazardous compounds; by its toxicity they are included by European Union and US Environmental Protection Agency (EPA) as a priority pollutant, PAHs represents the largest group of compounds with mutagenic, carcinogenic and teratogenicity properties. Their effect over environment and living organisms is usually widespread and permanent. At room temperatures, PAHs are colourless to yellow solids. The general characteristics are their high melting and boiling point, low vapor pressures, low water solubility and high lipid solubility. They are resistant to degradation, which is given by the complexity and stability of their molecular structures. The biodegradation rate decrease drastically with an increase of the number of aromatic rings. They are classified among the semivolatile organic compounds, having boiling points greater than 200ºC. Almost hundred different PAHs have been already identified but 16 are considered as priority because they are supposed to be more dangerous than others. In general, PAHs are not presented individually, they are always in mixtures. PAHs that have been extensively monitored are the compounds included in the United States Environmental Protection Agency (US-EPA) list of priority organic pollutants (the so-called 16 EPA PAHs) [1]. Since 2005, the European Union (EU) list of PAHs has also been included in the monitoring studies [2]. In beverages, like tea and coffee, the presence of these compounds could be produced by environmental pollution (e.g., fire, thermal reactions, or contaminated water, air and soil) or by cooking processes as roasting, smoked and grilling. In tea and coffee the main source of PAHs is roasting process [3].

The need for reliable data about the levels of PAHs in infusion and coffee samples is needed in order to establish new maximum allowed. In this sense, analytical laboratories play an important role, since they must have adequate methods for the analysis of PAHs in food and beverage. PAHs are presented in food samples at low levels and analyte enrichment is commonly required before chromatographic determination. In solid matrices an homogenization for the extraction of analytes is required followed by centrifugation of the mixture, the presence of fatty compounds difficult the extraction and a saponification process is needed [4]. The use of solid-phase extraction (SPE) for sample treatment is bound to substantially improve analytical sensitivity and throughput while minimising sample manipulation. For the analysis of PAHs in food samples, gas chromatography (GC) has been the most common analytical separation mechanism in combination with a number of sampling approaches and sample-preparation steps. The preference for GC over other systems [e.g., liquid chromatography or fluorescence detection] has been demonstrated by such factors as its greater selectivity, resolution, and sensitivity. To improve selectivity and to ensure the robustness of sample identification and quantification, GC is often used in combination with mass spectrometry (MS) to produce very powerful GC-MS coupled techniques, which are able to yield even lower limits of detection than GC alone [5].

A new analytical method for the isolation and determination of PAHs is reported combining continuous SPE system and GC-MS, including naphthalene, anthracene, fluoranthene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene. Samples were preconcentrated using a continuous SPE system and the analytes retained were eluted with ethyl acetate. A systematic overview is given of the advantages and disadvantages of several sorbents in the retention of PAH compounds and based on sensitivity, selectivity and reliability. Different parameters as amount of sorbent, eluents, flow system variables were investigated. The method provided good accuracy and precision in the determination of the PAHs at ng L^{-1} level in brewed infusions as white, black, green and red teas and roasted and natural coffee (caffeinated and decaffeinated).

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PHTHALATE DETERMINATION IN TEA SAMPLES BY SPE-ESI-LC-MS

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Phthalates are a group of chemical compounds widely use in industry and commerce due to their variety of uses. Because of their ability to increase the softness and flexibility of plastic, they are use as plasticizer in all of types of packaging. Tea is usually commercialized in tea bags, commonly made of silk, nylon and filter paper, containing tea leaves. The main drawback during the tea infusion preparation is that phthalates can migrate from the bag material to the infusion due to the high temperature of the water. Furthermore, certain phthalates and or their metabolites are suspected to be human carcinogenic agents and endocrine disruptors, which make their determination particularly important [1, 2].

A method for the determination of phthalates in tea infusion samples by Solid Phase Extraction-Liquid Chromatography-Mass spectrometry has been developed. The four phthalates studied were dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), diethyl phthalate (DEP) and dimethyl phthalate (DMP). Molecular Imprinted Polymer (MIP) was used as sorbent for SPE. The MIP was prepared via precipitation polymerization using DBP as template, methacrylic acid (MAA) as a monomer, ethyleneglycol dimethacrylate (EDMA) as crosslinking agent, acetonitrile as porogen and 2,2´-azobisisobutyronitrile (AIBN) as iniciator.

Phthalates were analyzed in the extract by high performance liquid chromatography (HPLC)-electrospray ionization-mass spectrometry, working in positive mode. Phthalates were separated by HPLC using a ZORBAX Eclipse XDB-C_8 (3.5 µm particle size, 2.1 mm i.d. x 50 mm length) column, working in gradient mode with acetonitrile-ultrapure water starting from 5% to 75% acetonitrile in 5 minutes, followed by isocratic elution for 27 min. The method was sensitive (LOD< 3 µg L^{-1}), precise (RSD<8%) and accurate.

The developed method was applied to the phthalate determination in several tea samples.

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SENSITIVE ELECTROCHEMICAL DETECTION OF SALMONELLA DNA BASED ON SILVER NANOPARTICLES AND HELICASE DEPENDENT ISOTHERMAL AMPLIFICATION

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Salmonella spp. are common pathogens that constitute a major cause of foodborne diseases in humans worldwide. Monitoring the presence of *Salmonella* in food at all stages in the "farm to fork" production chain is the only way for preventing foodborne outbreaks. A limitation of the traditional microbiological methods for *Salmonella* detection in food is that they require 5 to 6 working days to obtain a positive result. In order to overcome this, various rapid methods have been developed, including immunoassays and Polymerase Chain Reaction (PCR) assays. These approaches, however, are still far from being a routine procedure, perhaps hindered by the requirements of high-end instrumentation and skilled personnel.

Thus motivated, we propose here a simple and highly sensitive method for *Salmonella* genome quantification which combines helicase dependent isothermal reaction for target amplification and silver nanoparticles labelling for electrochemical detection. Indium Tin Oxide (ITO) electrodes are modified with a thiolated oligonucleotide capture probe, and serve as sensing platform for the detection of Helicase Dependent Amplification (HDA) products, amplicons. Two different approaches will be evaluated. Firstly, we investigate whether HDA can be performed between a DNA oligonucleotide immobilized on silver nanoparticles (AgNP) as the primer and genomic DNA as template. As a result, amplicons labelled with AgNP can be directly entrapped onto the modified ITO-electrode where the attached nanoparticles can be easily read out by square-wave voltammetry after dissolving with acid (Figure 1). Alternatively, oligonucleotide-functionalized AgNP and unmodified amplicons are cohybridized onto the ITO surface in a sandwich format, performing the detection step in the same way. Both strategies will be comparatively evaluated in terms of amplification efficiency, selectivity and analytical performance.

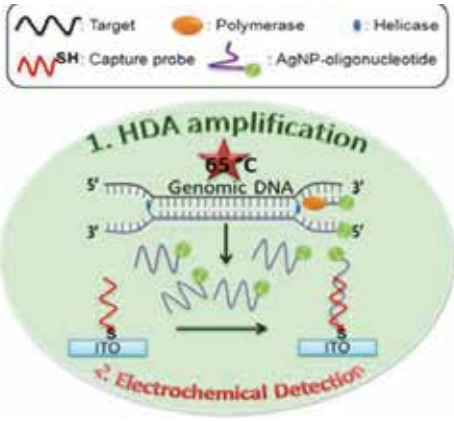

Figure 1: Schematic representation of the assay

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ACCIÓN DE LA LUZ ULTRAVIOLETA SOBRE EL FOTOINICIADOR IRGACURE 651 (2,2 METOXI, 2 FENILACETOFENONA): CONTINUACIÓN

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Los fotoiniciadores son compuestos químicos que pueden transformar la energía luminosa en energía química capaz de iniciar procesos de polimerización. Los fotoiniciadores comerciales, caso que nos interesa, absorben radiación ultravioleta y se utilizan para producir materiales que suelen entrar en contacto con los alimentos. Estos materiales se utilizan como recubrimientos, sellantes, adhesivos y en gran medida como tintas para imprimir envases. Estas tintas poseen formulaciones muy complejas ya que contienen colorantes, pigmentos, momómeros, oligómeros o prepolímeros llamados resinas que son capaces de sufrir procesos de polimerización; y además, contienen fotoiniciadores. Estas tintas se utilizan para imprimir la cara externa de los envases alimentarios y por tanto la no destinada a entrar en contacto con los alimentos. Se suponía que no presentaban ningún riesgo desde el punto de vista de la seguridad alimentaria hasta que surgieron los escándalos del ITX en alimentos infantiles y la benzofenona y 4 metilbenzofenona en panadería y bollería, todos fotoiniciadores. Por ello la preocupación al respecto ha ido aumentando. Teniendo en cuenta que existen fenómenos de trasferencia (migración) del fotoiniciador y de sus derivados, muchos de ellos producidos por acción de la luz UV en los procesos de curado de las tintas que impregnan los envases alimentarios (packging), el interés del conocimiento de estos procesos, desde el punto de vista de la seguridad alimentaria es crucial y por tanto la investigación de los mismos en diferentes alimentos.

Desde nuestro punto de vista hemos querido continuar el estudio [1] ya iniciado en [2] haciendo incidir radiación UV de diferentes lámparas y distintas longitudes de onda, sobre distintos fotoiniciadores. De entre las diferentes que se han probado, de la que nos ocupamos en este estudio, es la que mejor reproduce las condiciones que se dan en la industria de impresión de tintas sobre los envolventes o packaging alimentario, y se han estudiado diversos parámetros que la incidencia de esta radiación UV tiene sobre los fotoiniciadores. Entre los cuales el más utilizado es el Irgacure 651 (2,2dimetoxi,2 fenilacetofenona con nº CAS 24650-42-8) sobre el que basa este estudio y cuyos resultados se han determinado por HPLC inicialmente.

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DISCRIMINACIÓN DE ACEITES DE OLIVA VIRGEN ESTRA (AOVE) EN BASE AL CONTENIDO FENÓLICO Y PERFIL AROMÁTICO

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El Aceite de oliva virgen extra tiene una composición química muy variable [1]. Esta variabilidad depende sobre todo de la variedad de la aceituna, de las condiciones climáticas, agronómicas (riego, fertilización), del cultivo (cosecha, prácticas de maduración) y de la producción (almacenamiento poscosecha, batido, extracción) [2, 3]. Estos factores pueden afectar sustancialmente la composición de triglicéridos, ácidos grasos, esteroles, sustancias fenólicas, metales traza y compuestos volátiles [4].

Los compuestos volátiles en el aceite de oliva son producidos por la oxidación de ácidos grasos insaturados. Enzimas endógenas de la planta como la lipoxigenasa (LOX) son las responsables de la percepción de los aromas positivos del aceite de oliva, mientras que las enzimas exógenas a través de la actividad microbiana, así como de la oxidación química, son las responsables de los defectos sensoriales del producto [5]. Los principales compuestos volátiles presentes en el aceite de oliva virgen son los C6 y C5 aldehídos, alcoholes y ésteres formados a partir de 1,3-hidroxiperóxidos de los ácidos linoléico y linolénico. Por otra parte, el contenido en compuestos fenólicos es un factor importante a tener en cuenta al evaluar la calidad del AOVE [6], ya que estos compuestos tienen una potente actividad antioxidante y contribuyen significativamente a la estabilidad de los mismos contra la oxidación [7]. Estos compuestos han demostrado tener propiedades quimioprotectoras en seres humanos [8] siendo eficaces como anticancerígenos, antioxidantes y anti-inflamatorios, y contribuyendo a las propiedades sensoriales de estos aceites confiriéndoles amargura, acritud y astringencia [9]

Se analizaron 23 muestras de AOVE procedentes de cuatro términos municipales de la provincia de Huelva (Beas, Gibraleón, Niebla y Sanlúcar de Guadiana) mediante cromatografía de gases y líquida para la obtención de los perfiles aromático y fenólico, respectivamente.

La matriz de datos obtenida se analizó mediante técnicas de análisis multivariante. El análisis múltiple de la varianza puso de manifiesto que el origen de los aceites tiene un efecto significativo ($p < 0.001$). Siete compuestos fenólicos y siete compuestos volátiles fueron los que mostraron diferencias estadísticamente significativas entre los cuatro grupos de muestras. Estos resultados se confirmaron mediante técnicas de reconocimiento de patrones. Así mediante análisis discriminante se observó que las dos primeras funciones canónicas obtenidas a partir de todas las variables originales, así como a partir de las seleccionadas como significativas del MANOVA, permiten la diferenciación de los aceites procedentes de los cuatro municipios onubenses. Todas las muestras se clasificaron correctamente en su grupo.

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WATER BASED COLOURIMETRIC SENSORS FOR CO_2 DETECTION USING IONIC LIQUIDS FOR SMART PACKAGING APPLICATION

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In modern food packaging, the atmosphere inside the package is often controlled to inhibit the growth of bioorganisms and extend the shelf life of the food. Often, air is displaced by CO_2 during the packaging process for this purpose, and therefore, the CO_2 level inside meat packages can be used as indicator of freshness. For example, if the CO_2 concentration increases during storage it is a clear indicator that bacteria are growing inside the container as bacteria produce CO_2 through respiration. On the other hand, lower than expected CO_2 levels indicate the package is not well sealed and the modified atmosphere has been compromised. Despite this, an effective non-destructive method for determining the CO_2 concentration within the package has not, as yet, been reported [1].

To this end, the objective of the SmartPack project is to exploit the development and integration of a colorimetric CO_2 sensor in meat packages using the imaging and communications capabilities of SmartPhones for freshness detection.

Colour-based CO_2 sensors are normally based on the acidity of this molecule. However, a drawback of this approach in the food packaging industry arises from the long-term instability of the sensors, due to decomposition of the transfer agent (quaternary ammonium hydroxides). In this project, we avoid the use of these compounds. Instead, CO_2 sensors are prepared using a water-based formulation of thymol blue sodium salt as indicator, glycerol as plasticizer and sodium bicarbonate as buffer in a matrix of hydroxyethyl cellulose. The lifetime of the sensor is therefore increased. Furthermore, this composition creates an easily printable ink. Moreover, the ionic liquid 1-ethyl-3-methyl-imidazolium chloride is included in the matrix making the sensor more selective to CO_2 than other gases due to its higher solubility in this matrix.

This new CO_2-sensitive ink has been optimised and characterized. The next steps include testing of the printed sensors using a digital camera to progress the study towards the intended application as a smart packaging tool.

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PCBs IN TWO OYSTER SPECIES FROM GALICIAN RIAS, NW SPAIN

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Organochlorine compounds (OC), such as polychlorinated biphenyl compounds (PCBs), are bioaccumulated and biomagnified about 200-70000 times along the food chain. Due to their high chemical stability and dielectric properties, they have been used for years in hundreds of industrial applications as hydraulic equipment, pigments and plasticizers.

Bivalve molluscs have been considered as valuable sentinel organisms reflecting the pollution loading of marine environment. Both mussel and oyster were selected as bioindicators in National and International Oceanic Watch Programs in order to document and monitor anthropogenic contamination in coastal environmental [1, 2].

The main path of PCBs in humans is through the diet, mainly seafood and fish. Maximum permissible levels of six indicator sum PCBs (PCBs 28, 52, 101, 153, 138 and 180) have been established by European Commission (EC no 1259/2011) in order to limit dietary exposure to PCBs from fishery products to consumers.

This work presents the levels of ten indicator PCB congeners (IUPAC Nº: 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180) in two species of oyster (*Crassostrea gigas* and *Ostrea edulis*) coming from both intertidal beds and raft collected during the period 2011 to 2014 in the producer Galician Rías, Rías Altas (Barqueiro, Viveiro and Ortigueira), Ría Central (Ferrol) and Rías Bajas (Muros-Noia, Arousa, Pontevedra and Vigo). Three biometric parameters, lipid content, condition index and shell size, have also been investigated.

The aim of this work is to investigate the suitability of the oyster as an indicator of contamination in the Galician estuaries and to know distribution levels of PCBs in terms of food security.

One-way ANOVA revealed a great inter-species variability of shell length and lipid content ($p=0.000$ and $p=0.000$) in studied oyster samples. Only, lipid content was found to have a significant relationship with PCB28, $p=0.046$. No other significant differences between biometric parameters and PCB congeners appeared. PCBs levels in oyster varied from 5.58 to 179.49 ng g^{-1}, d.w. These values are consistent and even lower than previous studies in oyster from other marine environment [1, 3].

Geographical variations in PCBs have been studied by using multivariate statistical techniques. Polluted Rías were clearly separated from unpolluted ones. Oyster samples coming from Ría of Ferrol presented the highest values of PCBs (147.36 ng g^{-1}, d.w.). Samples from Rías Muros-Noia and Arousa were the least contaminated ones (8.12 ng g^{-1}, d.w. and 8.80 ng g^{-1}, d.w.). These patterns were similar to those found in the study of Galician wild mussels [3].

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P35

DETERMINATION OF TOTAL POLAR MATERIALS IN FRYING OILS BY NEAR INFRARED SPECTROSCOPY USING CHEMOMETRIC TECHNIQUES

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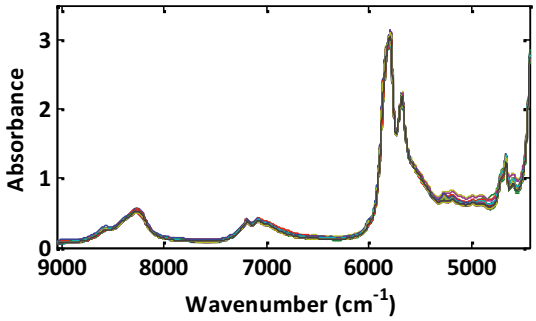
Frying with edible oils is a rapid and popular method of cooking. However, during frying food, the oil is continuously and repeatedly subjected to high temperatures taking place to a series of degradation reactions which generate the formation of a wide range of breakdown products, both volatile and nonvolatile. The non volatile products are accumulating in frying oil, are absorbed by the food and it has negative effects on human health. Spanish Law includes the Standard of quality for oils and fats heated to human consume and in which it is established a maximum level of 25% (w/w) for TPM.

A rapid and direct determination of TPM in frying oils by near infrared spectroscopy using chemometric tools was developed and applied to the analysis of samples. A total of 107 vegetable oils, that contain sunflower and olive oil (extra virgin and virgin) were analyzed. These oils concern 4 groups: i) 19 fried oils were received by Centro Superior de Investigación en Salud Pública (CSISP) of Valencia from restaurants and caterings, ii) 41 fried oils from home, iii) 5 edible oils samples without use and, iv) 42 mixed samples of three above groups. A procedure based on dielectric constant measurements to determine TPM content in these frying oil samples was used as a reference method.

Calibration models was applied to NIR spectral data and TPM values, in order to find the NIR region and the data pre-processing method that provides the best prediction of TPM. These models were created using Forward Stepwise Multiple Linear Regression (FSMLR) and Partial Least Square (PLS) Regression. Before building MLR and PLS models, data set was divided into a calibration and a validation subset with 75 and 32 objects, respectively, by the Kennard-Stone (K-S) algorithm for the calibration set selection. For variable selection in MLR model, stepwise variable selection was used and in PLS regression, interval PLS (iPLS) that selects a subset of variables which will give superior prediction compared to using all the variables in the data set was used.



NIR transmission spectra of fried oil samples

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P36

NEW SPECTROPHOTOMETRIC IMMUNOSENSOR KITS FOR THE DETERMINATION OF OCHRATOXIN A , FUMONISIN B1 AND DEOXYNIVALENOL MYCOTOXINS IN WINES AND CEREALS

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Ochratoxin A (OTA), fumonisin B1 (FB1) and deoxynivalenol (DON) are three of the most important mycotoxins that occur in a variety of foodstuffs such as cereals, cereal products, dried wine fruits, roasted coffee, wines, grape juices or processed cereal-based foods for infants and young children. Owing to its high toxicity in human and animals, the European legislation demands exhaustive analytical controls with the objective of protecting the consumer´s health, by keeping these contaminants in levels which are toxicologically acceptable. The maximum allowed concentration levels in European Union are in the range 0.5-10 μg Kg^{-1} (OTA), 200-4000 μg Kg^{-1} (FB1), and 200-1750 μg Kg^{-1} (DON).

We have developed three new spectrophotometric immunosensors for these mycotoxins, which allow determinations from extracts in about 50 min. Reproducibility is about 6-7 %RSD. Sensitivities are: LOD=0.52 μg kg^{-1} (in cereals), LOD=0.16 μg kg^{-1} (in wines) and EC_{50}=0.15 ng mL^{-1} for OTA; LOD=6.0 μg kg^{-1} and EC_{50}=0.15 ng mL^{-1} for FB1; LOD=5.3 μg kg^{-1} and EC_{50}=8.3 ng mL^{-1} for DON. The LODs (limits of detection) in foods were calculated from the concentrations of the mycotoxins producing a 10% inhibition against the antibody. EC_{50} relates to incubation concentrations of the mycotoxins in wells.

All the immunosensors were statistically validated with certified reference materials and by using official AOAC high-performance liquid chromatography (HPLC) methods. The relative errors were in all cases lesser than about 9%.

The company CAPHER IDI SL (capher@capher.es) commercially offers three kits, with all reagents and consumables included, for the selective determination of OTA, FB1 and DON in concentrations below the legislated. Thanks to improved separations and procedures, these spectrophotometric immunosensors consume smaller volumes of reagents, have faster incubation times, and have improved analytical performance compared with other kits on the market (eg. ELISA).

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NEW ELECTROCHEMICAL IMMUNOSENSOR KITS FOR THE DETERMINATION OF OCHRATOXIN A, FUMONISIN B1 AND DEOXYNIVALENOL IN WINES AND CEREAL-BASED FOODS

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Ochratoxin A (OTA), Fumonisin B1 (FB1), and Deoxynivalenol (DON) are three of the most important mycotoxins due to its occurrence in a variety of foods and high acute and chronic toxicity effects in human and animals. The IARC (International Agency for Research on Cancer) has classified OTA and FB1 as a Group 2B carcinogen (possibly carcinogenic in humans). FB1 (mainly in maize) and DON contamination are mostly found in cereals and cereal-based foods, while OTA can occur in cereals, wine, grape juice, dried wine fruit, cocoa, spices, meat products or coffee.

To protect public health, the maximum levels from legislations of European Union (EU) and other countries are very restrictive. The EU maximum allowed concentration levels are in the range 0.5-10 μg Kg^{-1} (OTA), 200-4000 μg Kg^{-1} (FB1), and 200-1750 μg Kg^{-1} (DON).

We have developed electrochemical immunosensors for the quick, selective, and sensitive determination of OTA, FB1 and DON mycotoxins in concentrations below the legislated (EU). After extraction from cereals or wines, determinations are carried out in approximately 60 min. The cross-reactivities between different mycotoxins are very low (in a range of 1.6-6%). The detection limits (10% inhibition) are: LOD=0.29 μg Kg^{-1} (OTA in cereals), LOD=0.18 μg L^{-1} (OTA in wines); LOD=6.0 μg Kg^{-1} (FB1 in maize foods); and LOD=5.3 μg Kg^{-1} (DON in cereals). The reproducibility of these electrochemical immunosensors is in the range 9-12 %DSR. All these immunosensors were validated with certified reference materials and statistically successfully compared with official AOAC (Association of Official Analytical Chemists) high-performance liquid chromatographic (HPLC) analytical methods.

From this research, the company CAPHER IDI SL (capher@capher.es) has developed and markets three electrochemical immunosensor kits for the determination of OTA, FB1 and DON in foods. These commercial kits allow faster and more sensitive determinations compared with other (eg. ELISA) kits on the market.

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ANÁLISIS DE HIDROCARBUROS AROMÁTICOS POLICÍCLICOS PARA EL CONTROL DE ZONAS DE PRODUCCIÓN DE MOLUSCOS. ALTERNATIVAS ANALÍTICAS EN FUNCIÓN DE LOS OBJETIVOS

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Los hidrocarburos aromáticos policíclicos (PAH) son un grupo de contaminantes ampliamente difundidos en el medioambiente. Su estudio resulta de gran interés por las propiedades cancerígenas y mutagénicas de algunos de ellos [1]. Para hacer el seguimiento de la presencia de estos compuestos en el medio marino, se deben implantar programas de control que permitan conocer su comportamiento y estudiar su distribución espacial y temporal [2,3]. Estos programas, basados en el análisis de organismos marinos como concentradores e integradores de la contaminación, tienen especial relevancia cuando se trata de moluscos bivalvos con destino al consumo humano. A nivel europeo, existe normativa que establece los contenidos máximos permitidos de PAH en productos alimenticios [4,5].

Además de controles programados en fechas determinadas, en ocasiones es necesario afrontar episodios de contaminación marina accidental debido a vertidos puntuales o catástrofes. En estos casos, con frecuencia es necesario realizar el análisis de un elevado número de muestras con la exigencia de obtener rápidos resultados para gestionar adecuadamente el accidente. En este trabajo se describen y comparan dos diferentes aproximaciones analíticas para dar solución a cada una de estas situaciones.

El método de elección para el análisis de muestras programadas, comprende su secado mediante liofilización (tratamiento que permite su conservación a largo plazo). Las muestras secas son extraídas mediante extracción acelerada con disolventes (ASE®) empleando la mezcla acetona:hexano. Posteriormente se realiza una etapa de limpieza mediante cromatografía en columna con adsorbentes, antes de la separación y cuantificación de los compuestos de interés mediante determinación por cromatografía líquida con detector de fluorescencia de longitud de onda programable (HPLC-FD).

Para realizar análisis rápidos de un elevado número de muestras, resulta necesario recurrir al análisis en fresco de las mismas. Se presenta la elección y aplicación de un método QuEChERS, que combina una etapa de extracción y partición con acetonitrilo según la norma EN 15662, con la purificación dispersiva SPE mediante adsorbentes basados en dióxido de zirconio (ZrO_2). La determinación analítica se realiza por medio de HPLC-FD.

Se comparan ambos métodos tanto en su desempeño analítico (precisión, exactitud) como en su conveniencia de acuerdo al tiempo de realización de los ensayos, capacidad de procesado de muestras y coste económico.

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DETERMINATION OF THE MINERAL COMPOSITION OF CAIGUA (*CYCLANTHERA PEDATA*) AND EVALUATION USING MULTIVARIATE ANALYSIS

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Caigua (in Brazil "*maxixe do reino")* is a fruit that is generally consumed either cooked or even raw as salad. This fruit has been used as a food and also in folk medicine. In this work, the mineral composition of Caigua was determined for the first time. Twenty-nine samples from five farms located in the southwestern region of Bahia, Brazil were acquired and analyzed using inductively coupled plasma optical emission spectrometry. The elements determined in this fruit included calcium, magnesium, sodium, potassium, phosphorus, manganese, iron, zinc, copper and vanadium. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to evaluate the obtained results. The average concentrations of the determined elements (expressed as mg kg^{-1}) were as follows: 9.09 for sodium, 1519 for potassium, 194 for phosphorus, 119 for calcium, 84 for magnesium, 0.74 for manganese, 2.11 for iron, 0.13 for copper, 1.27 for zinc and 0.15 for vanadium.

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VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY BASED METHOD FOR THE ASSESSMENT OF THE CO-OCCURRENCE OF MYCOTOXINS IN MAIZE SILAGES FROM DAIRY FARMS IN NW SPAIN.

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Mycotoxins are secondary metabolites naturally produced by filamentous fungi that can cause a carcinogenic, a teratogenic, an estrogenic, a nephrotoxic, a neurotoxic, a hepatotoxic and/or an immunosuppressive response when ingested by farms animals. A broad range of mycotoxins is reported to be present in concentrates, pasture forage and in preserved feeding stuffs, such as silage, which is one of the most important feed sources intended for ruminants. Due to their very chemical and physical resistance, mycotoxins can be transmitted into products derived from the animals. Therefore, the European Union (EU) has adopted rules limiting their maximum levels in feeding stuffs particularly aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, fumonisins, and, more recently, T-2 and HT-2 toxins. In addition, the European Food Safety Authority (EFSA) recommends developing multi-mycotoxin methods because the simultaneous occurrence of mycotoxins in feed is commonplace.

In this way, the objective of this study was the validation of a new efficient and rapid multi-analyte method for the simultaneous detection and quantification of mycotoxins in silage, by reverse-phase liquid chromatography coupled to electrospray ionization triple quadrupole mass spectrometry (LC-HESI-MS/MS). A simple liquid/solid extraction was performed with 1 g of silage and 20 mL of a mixture acetonitrile/water (84:16 v/v) containing 1% acetic acid, with clean-up on Mycospin 400 columns as well as without any clean-up.

Whereas the signals for fumonisins were strongly enhanced, all the remaining target mycotoxins showed high-suppressed signals in presence of matrix. These observed matrix effects emphasized the need to quantitate mycotoxins by means of matrix-matched calibrations.
As regards validation, an alternative method based on the ISO 11843 and on a single factor balanced design was implemented. The achieved average recoveries from spiked samples at three levels ranged from 65 % to 112 % with relative standard deviations (rsd) below 11%. Instrumental Detection Limits (IDLs) and Instrumental Quantification Limits (IQLs) were between 0.2-27.7 μg kg^{-1} and 0.5-92.2 μg kg^{-1}, the lowest values corresponding to aflatoxins. The calculated repeatability and within-lab reproducibility ranged from 3.9 to 11.2 % and from 6.9 to 23 %, respectively. Finally, the decision limit and detection capacity, CCα and CCβ, were calculated for all mycotoxins having regulated/recommended contents in feed. These values were between 10.2 and 5955 μg kg^{-1} and 15.2 and 7367 μg kg^{-1}, respectively.

The validated method was applied to 266 samples collected during two years in 32 dairy farms from Galicia (NW Spain). Zearalenone, deoxynivalenol, fumonisin B1, roquefortine C and mycophenolic acid were quantified in 16 % of the samples. DON and ZEA were the most abundant mycotoxins, but with concentrations always lower than the recommended maximum values in feed.

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DIFERENCIACIÓN GEOGRAFICA DE CAFÉ VERDE A PARTIR DE SU CONTENIDO METÁLICO MEDIANTE TECNICAS DE RECONOCIMIENTO DE PATRONES

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El café es un producto muy consumido en todo el mundo, habiéndose convertido en el segundo producto de comercio del mundo, después del petróleo. Por lo tanto, su cultivo y elaboración repercute de forma importante en la economía de numerosos países productores, principalmente en Sudamérica, América Central, África e Indonesia [1]. Las principales especies de café que se comercializan son *Coffea arabica* L. y *Coffea canephora*, conocidos como café arabica y robusta, respectivamente. El 90% de la producción mundial es de café de tipo arabica [2].

La caracterización de alimentos es un tema muy importante desde el punto de vista comercial, puesto que los consumidores están interesados en productos de una determinada variedad o procedencia geográfica o que hayan sido elaborados bajo unos estándares de calidad concretos. En este sentido, las técnicas de análisis multivariante combinadas al reconocimiento de patrones son herramientas muy útiles. En el caso del café, la aplicación de técnicas de reconocimiento de patrones a la composición química puede emplearse para la diferenciación de variedades u origen geográfico [2, 3].

En este trabajo se propone un método de determinación de Ca, Cu, Fe, K, Mg, Mn, Na, y Zn en muestras de café verde de tres países productores como son Brasil, Colombia y México. Las muestras se digieren con una mezcla de 1:3 de peróxido de hidrógeno y ácido nítrico en un horno microondas y el contenido en los citados elementos se determina mediante espectroscopia de emisión atómica de plasma inducido acoplado. El contenido metálico se emplea como datos de entrada para obtener modelos de clasificación basados en análisis discriminante lineal y modelado suave independiente de analogía de clases, obteniéndose capacidades de predicción del 97 y 94%, respectivamente.

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DIFERENCIACIÓN DE DENOMINACIONES DE ORIGEN ESPAÑOLAS DE PIMENTÓN A PARTIR DE MEDIDAS DEL COLOR Y RECONOCIMIENTO DE PATRONES

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El pimentón es un condimento culinario obtenido de algunas variedades de *Capsicum annuum* L. que se emplea para aportar color y sabor a numerosos alimentos. Desde el punto de vista de la industria alimentaria se suele encontrar en carnes adobadas, sopas, salsas, quesos procesados y aperitivos, y su uso está muy extendido también en la cocina tradicional de varios países. El pimentón contiene capsorubina, capsantina y unos veinte pigmentos carotenoides más que son responsables de su color, así como capsacinoides, como capsaicina e hidrocapsaicina, que le confieren distintos grados de picor. En España, el pimentón se produce principalmente en dos áreas, Murcia y La Vera (Extremadura), ambas reconocidas por la Unión Europea como Denominaciones de Origen Protegidas. Los productos de ambas áreas se obtienen de materias primas de distintas variedades cultivadas en suelos distintos y bajo condiciones climáticas diferentes. Los procedimientos de elaboración, especialmente el secado de los frutos, también difieren. Todos estos factores contribuyen a diferencias en la composición del producto final, pudiendo influir en propiedades como su color y aroma [1].

El color es uno de los atributos sensoriales más importantes de los alimentos, puesto que su apariencia influye en las decisiones del consumidor. La industria alimentaria está generalmente interesada en controlar y asegurar la estabilidad de esta característica. En cuanto a la industria del pimentón, la medida de color se realiza en el momento de la molienda de acuerdo a la escala ASTA, debiéndose cumplir unos requerimientos mínimos para este parámetro [2]. Otra posibilidad es la medida del color en el espacio CIELAB a partir de la radiación reflejada o transmitida por el alimento. A partir de espectros de absorbancia pueden obtenerse los valores triestimulo XYZ que se transforman matemáticamente en las coordenadas L*, a* y b* del espacio CIELAB, que dan cuenta de la luminosidad, la tonalidad amarillo-azul y la tonalidad rojo-verde, respectivamente [3].

En este trabajo se realiza la medida de espectros de absorción UV-Vis de extractos de pimentón en acetona para obtener los parámetros ASTA, L*, a* y b*. Posteriormente se emplearán estos parámetros para la diferenciación de las Denominaciones de Origen Protegidas de pimentón español mediante la aplicación de técnicas de reconocimiento de patrones tales como análisis discriminante lineal, máquinas de vectores soporte y redes neuronales artificiales. Además se proponen modelos de clasificación basados en los espectros completos, previa reducción del número de variables mediante análisis de componentes principales, empleando las mismas técnicas de reconocimiento de patrones.

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ESTUDIO DE LA BIODISPONIBILIDAD DE ELEMENTOS TRAZA EN ARROZ

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El arroz es uno de los cereales más consumidos en el mundo, siendo el alimento principal para la población de muchos países. Este alimento es la fuente de varios elementos traza esenciales para el hombre, como son Ca, Cr, Cu, Mg, Mn, P, K, Mo, Na, Zn S, Fe y Se, pero también de elementos tóxicos como el As, Cd, Pb, Al y Hg. Para evaluar la posible toxicidad de un elemento en un alimento, no sólo es importante conocer el contenido total sino también la biodisponibilidad del mismo.

Este trabajo se ha centrado en el estudio de la biodisponibilidad de As, Cd, Cr, Ni y Pb en diferentes muestras de arroz. Para estimar la biodisponibilidad de estos elementos se ha empleado un método de digestión *in Vitro* utilizando membranas de diálisis (1,2). Con este método se simula el proceso de digestión gástrica (pepsina, pH=2) seguido del proceso de digestión intestinal (pancreatina y sales biliares, pH=7,5) empleando membranas de diálisis (10 kDa) para simular el proceso de absorción que tiene lugar en esta etapa en el intestino.
Para realizar este estudio se escogieron 5 muestras de arroz de diferentes tipos y marcas comerciales, adquiridas en supermercados locales

Previa a la determinación del contenido total de los elementos estudiados en las muestras de arroz se ha realizado una digestión ácida asistida por microondas.
Tanto la determinación del contenido total del elemento en el digerido ácido de la muestra de arroz y en la fracción absorbible (dializada) se realizaron mediante Espectrometría de Masas con Plasma Acoplado por Inducción (ICP-MS).

Se ha detectado As y Cr en todas la muestras estudiadas pero solo en una se ha detectado Cd y Ni, y en otra diferente Pb. El contenido total de As encontrado varía entre 0,2 y 0,3 µg/g y los porcentajes de dializabilidad obtenidos entre un 40 y un 50%. En el Cr, el contenido total está entre 0,2 y 0,3 µg/g en cuatro de las muestras analizadas y presenta una concentración de 6,9 µg/g en una muestra compuesta por una mezcla de arroces (arroz largo vaporizado, rojo y de gramínea). La biodisponibilidad del Cr varía con el tipo de muestra (incluso en muestras en las que el contenido total es similar) de un 4 a un 100%. Hay que resaltar que el valor mas bajo (4%) corresponde a la muestra con el contenido en Cr mas elevado.

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Los autores desean a gradecer a la Xunta de Galicia (Grupo de Referencia Competitiva 6RC2014/2016) y a la Unión Europea (*European Regional Development Funds* 2007–2013 FEDER), *Infrastructure Program* UNST10-1E-491 (Ministerio de Economía y Competitividad) la financiación recibida.

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ASSESSMENT OF OLIVE OILS' HYDROPHILIC PHENOLIC COMPOUNDS BY THE NEW KINETEX BIPHENYL CORE-SHELL COLUMN

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Despite of its well known fatty acid profile, olive oil is also an important source of hydrophilic phenolic compounds, which are associated to stability, sensory and nutritional properties of the oil. Several methods have been proposed for the identification and quantification of hydrophilic phenolic compounds in olive oil by means of RP-HPLC [1, 2], being silica-based octadecyl (ODS) bonded phases among the most widely used of all the commercially available phases. So far, very little is known about the performance of other types of stationary phases on the separation of these compounds. Thus, in this study, three different columns were used to address separation of the hydrophilic phenolic extract of an olive oil sample, applying the same HPLC conditions (gradient elution between H_2O with 0.5% acetic acid and Acetonitrile). The columns used were (A) a Kinetex Core-shell Biphenyl, (B) a LiChrospher RP-18, and (C) a Spherisorb ODS2.

In Figure 1 the phenolic compounds separation profile is compared with the three used columns. In terms of peak capacity (P_c), column A shows 15% and 45% higher peak capacity than B and C, respectively, proving that better peak shapes (lower peak width) may be obtained. As for selectivity (α), between peaks 2 and 3, a better separation was observed by column A, with a value of 1.43, while for columns B and C values of 1.38 and 1.37, respectively, were obtained. Regarding the retention factor (k), also the best results were obtained for column A, with a value of 1.46 for peaks 2, comparing to column C where for the same peak a value of 1.94 was obtained. Overall, a much better resolution was able to be obtained when applying the Kinetex Biphenyl column. As for the quantification of the identified hydrophilic phenolic compounds, better results were also observed with the use of column A, with values of 6.70 mg/L, 50.82 mg/L and 61.49 mg/L for peaks 1, 2 and 3, respectively, in comparison to the results obtained for column C (3.83 mg/L, 31.58 mg/L and 49.31 mg/L for peaks 1, 2 and 3, respectively). Regarding the quantification with column B, similar results to column A were obtained.

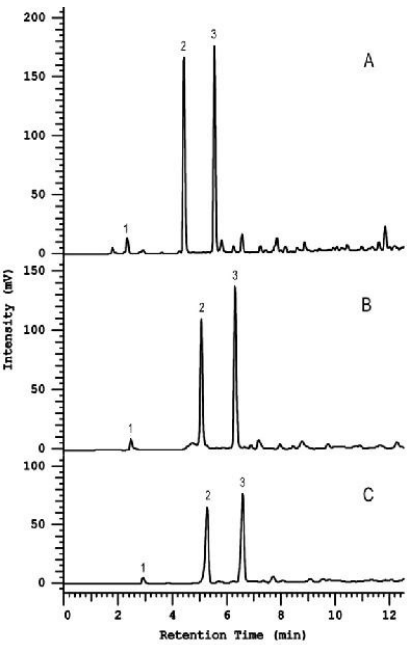

Figure 1. Chromatographic separation of olive oil's phenolic extract with (A) Kinetex Biphenyl column, (B) LiChrospher RP-18 column, and (C) Spherisorb ODS2 column, with gradient elution of H2O/ACN. Peak identification: 1- Dihydroxyphenylglycol; 2- Hydroxytyrosol; 3- Tyrosol.

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SPANISH RED WINES ANALYSIS BY UHPLC-HRMS/MS (ORBITRAP) ANDCLASSIFICATION ACCORDING TO GEOGRAPHICAL ORIGIN BY TARGETED AND UNTARGETED APPROACHES

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The quality of wines, and their commercial and economic values, depends on several issues such as geographical origin, grape variety, vintage and wine making conditions, and climate [1]. It is important to protect the quality properties of wines for both the consumers and the producers, considering the adulteration and false labelling cases that may occur in this excellence product area. Spain, together with Italy and France, is one of the main wine producers in Europe and in the world, and wine sector is of primary relevance because of economic, industrial and cultural matters, principally for these countries. Different analytical methods to authenticate and classify wines according to their geographical origins and grape varieties have to be always kept up-to-date to avoid frauds [2]. In the present work, several red wine samples from different regions of Spain were analysed. The work aimed at exploring a suitable method to classify wines according to their geographical origin based on untargeted (metabolic fingerprint) and targeted (polyphenolic profiles) analytical approaches.

147 Spanish red wines from 7 different areas were analysed by UHPLC-HRMS/MS (Q-ExactiveOrbitrap, Thermo Fisher Scientific). Separation was performed by standard gradient elution in a Supelco Ascentix Express C18 (150x2.1 mm, 2.7 µm) porous-shell column using 0.1% formic acid water and acetonitrile solutions as mobile phases. Full scan MS (m/z 100-1,500) at a resolution of 70,000 FWHM (full-width half-maximum) and data dependent MS/MS product ion spectra at a resolution of 17,500 FWHM were used for both metabolic fingerprint and polyphenolic profile acquisition. A chromatographic separation in less than 22 min employing a conventional reversed-phase method was proposed for the wine characterization by UHPLC-HRMS/MS analysis. The only sample preparation required was a filtration of the red wine samples prior injection. Blank (ACN) samples and two standard mixtures of polyphenols were employed as quality controls. Data obtained was treated by means of chemometrics in two different ways following the untargeted and targeted approaches.

On untargeted approach, full scan MS raw data was considered as metabolic fingerprint to be treated by principal components analysis (PCA). After correcting and improving MS signal quality by using specific filters, full scan MS metabolic fingerprints allowed wine classification depending on their geographical origin. An interesting pattern distribution regarding the different wine production areas was observed. On the targeted approach, polyphenolic signals were employed for wine characterization. For that purpose, MS data was processed by ExactFinderv2.0 software (Thermo Fisher Scientific) by applying a customized target database list of polyphenols. Retention time, accurate mass errors, isotopic pattern matches, and product ion scan spectra were used to confirm the identity of compounds when necessary. After further processing of polyphenolic profiles by removing signals with peak scores lower than 0.65, the most remarkable polyphenols of each wine production area were identified and selected to achieve characterization of Spanish wines by PCA.

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SÍNTESIS Y APLICACIÓN DE POLÍMEROS DE IMPRONTA MOLECULAR PARA EL ANÁLISIS DE MICOTOXINAS DE LA *ALTERNARIA* EN MUESTRAS DE TOMATE

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Las micotoxinas son metabolitos secundarios producidos por ciertas especies de hongos. Estos compuestos aparecen en condiciones favorables de crecimiento del hongo responsable y la contaminación del producto puede ocurrir en cualquier punto de la cadena alimenticia, desde la cosecha, pasando por la recolección, almacenaje, transporte, elaboración y conservación del alimento. Entre todas las micotoxinas conocidas, destacan aquellas producidas por la especie *Alternaria alternata* aisladas con frecuencia de una gran variedad de productos alimenticios como vegetales frescos y procesados, cereales o aceites, entre otros. Las toxinas más representativas producidas por este hongo incluyen el alternariol (AOH) y el alternariol monometiléter (AME) [1]. Estudios realizados in vitro, han demostrado la capacidad genotóxica, mutagénica, carcinogénica y citotóxica del AOH y del AME. Actualmente, la UE no tiene establecidos límites máximos para ninguna de las micotoxinas de *Alternaria spp*. aisladas en alimentos. Los métodos actuales para el análisis de AOH y AME se basan en el empleo de cromatografía líquida acoplada a detección por espectrometría de masas, fluorescencia o espectroscopia de absorción. Dada la complejidad de las matrices de alimentos, generalmente es necesaria una etapa limpieza de muestra mediante extracción sólido-líquido o extracción en fase sólida (SPE). Una alternativa a este problema consiste en utilizar polímeros de impronta molecular (MIPs) para llevar a cabo la extracción selectiva de los analitos previa al análisis cromátográfico. Estos polímeros sintéticos son capaces de reconocer selectivamente la molécula de interés debido a la presencia de cavidades complementarias (en términos de forma, geometría y grupos funcionales) a ésta generadas durante el proceso de polimerización.

En el presente trabajo se presenta la síntesis de MIPs selectivos a AOH y AME en forma de partículas esféricas, empleando gel de sílice como molde sacrificable. Los polímeros se han sintetizado empleando como molécula plantilla un análogo sintético al alternariol [2], N-(2-aminoetil)metacrilamida (EAMA) como monómero funcional, metacrilamida (MAM) como monómero diluyente y dimetacrilato de etilenglicol (EDMA) como entrecruzante.

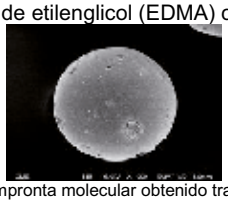

Figura 1. Micrografía SEM del polímero de impronta molecular obtenido tras la disolución del molde de gel de sílice.

Se han optimizado las distintas variables del método MISPE para la determinación de AOH y AME, concretamente la naturaleza del disolvente de carga (buffer fosfato 100 mM pH 8.5), lavado (acetonitrilo/agua 20:80 v/v, 3 mL) y elución (metanol/TFA 99:1 v/v, 1 mL) para la extracción selectiva de las micotoxinas. También se ha evaluado el volumen de ruptura de los cartuchos MISPE y la reactividad cruzada. Los análisis se han realizado empleando HPLC-FLD (λexc = 258 nm; λem = 440 nm). El método se ha aplicado a la determinación de las micotoxinas en muestras de tomate triturado salpicadas con AOH y AME. La validación del método MISPE-HPLCFLD se ha realizado de acuerdo a la Directiva Europea 2002/657/EC.

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A QUECHERS APPROACH TO THE ANALYSIS OF RESIDUES OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN MILK BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory, analgesic and antipyretic drugs in medicine and veterinary. Their action mechanism is based on the blocking of the biosynthesis of prostaglandins. NSAIDs are highly effective and extensively used, but they have some adverse side effects, such as hepatotoxicity, renal disorders or allergic reactions.

In several countries, to assure food safety and protect consumers, maximum residue limits have been established for some authorised NSAIDs. Therefore, high throughput and reliable analytical methodology is required for the effective control of NSAIDs in food from animal origin. Liquid chromatography (LC) coupled to mass spectrometry (MS) is currently the technique of choice in confirmatory analysis of NSAIDs residues.

We present a new method for the determination of representative NSAIDs in milk based on QuEChERS methodology and LC-MS/MS. To the best of our knowledge this is the first time that this strategy has been applied to the analysis of NSAIDs in milk. The method is straightforward, reliable, and well suited for high throughput confirmatory analysis.

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INTERNAL STANDARDS FOR PESTICIDE RESIDUE ANALYSIS IN FOOD COMMODITIES: AN APPROACH BASED ON PRINCIPAL COMPONENT ANALYSIS

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The most common techniques for analysis of pesticide residues in food samples are liquid chromatography (LC) and gas chromatography (GC), both with mass spectrometry (MS) detection. At LASPB, the quantification of pesticides is based on a calibration procedure with surrogate matrix matched standards (SMMS), which also includes the use of two internal standards (IS). Some representative matrices are used to prepare the SMMS for the quantification of different sets of food commodities.

The objective of this work is increasing the number of IS used in the LC-MS/MS and GC-MS/MS routine analysis methods at LASPB for the determination of more than 240 pesticides in a wide range of food samples. The final aim is selecting the best suited IS for each pesticide in each food commodity.

Eighteen compounds were tested as potential IS. First, the experimental conditions for LC-MS/MS and GC-MS/MS detection were established and the compounds that did not show a proper behavior in both techniques were discarded. Then the behavior of the IS candidates in 35 different sample matrices, including vegetables, fruits, nuts, honey, etc. was assessed. To handle and interpret all the results obtained, Principal Component Analysis (PCA) was applied to the complete set of data. This chemometric study allowed clustering sets of food commodities with similar behavior pattern and helped in the selection of the most suitable IS according to the pesticides to be determined and the sample matrices of interest.

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CARACTERIZACIÓN DE ESPECIES CINC EN RESIDUOS PROCEDENTES DE LA INDUSTRIA CERVECERA

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Actualmente, existe un gran interés en reducir la contaminación procedente de las actividades industriales, por lo que muchos estudios se enfocan hacia el aprovechamiento de dichos residuos. La industria cervecera genera cantidades relativamente grandes de subproductos y residuos; siendo los principales el bagazo y la levadura [1]. Éstos pueden ser reutilizados como productos agrícolas, lo que hace que, en comparación con otras industrias, la industria cervecera se considere más respetuosa con el medio ambiente. La levadura y el bagazo son residuos empleados tanto para alimentación animal como humana. La levadura de cerveza es un suplemento nutricional muy consumido, por sus propiedades beneficiosas para la salud. El bagazo se emplea en forma de pienso para ganado y como constituyente de algunos alimentos, por ejemplo ciertos tipos de pan. Estos residuos proporcionan un aporte en minerales y un elevado contenido en proteínas y fibra.

El cinc es un elemento esencial para la salud humana y animal [2], ya que muchos procesos metabólicos requieren la presencia de cinc para el buen funcionamiento de las enzimas. Uno de los problemas que presenta el cinc es su baja disponibilidad lo que hace interesante el desarrollo de suplementos nutricionales enriquecidos en cinc. En este sentido, en este trabajo se llevó a cabo la caracterización de las formas de cinc en residuos de la industria cervecera como fuente potencial de especies activas de cinc. La determinación de la concentración de cinc mediante FAAS puso de manifiesto que el cinc se encuentra tanto en el bagazo como en la levadura en elevadas concentraciones (73 mg kg -1 y 61 mg kg -1, respectivamente). Debido a los elevados niveles de cinc encontrados se procedió a la caracterización de las fracciones en que se encuentra mediante la aplicación de diferentes tipos de enzimas, proteolíticas (proteasa, pepsina), no proteolíticas (driselasa), celulasas y amilasas [3]. Los mejores porcentajes de extracción se obtuvieron mediante el empleo de driselasa y amilasa para la extracción del cinc en la levadura (70%) y bagazo (50-60%), respectivamente. La utilización de la sonda de ultrasonidos permitió reducir drásticamente los tiempos de extracción, desde 24 horas (incubación) a 2 minutos (empleando la sonda de ultrasonidos). Como una primera aproximación a la forma química en que se encuentra el cinc en los extractos, éstos se analizaron mediante SEC-ICP-MS para determinar el intervalo de pesos moleculares en que se encuentran los compuestos de cinc presentes y posibles candidatos para la preparación de productos funcionales de cinc. Con el fin de completar el estudio, se evaluó la bioaccesibilidad del cinc a través de un proceso de simulación buco-gastrointestinal lo cual se hizo para muestras comerciales: levadura de cerveza y pan de bagazo, ambos productos alimentarios procedentes de residuos de la industria cervecera.

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BIODISPONIBILIDAD DE ELEMENTOS TRAZA EN FRUTOS SECOS

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Se ha evaluado la biodisponibilidad de elementos traza (Ca, Mg, K, P, Co, Li, Cr, Mn, Fe, Cu, Zn, Se, Mo, Al, Sr, Ba, Tl, Ni, V, Hg, Cd, As y Pb) en frutos secos a través de la fracción dializable tras un procedimiento de digestión *in vitro*. Dicho proceso implica dos etapas sucesivas de digestión gástrica (empleo de pepsina como enzima proteolítica) y digestión instestinal (empleo de pancreatina como enzima proteolítica y sales biliares). Durante esta última etapa se simula igualmente el proceso de absorción intestinal empleando membranas de diálisis (tamaño de corte molecular de 10 kDa) y siendo la fase aceptora una disolución 0.15 N de PIPES. La determinación de elementos traza en la fracción dializable (análisis de la disolución de diálisis) se llevó a cabo mediante espectrometría de masas con plasma acoplado por inducción (ICP-MS) bajo condiciones óptimas (empleo de una calibración en 0.075 N PIPES, dializados diluidos 1:1). El contenido total de elementos traza en las muestras de frutos secos se realizó también por ICP-MS después de someter las muestras a un procedimiento de digestión ácida (empleo de ácido nítrico y peróxido de hidrógeno) asistido por energía de microondas. Las muestras de frutos secos se analizaron también para contenido total de proteína, grasa y carbohidratos (datos empleados a posteriori para establecer correlaciones entre la fracción biodisponible de los elementos traza estudiados y la composición nutricional de los distintos frutos secos). El procedimiento se aplicó a un total de 17 muestras de frutos secos (almendras, avellanas, nueces, piñones, castañas, anacardos, pistachos, y semillas de calabaza y de girasol), y también a otros alimentos considerados como frutos secos (cacahuetes y bayas Goji).

El porcentaje de biodisponibilidad varió enormemente en función del tipo de fruto seco y del elemento traza considerado. Elementos esenciales tales como Mn, Cu y Zn resultaron ser biodisponibles en todos los frutos secos (valores de dializabilidad entre 2 y 30% en función del tipo de fruto seco). Por el contrario, el Fe sólo se encuentra biodisponible en las nueces de Macadamia y en los cacahuetes (2 y 6%, respectivamente), mientras que el Se solo es biodisponible en las nueces de Brasil (20%). En cuanto a los elementos tóxicos, su biodisponibilidad ha sido muy pequeña en la mayoría de los frutos secos estudiados. Únicamente se encontró Hg biodisponible en avellanas crudas y almendras de la variedad Marcona (1 y 2%, respectivamente), Pb biodisponible (45%) en las bayas Goji, y As biodisponible en avellanas crudas (75%), bayas Goji (33%) y pistachos (51%). En cuanto al Cd, se encontraron valores biodisponibles en todos los frutos secos, entre 2% (semillas de calabaza) y 85% (nueces de Macadamia).

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IDENTIFICACIÓN DE LA PRESENCIA Y DETERMINACIÓN DE LA PROPORCIÓN DE ACEITE DE PALMA EN MEZCLA CON OTROS ACEITES VEGETALES. APLICACIÓN DE LA ESTRATEGIA DE "HUELLAS DACTILARES" CROMATOGRÁFICAS

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En la industria alimentaria es muy común la mezcla de diferentes aceites vegetales en la elaboración de alimentos con objetivo de abaratar costes y tener un producto que presente mejores características de estabilidad y de calidad.

El Reglamento (UE) Nº 1169/2011 sobre la información alimentaria facilitada al consumidor, que entró en vigor de forma definitiva en diciembre de 2014, establece que en los alimentos deberá indicarse qué tipo de aceite vegetal ha sido usado para la elaboración del producto. Por tanto, aquellos alimentos que contengan mezcla de aceites vegetales podrán seguir agrupándose bajo la designación "mezcla de aceites vegetales" pero indicando siempre el tipo de aceite que contiene (información cualitativa). De esta forma se pretende evitar que el consumidor pueda estar desinformado sobre los aceites vegetales que contienen los alimentos, Un paso más en la información supondría el facilitar los datos cuantitativos sobre la composición de la mezcla de aceites, aunque en la actualidad este dato no es preceptivo.

En este sentido, es necesario disponer de métodos analíticos eficientes para ambos objetivos, es decir, para la identificación de los aceites vegetales presentes, y para la cuantificación de la cantidad de cada uno de ellos.

En este trabajo se presentan varios modelos de clasificación de aceites vegetales, discriminando aquellos que contienen aceite de palma de los que no, y un modelo de calibración multivariante aplicado a la determinación de la proporción de aceite de palma con otros aceites vegetales (semillas, girasol, cacahuete, canola, colza, uva, maíz, sésamo y soja). Previamente al análisis, las muestras fueron sometidas a un proceso de transesterificación en el que tiene lugar la rotura de las moléculas de triglicéridos generándose los esteres metílicos de los ácidos grasos y la liberación de aquellas moléculas de esteroles que están enlazados con otras. Una vez separada la fracción transesterificada, ésta se somete a análisis cromatográfico para obtener las correspondientes "huellas dactilares" cromatográficas.

La técnica analítica empleada es la cromatografía líquida de altas prestaciones, en la modalidad de fase normal, acoplada a un detector de aerosol de partículas cargadas en corona (HPLC-CAD). Los cromatogramas obtenidos para cada mezcla constituyen una "huella dactilar" cromatográfica característica de cada mezcla de aceites. Los modelos quimiométricos se establecieron a partir de los vectores de datos que determinan el cromatograma completo, sin selección previa de variables.

En la comunicación se presentará el método analítico aplicado y la metodología seguida para establecer el análisis multivariante así como los resultados obtenidos sobre un conjunto de muestras.

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HEADSPACE-SINGLE DROP MICROEXTRACTION COMBINED WITH UV-VIS MICRO-SPECTROPHOTOMETRY: A SENSITIVE APPROACH FOR THE DETERMINATION OF SULPHITE PRESERVATIVES IN FOODS

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Sulphur dioxide (E220) and its precursor salts (sodium sulphite, E221; sodium hydrogen sulphite, E222; sodium metabisulphite, E223; potassium metabisulphite, E224; calcium sulphite E226; calcium hydrogen sulphite E227; potassium hydrogen sulphite, E228) are important stabiliser and preservative in food industry. Nevertheless, they are banned in fresh fruits and vegetables [1] because they would be the main source of SO_2 intake, causing health problems (1% of people are sulphite sensitive and it is multiplied by 5 in asthmatic patients [2]). When fruit or vegetables are fraudulently immersed in a solution containing a sulphiting agent (200-300 ppm as SO_2), a fresh appearance is provided and only an small amount of SO_2 (<10 ppm) can be subsequently found. As a consequence, this SO_2 in fruits and vegetables is present as free (SO_2, H_2SO_3, HSO_3^- and SO_3^{2-}) and can be easily extracted by shaking with water, but it may also lead to problems from instability of sulphite in aqueous solution [3].

The optimised Monier–Williams method, AOAC Method 990.28, based on a titration is the most used for determining SO_2 in foods. However, it is not suitable for residual concentrations [4]. In addition, although many methods have been developed for this purpose, very few are aimed to processed fruits and vegetables. Spectrophotometric methods using different reagents are the most usual. When an increase in sensitivity is required, as in this case, liquid-phase microextraction techniques could be implemented in sample preparation. In particular, headspace single-drop microextraction (HS-SDME) provides high enrichment factors when a species volatile, such as SO_2, is extracted. An aqueous extractant with derivatising reagents can be used in these determinations [5].

In this work, a new analytical approach based on HS-SDME combined with UV–vis microspectrophotometry for determination of sulphite preservatives at trace level (E220-228) in treated vegetables and fruits is presented. Sulphites contained in 0.5 g of sample (pureed or sliced) were extracted by strirring with 10 mL of degasified water for 5 min. 8 mL of this extract were placed in a closed vial for microextraction and subjected to agitation. Then, 1 mL of a diluted HCl solution was injected through the septum for SO_2 generation. A 2.5 µL drop containing 200 mg L^{-1} of DTNB [5,5′dithiobis (2-nitrobenzoic acid), also named Elman's reagent] in phosphate buffer was exposed to the headspace for 3.5 min. The drop was subsequently retracted back into the syringe and placed onto the pedestal of the UV–vis micro-spectrophotometer for absorbance measurement at 410 nm.

After full optimisation of factors influencing microextraction and reaction conditions between SO_2 gas and DTNB in the drop, analytical characteristics were obtained.
A high sensitivity was reached (enrichment factor 380, limit of detection 0.06 µg g^{-1}, limit of quantification 0.2 µg g^{-1}), thereby enabling the determination of low levels of free SO_2 in fruits and vegetables with an appropriate precision (repeatability expressed as RSD of 5 %) [6].

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ASSESSMENT OF ARSENIC BIOACCESSIBILITY IN RAW AND COOKED EDIBLE MUSHROOMS BY A PBET METHOD

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Food and drinking water are the principal routes of exposure to arsenic (As) for humans. The capacity of some mushroom species to accumulate arsenic may represent a serious risk to consumer health nonetheless; the consumption of edible mushrooms has increased considerably worldwide in recent years due to their nutritional properties.

Currently, European Union legislation does not establish maximum levels of arsenic in food. A complete food safety assessment should always evaluate the intake of arsenic from food on the basis of the product as ingested by the consumer. The inclusion of bioaccessibility data when assessing exposure can further refine and improve the risk assessment process. A limited number of arsenic bioaccessibility studies has been conducted, mostly concerning conventional food items. There is thus a lack of data on the bioaccessibility of arsenic in edible mushrooms.

Therefore, the present study focused on arsenic bioaccessibility to assess the potential health risks involved in the consumption of mushrooms. The present study reports arsenic analysis in *Lentinula edodes*, *Agaricus bisporus* and *Pleurotus ostreatus* before and after being cooked. Furthermore, arsenic in raw and cooked mushroom was determined in the gastric and gastrointestinal bioaccessible fractions obtained after simulating human digestion by means of an *in vitro* physiologically based extraction test (PBET). Several certified reference materials (SRM 1568a, SRM 1570a, CRM 7503-a, BC211 and IPE-120) were analysed to evaluate the proposed methods. Arsenic content was 1393, 181 and 335 μg As kg^{-1} for *L. edodes, A. bisporus* and *P. ostreatus*, respectively, and decreased by between 53% and 71% in boiled mushroom and less than 11% in griddled mushroom. High bioaccessibility was observed in raw, boiled and griddled mushroom, ranging from 74% to 89% and from 80% to 100% for gastric and gastrointestinal extracts, respectively, suggesting the need to consider the potential health risk of consumption of the mushrooms analysed.

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ASSESSMENT OF TOXIC AND NON-TOXIC ARSENIC COMPOUNDS IN FOODSTUFFS

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Food and drinking water are the principal routes of exposure to arsenic (As) for humans. More than 50 different naturally occurring As-containing compounds have been identified [1]. Regarding the toxicological aspects of arsenic in food, inorganic arsenic (iAs, (arsenite or As(III) and arsenate or As(V)) is considered to be the most dangerous form and is classified as a nonthreshold, class 1 human carcinogen. The organic forms are mainly considered to be non-toxic. Therefore, species-dependent differences in toxicity must be considered when establishing the maximum tolerated levels in food directives. Currently, no such maximum levels have been fixed for iAs in EU legislation in food matrices. The EFSA published in 2009 and 2014, two reports about the dietary exposure to As in the European population [1,2]. Both reported the urgent need for further data on As species, particularly iAs data, in food groups that provide a significant contribution to the dietary exposure to reduce the uncertainty of the exposure assessments to iAs. As a general recommendation, dietary exposure to iAs should be reduced.

Therefore, the present study reports suitable analytical methods for determination of As species: iAs, MA, DMA, AB, TMAO, AC and arsenosugars in foodstuffs. The study was focused on extraction and quantification of iAs. Arsenic speciation was carried out via HPLC with both anionic and cationic exchange with ICPMS detection (HPLC-ICPMS). A wide variety of food samples such as rice and rice products, infant cereals, cereal-based food, seaweeds, mushrooms, fish, bivalves and crustaceans were analyzed. For quality control, several (CRMs) were used to evaluate the accuracy of the methods.Higher concentrations of total arsenic were found in marine foods than in terrestrial foods. However, toxic iAs was the major species in several terrestrial foods such as rice and rice products, infant cereals, cereal-based food or mushrooms while AB was predominant in fish and seafood and arsenosugars in seaweeds. Furthermore, the toxicological intake limit for inorganic arsenic was estimated in each analyzed food samples and was calculated as a Benchmark Dose Level (BMDL), which was established in 0.3–8 µg/kg bw per day for cancers of the lung, skin and bladder as well as skin lesions ($BMDL_{01}$) [1, 2].

The analytical methods used may contribute to increase the availability of reliable results on iAs in foodstuffs. Furthermore, the present results may be useful in ongoing discussions in the European Commission and the CODEX Alimentarius for establishing and implementing future maximum levels of iAs in food commodities.

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HPLC-MS/MS METHOD FOR THE CONTROL OF ANTIBIOTICS RESIDUES AND METABOLITES IN PIGS URINE DURING TREATMENT AND SUPPRESSION AT FARM

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Antibiotics are widely used in food animal production for therapy and prevention of bacterial infections and for growth promotion [1, 2]. Evidence that antibiotic use in food animals can result in antibiotic-resistant infections in humans has existed for several decades. The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products [2, 3]. The pathway of veterinary antibiotics and their metabolites entering the environment is different from human pharmaceuticals not only through direct application in aquaculture [4], but also through manure application [5-6]. The knowledge on the excreted levels is important to increase the scientific evidence concerning the environmental persistence and ecotoxicity of antibiotics [7], the chances for antibiotic-resistant bacteria and resistance genes in animal urine [8], but also because it can contribute to the understanding of what happens during the antibiotics treatment and suppression steps in animals at farm. For all these reasons, there is an evident interest of both official organisms and food industry to control the presence of these substances in farms and foods of animal origin.

Once absorbed, the antibiotics are distributed to tissues by blood flow and excreted finally by the animals as parent compounds or metabolites and end up in the urine and feces. Therefore, plasma and urine are appropriate samples (invasive and non-invasive) to monitor antibiotic residues and their metabolites in live animals. Therefore in this study, two analytical methods were developed and optimized for the determination of two veterinary antibiotics (tiamulin and doxycycline) and their main metabolites in plasma and urine of pigs treated with Denagard® and Doxivet, commercial formulas containing the target antibiotics. The developed methods were based on solid phase extraction (SPE), followed by instrumental analysis using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Using the optimised conditions established in this study, good recoveries were obtained for the selected compounds. The optimised methods had an acceptable linearity, good precision and they allowed the detection of the target antibiotics at levels lower than those published by other authors. Analysis of 40 urine and 20 plasma samples from commercial pig farms where Denagard® and Doxivet have been applied were studied in order to set the levels of the target analytes and their metabolites during the treatment as well as their residues during the suppression periods. Results showed that suppression period set by the manufacturer were sufficient to remove both antibiotics from the animal although traces of both antibiotics and their metabolites are still detected 8 days after the last administered dose.

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CLASIFICACIÓN DE VINOS BLANCOS BASADA EN EL PERFIL ELECTROFORÉTICO OBTENIDO MEDIANTE ELECTROFORESIS CAPILAR CON DETECCIÓN ELECTROQUÍMICA

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Los polifenoles son un grupo de sustancias químicas de gran interés en la actualidad debido a sus propiedades anticancerígenas, bactericidas y antiinflamatorias. Están presentes en una gran cantidad de alimentos, entre los cuales se encuentran principalmente las frutas, verduras y sus derivados. El análisis de este grupo de compuestos se hace especialmente relevante en vinos, en los cuales se encuentran en elevada concentración y cuyo consumo de forma moderada está relacionado con la prevención de enfermedades cardiovasculares, neurodegenerativas o cáncer. Además estos compuestos desempeñan un papel importante en las propiedades organolépticas del vino por ser el origen del color, de la astringencia y de la acidez, características que, entre otras, a su vez determinan la calidad de los vinos.

Para tener un conocimiento pormenorizado de las diferentes especies de polifenoles presentes en el vino es imprescindible emplear técnicas de separación analíticas, especialmente HPLC así como electroforesis capilar, siendo la detección UV-visible el modo de detección utilizado mayoritariamente. La información analítica obtenida de esta forma no solo permite estimar la concentración y la distribución de diversos polifenoles individuales en la muestra de vino sino que también puede reflejar diferencias más o menos evidentes en el contenido de polifenoles según las características del vino (procedencia, tipo de uva, año de elaboración…) que pueden ser aprovechadas para fines de clasificación a través del uso de técnicas quimiométricas.

En este trabajo se propone una metodología para el análisis del contenido de polifenoles en vinos blancos, sin necesidad de tratamiento previo de muestra, basada en la separación por electroforesis capilar de zona con detección amperométrica, empleando para este fin electrodos de trabajo modificados con nanotubos de carbono. La separación de los compuestos de interés se completa en 25 minutos, fijando las siguientes condiciones: tampón borato 200 mM de pH 9,4 conteniendo un 10 % v/v de metanol como electrolito de separación, un voltaje de 27,5 kV y columnas capilares de 25 µm de diámetro interno. El potencial de detección se fija en 0,60 V, al cual es posible obtener señales amperométricas con notable estabilidad y reproducibilidad (RSD < 12% para una misma muestra de vino; n=3) gracias a la presencia de los nanotubos de carbono en el electrodo de trabajo. De este modo es posible procesar un elevado número de muestras sin tratamiento previo gracias a la mejora en la vida útil del electrodo.

Los perfiles electroforéticos obtenidos empleando la metodología propuesta en el análisis de muestras de vinos elaborados con uvas *airén* o *verdejo* muestran zonas de señales características que se han utilizado como variables para la construcción de un modelo de clasificación mediante análisis quimiométrico (Análisis de Componentes Principales y Análisis Discriminante). Este modelo es capaz de separar las muestras de vino analizadas (n=29) en dos grupos, en función de la diferente variedad de uva, con un porcentaje de asignación correcta del 97% al realizar su validación cruzada.

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ANALYSIS OF NUCLEOTIDE MONO- AND DIPHOSPHATES IN HUMAN MILK BY CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY (CE-MS). EFFECT OF PASTEURISATION OR HIGH-PRESSURE PROCESSING ON THE FREE NUCLEOTIDE CONTENTS

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Nucleotides are semi-essential dietary nutrients and are present in the milk of mammals. It is well known that nourishment with human milk increases the immune response in infants. Human milk is the best source of nucleotides for infants and hence the supplementation of infant formulas with concentrations within the ranges found in human milk has been recommended by the Scientific Committee for Food of the European Community.

Human milk banks are institutions that collect and store milk from donor mothers and are responsible for ensuring the safety and quality of the human milk deposited there. They must perform different treatments to inactivate any pathogenic microorganisms and part of the commensal flora that are potentially present in milk from donors. The most commonly used pasteurisation method in human milk banks is low-temperature long-time pasteurisation, also known as Holder pasteurisation (HoP). However, this process results in a variable loss of certain components. In recent years, high-pressure processing (HPP) has received much attention as a novel food preservation method. HPP is a minimal processing technology and serves as a cold-pasteurisation method that does not impair the nutritional and sensory characteristics of foodstuffs.

In the present work we report a simple and efficient analytical method for the determination of nucleotide mono- and diphosphate in human milk. It involves centrifugal ultrafiltration (CUF) as sample treatment and capillary electrophoresis-electrospray mass spectrometry (CE-MS) for separation and quantification. The target compounds were adenosine 5'-monophosphate, cytidine 5'-monophosphate, guanosine 5'-monophosphate, uridine 5'-monophosphate and inosine 5'-monophosphate and their corresponding diphosphates.

The CE-MS method provided instrumental limits of detection ranging between 15 and 26 ng mL^{-1} (S/N=3) for nucleotide monophosphates and between 99 and 171 ng mL^{-1} for diphosphates. The recoveries ranged from 87 % to 110 %. The optimized method, applied to the analysis of human milk samples, included their dilution (1:5, v/v) with ultrapure water followed by a centrifugal ultrafiltration treatment. Under these conditions, no matrix effects were found. Method validation according to the European Union Decision 2002/657/EC is reported.

Here we also describe the application of the proposed method to the study and comparison of the effects of Holder pasteurisation and high-pressure processing on the free nucleotide contents in milk samples from a human milk bank. The results showed concentration values between 0.5-10 µg mL^{-1} for nucleotide monophosphates, with higher concentrations for milk samples treated by pasteurisation. In human milk, no nucleotide diphosphates were detected. The effect of freezing time on the content of free nucleotides was also assessed.

The results obtained shown that high-pressure processing (HPP) could be a suitable alternative to traditional Holder pasteurisation (HoP) in the treatment of human milk.

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FAST AND GREEN ANALYSIS OF MINERAL ELEMENTS IN ARTICHOKES BY INFRARED SPECTROSCOPY AND X-RAY FLUORESCENCE

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Near infrared (NIR) and X-ray fluorescence (XRF) spectroscopy were investigated to predict the concentration of calcium, potassium, magnesium, iron, manganese and zinc in artichoke samples.

For this study, sixty artichokes were purchased from different Spanish areas (Benicarló, Valencia and Murcia). NIR and XRF spectra, combined with partial least squares (PLS) data treatment, were used to develop chemometric models for the prediction of mineral concentration. To obtain reference data, samples were mineralised and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). NIR and XRF spectra were obtained from the lyophilised samples, without any more pre-treatment of samples. For build PLS models, Kennard-Stone algorithm was assayed to selected two sets of samples, one for calibration and another for external validation of the model.

Coefficients of determination obtained for the regression between predicted values and reference ones for calcium, potassium, magnesium, iron, manganese and zinc were 0.61, 0.79, 0.53, 0.77, 0.54 and 0.60 for NIR and acceptable relative prediction errors of models were obtained with average values of 18.6% for calcium, 3.9% for potassium, 8.0% for magnesium, 10.4% for iron, 14.0% for manganese and 18.5% for zinc. These results demonstrate a poor predictive capability of the PLS-NIR models developed to predict the concentration minerals in artichokes, thus reducing the capability of this methodology as a green alternative for the direct determination of the aforementioned elements in dried samples without a previous sample digestion nor the use of plasmogen gases nor an expensive instrumentation.

However, for XRF methodology, coefficients of determination obtained for the regression between predicted values and reference ones for calcium, potassium, magnesium, iron, manganese and zinc were 0.96, 0.93, 0.80, 0.79, 0.76 and 0.90. The differences found in the external validation set between predicted concentrations and reference values vary from an average value between 8.4% for calcium, 2.5% for potassium, 4.4% for magnesium, 14.7% for iron, 14.1% for manganese and 13.2% for zinc. These results demonstrate a good predictive capability of the PLS-XRF models developed to predict the concentration of calcium, potassium, magnesium, iron, manganese and zinc in artichokes.

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FEASIBILITY STUDY OF DIFFERENT MASS SPECTROMETRIC METHODS FOR DIRECT VIRGIN OLIVE OIL ANALYSIS

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The composition of vegetable oils makes it a challenging matrix for any type of instrumental analysis. Triglycerides and a reduced percentage of free fatty acids are their main components, but a series of minor polar compounds are also present and their distributions may be characteristic of different types of oils. Amongst vegetable oils, those with high market prize, such as virgin olive oils (VOO) may be adulterated with lower priced oils (eg. seed/refined oils). These results a need for authentication of VOO samples by fast, straightforward, and accurate methods enabling the determination of its origin, quality parameters, adulterations or even its origin. Ideal analytical methods require minimal or no sample preparation. Available methods for characterization of vegetable oils include optical techniques such as fluorescence and vibrational spectroscopy , mass spectrometry and also other spectrometric techniques such as nuclear magnetic resonance spectrometry. In this communication, the performance of different mass spectrometric methods has been examined for the direct analysis of VOO for quality control purposes. Different experiments has been examined, including: (1) direct measurement of untreated olive oil using ambient mass spectrometric methods such as low-temperature plasma mass spectrometry (LTP-MS) or paper spray mass spectrometry (PS-MS); or alternatively (2) the use of atmospheric pressure ionization mass spectrometry (direct infusion) using either electrospray or APCI sources, combined with a minimum sample work-up consisting on either a simple olive oil dilution (from 1:10 to 1:1000) with appropriate solvents or a quick liquid-liquid extraction to shift the measurement towards a specific part of the composition of the edible oil (i.e. polyphenol rich fraction or lipid/fatty acid profile). The later strategy -using methanol/water as solvent- was found the more appropriate given the complexity of matrix, as it provided useful composition information whereas no major instrument service was required after a batch of experiments. Main components identified were different classes of phenolic acids and polyphenols, which are present in virgin olive oil in the mg per litre range.

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ANALYSIS OF FLUOROQUINOLONES IN MILK USING TOTAL FLUORESCENCE

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In the past few years, the public concern about the utilization of antibiotics in food-producing animals has increased due to the transfer of antibiotic-resistant bacteria to man. This is an increasingly prominent problem because antibiotics are used in animals both to treat infections and as growth promoters and so antibiotic-resistant strains can emerge in both healthy and sick animals. The misuse of antibiotics can produce allergic hypersensitivity reaction in some person and could hide the existence of pathogens in foodstuffs. Important group of antibiotics are quinolones. They use in the treatment of respiratory diseases and enteric bacterial infections in humans and in food-producing animals such as cattle, swine, turkey and chicken. Residues of these antibiotics may persist in edible tissues or foodstuff, such as milk and eggs. There are quinolones regulated in bovine milk, so the development of analytical methods that are sensitive enough to monitor and determine these drugs in bovine milk is demand.

Focus of this work is to develop a simple and a rapid analytical procedure for the simultaneous determination of flumequine and danofloxacin in milk by building a stable and reliable model. It involves a multi-way analytical methodology based on excitation emission matrix fluorescence data and multivariate calibration tools, i.e. N-PLS and unfolded PLS.
One of the main difficulties in the development of an analytical method for a complex matrix is the sample treatment.

The determination was performed in an ethanol/water medium (20%) at pH 4.8, provided by adding a sodium acetate/ acid acetic buffer solution. The matrix calibration was constructed by a combination of a factor design with two levels per factor, a central composite design. In order to ensure accurate results, the calibration matrix was implementing a milk sample containing no fluoroquinolones (i.e. milk blank). The limits of the experimental space for the design were as follows: flumequine, 0.0-500.0 μg L^{-1} and danofloxacin 0.0-80.0 μg L^{-1}. Whey dilution was varied from 1:4 to 1:20. The ability to construct the calibration validation sets directly from the whey samples itself avoids the need to consider matrix interferences or to pre-treat the sample.

The proposed chemometric methods were applied to milk samples fortified with the two compounds of interest. Untreated freshly milk purchased at different supermarkets was spiked with both drugs With the aim to generate the possible interactions between analytes and the milk matrix, the samples were maintained at ambient temperature during 30 min, and then diluted with ultrapure water. The concentration values of the analytes in samples were assigned by a home-made program that generates random numbers, within a desired interval. The whey samples prepared in the established chemical conditions were analyzed by using the PLS optimized models. The results obtained with the proposed method and a previously reported HPLC method [1] were compared. Recoveries ranged from 96 to 104 % and were thus acceptable for quantitative analysis. The study demonstrates an application of the total fluorescence spectroscopy combined with multivariate regression methods to the simultaneous quantification of two quinolones for veterinary use in raw milk. This new method is useful for the determination of fluoroquinolones in complex matrix with unknown background fluorescence, such as whey, without the need of tedious preseparation.

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HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF POLAR PESTICIDES IN OLIVE OIL AND OLIVES

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The presence of polar pesticides in virgin olive oil is unlikely given their different physicochemical properties and, thus the relative preference of polar pesticides towards the aqueous-phase (byproduct) during virgin olive oil production instead of the oil phase. With the aim to estimate the behaviour of these pesticides, and the extent of their transfer to olive oil during olive oil production (processing factor), a methodology for the determination of these polar and challenging compounds have been addressed in both virgin olive oil and olive matrices. The proposed methodology is based on liquid partitioning with methanol, adapted from QuEPPe-Method method for polar pesticides [1], followed by HPLC-MS determination using hydrophilic interaction liquid chromatography (HILIC) -with a 1.8 µm particle size HILIC column- coupled to electrospray time-of-flight mass spectrometry (HILIC-TOFMS). The selected polar pesticides included in the study were: amitrol, cyromazine, diquat, paraquat, mepiquat, trimethylsulfonium (trimesium, glyphosate counterion) and Fosetyl aluminium. Identification of the targeted species were undertaken by retention time matching, and accurate mass measurements of the ions of interest. Both recovery rates and matrix effects were studied in both olive oil and olives matrices. The results in terms of extraction efficiency were satisfactory in most cases except for some particular pesticide/commodity combinations, such as the extraction of diquat and paraquat from olives, which yielded very poor recovery rates. Matrix effects were minor in the case of olive oil (ca. 20 % suppression average), while in olive matrix the suppression was more notorious (30-50 % suppression average). The selected approach (UHPLC HILIC) was found to be useful for the determination of the pesticides studied in olive oil and olives with limits of quantitation in the range from 0.0005 to 0.12 mg Kg^{-1}.

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DETERMINATION OF POLYPHENOLS BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR THE CHARACTERIZATION OF NATURAL EXTRACTS

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American red cranberries (*Vaccinium macrocarpon*) are important for the consumers because of their protecting effect against urinary tract infections by controlling urine acidity (by preventing bacterial proliferation) and inhibiting *E. coli* growth, which is the bacterium most responsible for urinary infections. The antibacterial activity of cranberries is attributed to its high content in different types of polyphenols such as flavonoles, anthocyanins, and phenolic acids and, more specifically, a type of proanthocyanidins (PAC) present in this fruit [1]. Nowadays there are some concerns that some of the products sold in the market labeled as derived from red cranberry extracts come from other fruit-based extracts like grapes or blueberries, which do not contain the adequate polyphenols to fight these infections. Therefore, it is important to develop analytical methods for the authentication of natural extracts and pharmaceutical preparations.

In a previous work, a conventional LC-ESI-MS/MS method for the determination of several polyphenols in fruit products and pharmaceutical preparations was proposed [2]. However, an analysis time up to 36 min was required and PACs were not considered. The aim of the present work is to develop a fast UHPLC-API-MS/MS method for the analysis of 29 polyphenolic compounds, including several PACs, in natural extracts to propose a fast and reliable method for characterization and authentication. Moreover, different API sources such as APCI and dopant assisted APPI in negative mode have been evaluated for the coupling of UHPLC to MS and compared to the previously studied ESI source. For UHPLC separation, a Syncronis C18 (100 x 2.1 mm, 1.7 µm) column and a Hypersil GOLD C18 (50 x 2.1 mm, 1.9 µm) column were evaluated using gradient elution with methanol and aqueous 0.1% formic acid solutions. Fruit-based natural extracts were only extracted with acetone/water/hydrochloric acid (70:29.9:0.1 v/v/v) in an ultrasound bath and centrifuged prior to UHPLC-API-MS/MS analysis.

Separation of all targeted polyphenolic compounds was achieved in less than 21 and 10 min for the Syncronis and Hypersil GOLD columns, respectively. No ion suppression due to the coelution of analytes was observed as no significant differences between the analysis of individual standards and a standard mixture were found (ANOVA, 95% confidence level, p values higher than 0.05). Regarding MS data, differences in the full-scan mass spectra obtained with each API source were observed. For instance, adducts with components of the mobile phase were in some cases the base peaks of the spectrum in APCI, whereas with ESI and APPI the deprotonated molecule was always obtained. Four dopants were tested for APPI (toluene, acetone, anisole and chlorobenzene), obtaining the best signal-to-noise ratio with acetone, which was proposed for further APPI studies. MS/MS spectra were also obtained and ion assignment for quantifier and qualifier ions was proposed. The applicability of the proposed UHPLC-MS/MS method was evaluated for the determination of polyphenols in cranberry-based natural extracts and pharmaceutical preparations. The connection between polyphenolic profiles and the natural extract fruit of origin through a Principal Component Analysis (PCA) was evaluated in order to achieve authentication of natural extracts.

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DETERMINATION OF BENZOPHENONES IN PACKAGED FOODSTUFFS BY FIELD-AMPLIFIED SAMPLE INJECTION-MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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Food products are produced and distributed worldwide, so leading to very stringent regulations to guarantee food quality and safety. The comparison of the various sources of food contamination with organic chemicals suggests that among the public, but also among experts, the perception of risk is often distorted. As reported by Grob *et al.* [1], if you ask educated consumers about the principal source of food contamination they will list pesticides in the first item, then environmental chemicals such as PCBs. Few would even mention food-packaging materials, although the amount of contaminant materials migrating from food packaging into food may well be 100 times greater than the contribution of pesticides or environmental pollutants. Moreover, it is difficult to compare the toxicity (primarily acute) of well-controlled pesticides with the potential (primarily chronic) toxicity of frequently not even identified compounds entering food from packaging materials.

Benzophenones (BPs) have been widely used in packaging materials as a main component of UV inks and plastic protectors. These compounds contain photo-sensible groups that start the polymerization process to cure the ink by UV radiation. These UV inks are used to print packaging materials such as multilayer laminates, rigid plastics, cardboard and paper. Although intermediate aluminum layers are commonly used to prevent the migration of ink components into food products, the unintentional transfer of printing-ink components from the outer printed surface onto the food-contact surface can occur when the printed material is rolled on spools or stacked during storage. Although there are no specific EU controls for migration for inks and their associated coatings, there is a Group tolerable daily intake (TDI) level for benzophenone and 4-hydroxybenzophenone of 0.01 mg/kg body weight/day. Moreover, an specific migration limit for benzophenone of 0.6 mg/kg has been established in specific legislation for food-contact plastics [2].

The aim of this work is to develop a micellar electrokinetic capillary chromatography (MECC) method for the analysis of fourteen benzophenones in packaged foodstuffs as well as in food simulants used in specific migration tests. Good electrophoretic separation of the 14 targeted benzophenones was achieved by using a fused-silica capillary of 60 cm (effective length 50 cm) × 75 µm i.d. and a 25 mM phosphoric acid-dihydrogenphosfate buffer solution (pH 2.5), containing 200 mM sodium dodecyl sulphate (SDS) and 30% of isopropanol as background electrolyte. In order to improve method sensitivity, the applicability of an in-line enrichment procedure, field-amplified sample injection (FASI), was evaluated. For that purpose, several parameters such as sample matrix composition for FASI injection, water plug hydrodynamic injection time and sample electrokinetic injection time were optimized in order to achieve the best sensitivity without losing the established MECC separation. Limits of detection down to low µg/Kg values with good run-to-run and day-to-day precision were obtained with the proposed FASI-MECC method. The applicability of the developed FASI-MECC method was evaluated by determining the targeted benzophenones in a typical food simulant (3% acetic acid solution) used for migration assays of plastics intended to contain clear drinks such as water, juices, soft drinks, milk, creams, etc, as well as in packaged foodstuffs of different origin such as juices, creams, wines, etc.

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AROMATIC CHARACTERIZATION OF NEW CIDER APPLE GENOTYPES BY HEADSPACE-SPME-FAST-GC

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After the establishment of the Protected Designation of Origin (PDO) "Sidra de Asturias" has occurred there has been an increase of demand of apple cider. In order to improve productivity and quality of Apple cider SERIDA has developed a breeding program of Asturian apple with three goals of breeding: 1) biotic resistance, 2) regular bearing and scab resistence, and 3) high polyphenol content and late ripening. The analysis of the volatile compounds of these new apples has a high importance to know their sensory qualities [1] and establish a pattern recognition on basis of the different volatile compounds.

In this work, a High Speed Gas Chromatography method using Solid Phase Microextraction (SPME) for determination of aroma profile of new cider apple genotypes was developed. Chromatographic conditions were optimized to afford the separation of the compounds studied in less than twelve minutes. Different commercial fibers were evaluated by comparing the different normalized extraction efficiencies. The best extraction efficiency was obtained using fibers of 65 µm PDMS/DVB. In order to establish the optimal conditions for SPME extraction and obtain the maximum sensitivity,a 2^3 full factorial design (FFD) was performed to investigate the effects of temperature, equilibration time and extraction time. NaCl amount stirring effect and sample volume were fixed according to univariate analysis results. The proposed method, obtaining analytical parameters according to employed method and used to determine the aromatic profile of 386 samples to different three goals of breeding, was validated.

A chemometric characterization of these cider apple genotypes was carried out by using of different exploratory and modelling techniques (SOM, PCA, SIMCA, and ANN). Exploratory techniques, as SOM and PCA, established two genotypes groups, those that come from agronomic goals of breeding (biotic resistance and regular bearing) and those that come from a technological goal breeding (high level of polyphenol and late ripening). Alcohols, as 1-hexanol, 1-butanol and 2-methyl-1-butanol, were related to genotypes with high content of phenolic compounds and late ripening, and other compounds such as ethyl esters and acetate esters were related to genotypes characterized by their biotic resistance and regular bearing. Models computed by SIMCA technique presented good sensitivity (93%), specificity (91%), and classification hits (96%), however prediction capacity computed by SIMCA and ANN methods was lower (70 and 76%, respectively).

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SEGUIMIENTO ANALÍTICO DE LA CALIDAD, COLOR Y CARÁCTER ANTIOXIDANTE DEL ACEITE DE OLIVA PROCEDENTE DE UN MISMO CULTIVO

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España sigue siendo actualmente la principal potencia mundial productora de aceite de oliva virgen. La mayor parte de esa producción tiene lugar en la comunidad andaluza pero, mientras que la demanda de aceites de alta calidad va en aumento, se ha detectado que los olivicultores de la zona situada en el Altiplano de Granada siguen priorizando la obtención de un alto rendimiento en el proceso de extracción a obtener un aceite de calidad superior –con el correspondiente detrimento de los atributos del producto obtenido–. El presente estudio es consecuencia de la preocupación que ha surgido en el lugar para la mejora de la calidad del AOVE allí producido.

Los análisis realizados se llevaron a cabo sobre 24 muestras de aceite de oliva obtenido mediante el sistema Abencor a partir de aceitunas recogidas en tres parcelas distintas de3 municipios de la zona del Altiplano de Granada, y en el periodo de tiempo comprendido entre noviembre de 2014 y enero de 2015.
Antes de realizar la obtención del aceite de oliva se procedió a determinar la resistencia al desprendimiento, el peso de los 100 frutos, el índice de madurez y el rendimiento tanto sobre materia seca como sobre materia húmeda mediante la extracción de su aceite por el método Soxhelt.

Sobre los aceites obtenidos se realizaron determinaciones referentes a la calidad, al color y al carácter antioxidante.

Las determinaciones realizadas referentes a la calidad fueron: acidez, índice de peróxidos, absorción en el ultravioleta y análisis organoléptico. Se siguieron los procedimientos detallados en el Reglamento (CEE) Nº 2568/91 relativo a las características de los aceites de oliva y sobre sus métodos de análisis.

Con respecto al color han sido cuatro las determinaciones realizadas. Se ha desarrollado dos índices de color (índice de verde e índice de amarillo) que se basa en medidas de absorbancia realizadas sobre aceite sin diluir, y se ha determinado la concentración total de colorantes carotenoides y clorofilas (Mínguez et al.,1991).

Para evaluar el carácter antioxidante de los aceites se ha determinado la concentración de polifenoles totales y el índice de amargor (K_{225}). La concentración de polifenoles totales se ha obtenido mediante un método colorimétrico (Vázquez Roncero et al.,1973). Este método consiste en hacer una extracción líquido-líquido de los polifenoles, añadir un reactivo que produzca color (reactivo de Folin-Ciocalteau) y la determinación de la absorbancia a 725 nm. Se han realizado dos calibrados distintos para poder referir los resultados a dos polifenoles. Los resultados se expresan en mg de ácido cafeico/kg de aceite y en mg de tirosol/kg de aceite. Para la determinación del índice de amargor se aislaron las sustancias causantes de este atributo mediante extracción en fase solida (SPE) con cartuchos de C18,y posteriormente se midió la absorbancia de la solución obtenida a 225 nm.

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GREENER DETERMINATION OF ARTIFICIAL SWEETENERS IN BEVERAGES BY HIGH TEMPERATURE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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The aim of this work is the development of a separation procedure for eight artificial sweeteners (acesulfame, alitame, aspartame, cyclamate, neotame, neohespiridine dihydrochalcone, saccharin, and sucralose) and one sweetener of natural origin (stevioside), currently being introduced in the market as a replacement of artificial sweeteners. Although, most of them are approved by the European Union (EU), their concentration in beverages is strictly regulated [1]. Therefore, there is a need for analytical methods, in order to check that the concentrations of these additives in beverages fulfill the regulation requirements.

The developed analytical method makes use of a high temperature liquid chromatography–tandem mass spectrometry (HTLC–MS/MS) approach, applying mobile phases constituted by water and small percentages of ethanol. The advantages of applying elevated temperature in LC separations includes that the viscosity of the eluent is decreased with increasing temperature that results in a lower backpressure of the separations systems and also the diffusivity and mass transfer of solutes are enhanced [2]. Thus, the amount of organic modifier in the mobile phase can be reduced significantly or even eliminated andmethanol or acetonitrile can be replaced by ethanol. Water and ethanol are both nontoxic solvents and analysis with these substances can be considered as “green chromatography”

The developed method permitted the analysis in 23 min (including column reequilibration) and consuming only 0.85 mL of a green organic solvent (ethanol). This methodology provided limits of detection (after 50-fold dilution) in the 0.05–10 mg/L range, with recoveries (obtained from five different types of beverages) being in the 86–110% range and relative standard deviation values lower than 12%. Finally, the method was applied to 25 different samples purchased in Spain, where acesulfame and sucralose were the most frequently detected analytes (>50% of the samples) followed aspartame and cyclamate. All sweeteners were below the concentration limit regulated by the EU, with the exception of cyclamate which exceeded the 250 mg/L concentration in one of the samples and was at the limit in three beverages.

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TIME-OF-FLIGHT ACCURATE MASS SPECTROMETRY IDENTIFICATION OF QUINOLINE ALKALOIDS IN HONEY

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Honey is a natural foodstuff also considered as a valuable source of bioactive compounds with positive effects in the health of consumers [1]. Particularly, some types of honeys have been reported as relevant sources of antioxidants and proposed to be useful for the reduction of glucose levels in blood. Additionally, honey has a reputation as a wound healing agent due to its antimicrobial activity. Although the above features have not been correlated with a single chemical species, it is recognized that certain families of compounds may be responsible for the healthy properties of honey. In this line, phenolic acids and flavonoids are considered as the most relevant antioxidant compounds present in honey [2]. Together with the volatile fraction, polyphenols have been proposed to determine the botanic and/or geographic origins of honey [3].

Alkaloids are also naturally occurring compounds displaying a range of biological activities. Quinolines are a particular family of alkaloids with medicinal properties, including antileishmanial activity [4]; moreover, the quinoline heterocyclic ring constitutes the basic skeleton of many synthetic drugs with anti-inflammatory, antibacterial, antihypertensive, antimalarial and even anticancer pharmacological properties [5]. Some examples of natural occurring bioactive quinolines are their carboxylic acids derivatives. These compounds are strong inhibitors of enzymes related to the regulation of glucose blood levels [6]. Also, 2-phenylquinoline, present in the bark of some tropical trees, is useful in the treatment of parasitic diseases [7]. In general, quinoline and isoquinoline derivatives have been reported to be present in some medicinal plants [8] and essential oils [9]. From plants, quinolines may be transferred to honey and, may therefore, be useful for botanic and/or geographic origin classifications purposes. However, conversely to other bioactive compounds, the determination of quinolines in honeys has received little attention.

The aim of this research is to further investigate the presence of quinoline alkaloids in honey samples from their accurate time-of-flight mass spectrometry (TOF-MS) spectra, acquired after gas chromatography separation following two different sample preparation strategies, and considering columns displaying different polarities for compounds separation. For those species, undergoing low fragmentation at the electronic ionization (EI) source, their accurate scan tandem MS/MS spectra are also acquired. In some cases, liquid chromatography (LC) combined with a hybrid QTOF MS system was also employed to complete the structural characterization of the compounds. Finally, commercial available standards, or in-house synthesized ones, of the most probable candidates were analyzed for retention times and spectra comparison with peaks existing in honey samples. Positive and tentative identified compounds were then explored in a set of 62 honeys.

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LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY SELECTIVE DETERMINATION OF OCHRATOXIN A IN WINE

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Ochratoxin A (OTA) is a natural mycotoxin produced by several families of fungi, particularly by those belonging to *Aspergillus* and *Penicillium* classes. Upon ingestion of contaminated food commodities, OTA has nephrotoxic and neurotoxic effects and is accumulated in adipose tissues with very low removal rates [1]. Furthermore, OTA has been classified as a potential carcinogen for human beings [2]. The existence of OTA residues in wine is of particular relevance in some geographic areas, such as the Mediterranean countries [3]; however, it is recognized as a worldwide problem, with quantificable levels found in wines produced in different areas, from Asia to South America and South Africa [4]. In this line, wine consumption has been rated as a significant source (ca. 10% of the total dietary intake) of human exposure to OTA [5] and, consequently, the maximum allowable concentration of OTA in this foodstuff has been established, with a limit of 2 ng mL^{-1} within the European Union [6]. On the basis of OTA toxicity and the existing regulation, there is a need for analytical procedures able to provide reliable data at this concentration level, displaying also a high throughput, suitable accuracy and moderate cost.

Liquid chromatography (LC) represents the most resorted technique for the determination of OTA in wine. Without doubts, the highest sensitivity is attained by combining LC with fluorescence detection (FLD) [7]. On the other hand, LC-FLD offers a limited selectivity; thus, it is normally preceded by specific clean-up approaches such as (1) immunoaffinity chromatography to purify wine extracts and/or for direct concentration of wine samples and, less often, (2) molecularly imprinted sorbents. The elevated cost of immunoaffinity sorbents has fostered the evaluation of alternative extraction and/or clean-up approaches, such as solid-phase microextraction and liquid-liquid microextraction, which, in general, are less selective and provide lower enrichment factors than the former technique [7].

The spread of LC tandem mass spectrometry (MS/MS), within food analysis laboratories offers an excellent alternative to LC-FLD for the determination of OTA in complex food stuff commodities. Recently, the accurate, scan mass capabilities of TOF MS systems have been implemented with a preceding Q MS analyzer. Thus, high resolution MS and MS/MS scan spectra are available increasing the reliability of analytes identification from their accurate (typical errors remain below 10 ppm) product ion (MS/MS) scan spectra.

The aim of this study was to establish whether hybrid QTOF-MS, following LC separation, could be used for the selective and quantitative determination of OTA levels in wines with different characteristics (dry and sweet wines) after a straightforward sample enrichment step based on the use of reliable, moderate cost, reversed-phase SPE cartridges. SPE conditions were optimized aiming to attain quantitative recoveries at the same time that interfering compounds, potentially disturbing the yield of ESI ionization, were removed from the final extract without increasing the complexity of the sample preparation process.

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COMBINACIÓN DEL ANÁLISIS QUIMIOMÉTRICO Y PERFIL METÁLICO PARA LA DIFERENCIACIÓN DE ACEITES DE OLIVA VIRGEN EXTRAS (AOVE) DE DIFERENTES ZONAS DE PRODUCCIÓN ESPAÑOLAS

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Los aceites crudos fabricados sin refinar, tales como los aceites de oliva virgen extra, podrían contener cantidades relativamente altas de metales traza. De hecho, uno de los criterios de calidad más importantes de aceite de oliva virgen extra es su contenido mineral. Es importante supervisar y controlar la presencia de estos elementos para preservar el alto valor nutricional y la alta calidad organoléptica de este producto. La presencia de elementos traza en los aceites vegetales comestibles puede ser debida a la composición del suelo y la contaminación medioambiental o a la sufrida durante los procesos de extracción y de conservación [1, 2]

La concentración de estos elementos es un criterio importante para la evaluación de su calidad en lo que se refiere a la frescura, mantenimiento de sus propiedades nutricionales y organolépticas y almacenamiento [2]. Se puede suponer que la distribución de elementos traza en los aceites de oliva virgen varía en función de su origen geográfico en consecuencia, un tratamiento estadístico adecuado de los datos de elementos traza podría permitir una caracterización geográfica de los diferentes aceites [1,3].

Para la realización de este estudio, se seleccionaron un total de 125 muestras de aceite de oliva virgen extra procedentes de diferentes áreas geográficas de la península.
El tratamiento de las muestras se realizó mediante una digestión ácida asistida por microondas y las disoluciones resultantes fueron analizadas mediante ICP-OES para los metales mayoritarios e ICP-MS para los traza.

Los resultados obtenidos fueros analizados mediante técnicas de análisis multivariante tales como ACP y LDA. Cuando se lleva a cabo el ACP, se observa una separación de las muestras procedentes de la provincia de Huelva del resto de los aceites que quedan dispuestos de forma heterogénea sin que se aprecie un patrón de agrupamiento. La aplicación posterior del análisis discriminante utilizando como variable de agrupamiento la provincia de procedencia muestra algo similar a lo obtenido con el ACP: las muestra de Huelva se separan del resto, pero en este caso se observa una tendencia de estas a discriminarse en dos grupos, uno que incluiría los aceites procedentes de la costa mediterránea y el otro en el que estarían incluidas las muestras procedentes de provincias del interior. Por ello se volvió a realizar el LDA utilizando como criterio de agrupamiento el mencionado anteriormente. Se aprecia en este caso una buena separación de los tres grupos de muestras de aceite: Huelva, Costa mediterránea e interior.

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ESPECIACIÓN DE ARSÉNICO EN MUESTRAS DE ARROZ. PRESENCIA DIFERENCIAL DE ESPECIES TÓXICAS DE ARSÉNICO EN ARROZ CON CÁSCARA Y PULIDO.

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Los alimentos y el agua potable son las principales fuentes de exposición a Arsénico (As) para los seres humanos [1,2], habiendo sido estimada la ingesta de este elemento en humanos ha sido estimada en el rango de 12 a 20 µg de As por día. Las formas inorgánicas de arsénico (arsenato (As (V)) y arsenito (As (III))) son las más tóxicas, mientras que las formas metiladas (MA y DMA) son consideradas moderadamente tóxicas [3,4], por ello, es importante determinar cuales están presentes en los productos alimenticios, información que complementa la concentración total de arsénico. Recientemente, se ha demostrado la presencia significativa de arsénico en este alimento y en sus productos de transformación, donde se encuentra fundamentalmente en forma inorgánica [5,6].

En este estudio, se ha optimizado y aplicado un procedimiento analítico diseñado para abordar esta problemática, tomando como base distintas variedades de arroz y su forma de presentación (con cáscara y pulido). La metodología resultante se apoya en la propuesta de Francesconi et al. [7] que utiliza una extracción suave del producto triturado con TFA (dejando la mezcla toda la noche para lograr una mayor recuperación de las especies) seguida de la especiación de las formas de arsénico de interés (As (III), As (V), DMA y MMA) mediante cromatografía liquida de intercambio aniónico y determinación del arsénico por ICP-MS. El procedimiento se ha validado utilizando un material de referencia certificado de arroz que contiene estas especies (IRMM-804).

Los resultados demuestran que las formas más abundantes son As (III) y DMA, especialmente en muestras de arroz con cáscara. Por otro lado se observa la ausencia de DMA y As (V) en las muestras de arroz pulido, lo que parece indicar una migración de estas especies hacia la cáscara del cereal. Este hecho es especialmente interesante en relación al As (V) por su mayor toxicidad.

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ESTUDIO SISTEMÁTICO DEL CONTENIDO DE FITOQUÍMICOS EN DISTINTAS VARIEDADES DE MANZANA MEDIANTE EL EMPLEO DE CROMATOGRAFÍA DE LÍQUIDOS ACOPLADA A ESPECTROMETRÍA DE MASAS DE TRIPLE CUADRUPOLO

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La manzana es una de las frutas más consumidas a nivel mundial con un alto poder antioxidante, pudiendo disminuir el riesgo de padecer diversas enfermedades, tales como disfunciones cardiacas, cáncer, diabetes, y desórdenes neurológicos [1]. Estas propiedades se deben básicamente a su contenido en fitoquímicos, aunque en función de la variedad, su contenido puede variar, siendo de 2 a 6 veces superior en la piel que en la pulpa [2]. Es por ello que es necesario realizar un estudio sistemático del contenido de fitoquímicos en distintas variedades de manzana, tanto en la pulpa como en la piel, con objeto de determinar las que presentan un mayor contenido de estas sustancias, así como la relación de los mismos entre piel y pulpa. En el presente trabajo se han analizado distintas familias de fitoquímicos como ácidos fenólicos, flavanoles, flavonoles, flavonas y dihidrochalconas. La determinación de estos compuestos se ha llevado a cabo mediante cromatografía de líquidos de ultra alta eficacia acoplada a un analizador de triple cuadrupolo (UHPLC-QqQ-MS/MS), y la extracción de los mismos se ha realizado aplicando una extracción sólido-líquido, utilizando una mezcla de metanol:agua (80:20 v/v) como agente extractante.

La metodología analítica se validó tanto en pulpa como en piel de manzana, obteniendo límites de cuantificación comprendidos entre 5 y 50 µg kg^{-1}, con valores de recuperación entre 60 y 119 % y con una precisión inter-día inferior al 25 %. Esta metodología analítica se ha aplicado a diversas variedades de manzana (granny, fuji, pink-lady, ambrosia, Golden (de origen español y francés), roja y royal), analizando tanto la pulpa como la piel. Este estudio revela que las variedades con mayor contenido en la pulpa fueron granny y fuji, con una concentración total de fitoquímicos superior a 700 mg kg^{-1} en peso seco (DW), mientras que la de menor contenido fue ambrosia (460 mg kg^{-1} DW), siendo los componentes mayoritarios el ácido clorogénico (ácido fenólico), epicatequina (flavanol) y floridzina (dihidrochalcona).

En cuanto a la piel se observó que su contenido era superior a la pulpa, detectándose compuestos como los glucósidos de kaempferol, quercetina e isorhamnetina, que no se habían detectado en la pulpa. Las variedades con mayor contenido fueron pink-lady y ambrosia con niveles superiores a 4000 mg kg^{-1} DW, mientras que la de menor contenido fue la variedad roja con niveles inferiores a 2000 mg kg^{-1} DW. Las familias predominantes en la piel fueron los flavonoles y flavanoles. Finalmente, el contenido de fitoquímicos se ha determinado en manzana fresca y de IV gama (variedad royal), observando que aunque los niveles eran superiores en manzana fresca, no lo eran de manera significativa cuando se llevó a cabo el correspondiente estudio estadístico. Teniendo en cuenta estos resultados, se puede indicar que la manzana debe comerse entera, ya que aunque la piel solo supone un pequeño porcentaje del peso de la fruta, contiene un mayor contenido de fitoquímicos, independientemente de la manera de presentación de la manzana.

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METAL CONCENTRATIONS IN COCKLES FROM GALICIAN COAST

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Heavy metals have a significant ecological impact due to their toxicity and accumulative behaviour; they play an important role in marine ecosystems, and constitute a potential hazard not only on the fauna but also on man. Thus metal monitoring in bivalve molluscs, which integrate metal loads in their tissues, is required by Regulation (EC) No 854/2004.
In addition, as the metal accumulation characteristics of the exploited molluscs can differ substantially, information of each species must be obtained to guarantee their safety consumption. This study completes previous reports made on the Galician coast concerning the monitoring of metal concentrations in different mollusc species. In this case we determine metal concentrations in cockles (*Cerastoderma edule*) from 27 sites in Galician coast during the period 2005-2014. When the whole area and sampling period were considered, 85% of the observations were below 0.03 for Hg, 0.05 for Cd, 0.28 for Pb, 0.25 for Cr, 2.9 for Ni, 2.0 for As, 1.1 for Cu and 11 for Zn (mg kg^{-1} wet weight). Maximum values found for metals with legally regulated limits (Hg, Cd and Pb) were much lower than the threshold values.

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OPTIMIZATION OF THE EXTRATION OF PHENOLIC COMPOUNDS FROM THE GUAVA FRUITS (*PSIDIUM GUAJAVA L.)* USING THE SIMPLEX LATTICE SURFACE FOR DETERMINATION BY SPECTROFOTOMETRY AND HPLC

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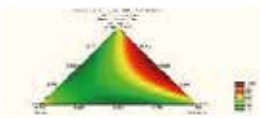
Phenolic compounds may be found in vegetables and are chemical structures that are composed of a hydroxyls and aromatic rings, in a simple or polymeric form, which confer antioxidant characteristic [1]. The extraction of bioactive compounds from permeable solid plant materials using solvents constitutes an important step in the manufacture of fitochemical-rich products [2,3]. For the Simplex *lattice* Design with 3 components, the space of the mixture is a triangle, with vertices corresponding to the pure components and the sides corresponding to binary mixtures. The aim of this work is to use the Simplex *lattice surface* for optimization of polyphenol extraction with subsequent determination of these by spectrophotometry and high performance liquid chromatography (HPLC). For the optimization were conducted six experiments. To a slurry of 0.5 g of pulp guava (Psidium guajava L.) lyophilized added 30 ml of the solvent. The simple was submitted at sonication for 30 minutes and centrifugation for 15 min. After the extract was dried by rotary evaporation. The resulting material was resuspended in 1.5 ml of 50% methanol (v /v), filtered and diluted before determinations. Table 1 shows the different compositions of solvent mixtures (%v/v) and results in the % extraction.The results were processed using the Statistica 7.0 software. Figure1 shows the obtained surface.

Tabel 1: Simplex Matrix

Experiment	*Water*	*EthilAcetate*	*Methanol*	*%Extaction*
1	100	0,0	0,0	21,65
2	0	100	0,0	51,81
3	0	0,0	100	54,22
4	50	50	0,0	24,09
5	50	0,0	50	25,30
6	0	50	50	100

Figure 1: Simplex Surface



For the determination of total phenolic compounds (TPC) and total flavonoid (TF) was used the official methodology adapted [3]. For the determination of phenolic acids by HPLC, the equipment used was a "Shimadzu" SIL 20 A with column C18 "Lichorspher 100" (250x 4 mm, 5µm. Mobile phase was: 1% acetic acid (A)/acetonitrile (B).ntra-day and inter-day precision were below 5% (n = 5) and the addition and recovery test ranged from 85 to 105% at 2 levels: 30 and 60 mg L^{-1}. The LOD (detection limit) was 0.22; 0.27 and 0.39 (mg L^{-1}) for: gallic, chlorogenic and caffeic acid respectively. The results for the determination of phenolic compounds with the best mix solvent (methanol: ethil acetate 1:1) are shown in Table 2.

Tabel 2: Phenolic acids, TPC and TFC in guava fruits (*Psidium guajava L.)*

Sample	***Gallic Acid*** *mg 100g^{-1}*	***Chlorogenic Acid*** *mg 100g^{-1}*	***Caffeic Acid*** *mg 100g^{-1}*	***TPC*** *mg EAG* 100g^{-1}*	***TF*** *mg EQ**100g^{-1}*
GG	23,86 ± 3,45	1,20 ± 0,02	2,34 ± 0,11	307,14 ± 3,80	138,10 ± 5,19
RG	25,95 ± 4,55	1,22 ± 0,02	1,68 ± 0,13	344,05 ± 2,80	153,20 ± 4,21

GG green guava RG ripe guava Values expressed as the means ± 95% confidence interval n=10 *gallic acid equivalents **Quercetin equivalents

The experimental design approach was successfully applied in the optimization of the conditions for the extraction of phenolic compounds in guava fruit.

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TRAZABILIDAD DE LOS ACEITES DE OLIVA VIRGEN EXTRAS (AOVE) DEL SUROESTE DE ESPAÑA ATENDIENDO A SU ESPECTRO DE RMN-^{1}H, AL CONTENIDO EN ÁCIDOS GRASOS Y ESTEROLES

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La composición del aceite de oliva virgen extra (AOVE) determina su calidad intrínseca y está influenciada por varios factores agronómicos y tecnológicos, como la variedad de aceituna [1], el clima, el grado de maduración [2,3], la época de cosecha [4] y el proceso de producción [5,6]. Sin embargo, el área geográfica es en gran medida responsable de las características específicas de aceite de oliva [7]. Distintos autores han estudiado la relación entre la zona geográfica y los índices de calidad así como la composición química de diferentes AOVE monovarietales, clasificándose dichos aceites de acuerdo a su origen geográfico en base a su composición química en ácidos grasos y esteroles [8] y sus espectros de ^{1}H NMR [9] aplicando técnicas de análisis estadístico multivariante.

El contenido de ácidos grasos y esteroles de 48 muestras de AOVE fue determinado mediante técnicas de cromatografía de gases. Así mismo se registraron los espectros de resonancia magnética nuclear de protón. Estas muestras, pertenecientes a tres variedades de aceitunas (Arbequina, Picual y Verdial), fueron procesadas en cooperativas de cuatro municipios de la provincia de Huelva.

Para establecer el potencial de estos compuestos en la trazabilidad geográfica de estos aceites, se utilizaron herramientas quimiométricas de discriminación. La aplicación del LDA a los resultados obtenidos, mostró una buena discriminación entre grupos cuando las muestras se representaron en el plano definido por las funciones canónicas principales.

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DETERMINATION OF CHLOROGENIC ACID AND OTHER CAFFEOYLQUINIC ACIDS IN VEGETAL EXTRACTS AND ANIMAL FEED FORMULATIONS

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Caffeoylquinic acids (QCAs) are esters of the caffeic acid and the quinic acid, that are naturally present in several plants and have been shown to possess a multitude of preservative and pharmacological activities, such as antioxidant, antiviral, antibacterial and anti-inflammatory among others. The most abundant CQA is chlorogenic acid. The interest in using natural products with therapeutic properties as additives has increased in recent years. By-products of vegetable processing are of interest to recover CQAs that can be used in animal feedstuff for their health-promoting properties. Commercial products containing CQAs from plant extracts can differ for the methodologies of preparation as for the different content in polyphenolic compounds and, therefore, analytical methods are demanded for quality control of raw materials and final products.

A method to determine chlorogenic acid and other CQAs in three sources (herbal extract, feed additive and finished feed) has been developed, validated and applied to these feed formulations [1]. It is based on ultra-high performance liquid chromatography (UPLC) coupled to quadrupole-time of flight mass spectrometry and uses, for the first time, focused ultrasound solid-liquid extraction (FUSLE) for analyte extraction. FUSLE variables such as extraction solvent, power and time were optimised by a central composite design. Under optimal conditions, FUSLE was performed with 8 mL of an 83:17 methanol-water mixture for 30 s at a power of 60%. Only two extraction steps were found necessary to recover analytes quantitatively. Pressurised liquid extraction (PLE) was also tested as extraction technique but it was discarded because cynarin, a dicaffeoylquinic acid, was not stable under temperature values used in PLE.

The separation of the CQAs isomers by UPLC was carried out in only seven minutes. Quantification was performed by selective ion monitoring (SIM) at m/z 353.06 and m/z 515.12 corresponding to the quantification ions of mono-CQAs and di-CQAs, respectively, and using a tolerance of 0.01 Da.

Matrix effect was studied for each type of sample. It was not detected for chlorogenic acid, whereas the cynarin signal was strongly decreased due to ionization suppression by matrix components. Therefore, the quantification by standard addition was mandatory for the determination of di-caffeoylquinic acids. Sensitivity, linearity, accuracy and precision were established. LODs of 0.07 μg g^{-1} and 0.04 μg g^{-1}, and LOQs of 0.2 μg g^{-1} and 0.1 μg g^{-1} were found for chlorogenic acid and cynarin, respectively. For finished feed samples, RSD values less than 9% and 22% were found for repeatability and intermediate precision, respectively. Excellent recovery values of chlorogenic acid from the different matrices, between 91 and 109 %, were obtained.

Finally, the method was used for raw material screening and for process and quality control in feed manufacture.

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APLICACIONES DE LOS POLÍMEROS DE IMPRONTA MOLECULAR EN EL ANÁLISIS DE MICOTOXINAS EN ALIMENTOS

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La seguridad alimentaria es uno de los temas de mayor trascendencia a escala mundial en nuestros días. La presencia de contaminantes de origen natural o antropogénico en los alimentos, especialmente en los denominados alimentos "orgánicos", es una fuente de creciente preocupación, tanto para los consumidores, como para las autoridades y los organismos reguladores. A este respecto, la contaminación de los alimentos por hongos toxicogénicos (especialmente del género *Aspergillus*, *Penicillinium* y *Fusarium*) o los productos de su metabolismo, conocidos por micotoxinas, es un problema de gran importancia sanitaria y económica, especialmente en países cálidos como España. Las micotoxinas se producen en condiciones favorables para el crecimiento del hongo responsable, ésto es, elevada actividad de agua y temperatura, y representan un peligro latente para la salud humana y animal. La contaminación del producto puede ocurrir en cualquier punto de la cadena alimentaria, desde la cosecha, a la recolección, almacenaje, transporte, elaboración o conservación. Por ello, existe una demanda creciente de métodos analíticos rápidos, baratos, sensibles, selectivos y con escasa intervención del operador para la determinación de micotoxinas en diversas muestras de alimentos [1].

En los últimos años, la aplicación de los polímeros de impronta molecular (MIPs) para esta aplicación ha despertado gran interés, especialmente entre las empresas que comercializan materiales para extracción en fase sólida [2]. Un MIP es un material sintético que se comporta como un receptor biomimético, reconociendo selectivamente determinado analito [3]. Para ello se forma una estructura macromolecular muy entrecruzada alrededor de una molécula "plantilla", la cual se extrae tras la polimerización, obteniendo cavidades en el polímero, complementarias en forma, tamaño y distribución de grupos funcionales a la molécula plantilla. Ello permite el reconocimiento selectivo de ésta, de manera similar a las enzimas o los anticuerpos. Se trata de materiales muy robustos y fáciles de preparar, con un bajo coste, lo cual ha permitido su implementación como materiales de reconocimiento molecular frente a distintas especies.

En la presente comunicación se mostrarán las distintas aproximaciones abordadas en nuestro grupo de investigación para la síntesis de MIPs selectivos a diferentes micotoxinas, tanto legisladas como no legisladas, incluyendo, zearalenona, alternariol y citrinina y algunos de sus derivados. Los polímeros se han sintetizado empleando moléculas plantilla sintéticas para evitar el efecto sangrado en el proceso de extracción en fase sólida. Además, se mostrarán distintas aproximaciones para la síntesis de los MIPs en forma de monolitos, partículas esféricas o nanopartículas magnéticas. Finalmente se discutirán las aplicaciones de estos materiales en extracción en fase sólida (MISPE) para la determinación de las micotoxinas seleccionadas, en distintas matrices (tomates, cereales, piensos) empleando cromatografía liquida.

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MONITORING THE EVOLUTION OF THE PROFILE OF VOLATILE ORGANIC COMPOUNDS (VOCs) FROM OLIVE OIL AT DIFFERENT RIPENING STAGE BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Many varieties of olive fruits are currently available and suitable for yielding “extra virgin” quality olive oil; the producers therefore need to know the optimal time for harvesting olive fruits in order to obtain high quality oils in terms of both: physic-chemical characteristics and sensory attributes. The evolution of aroma active compounds in olive fruits across different ripening stages to set optimal harvesting time is therefore of high interest.

Most of the quality flavour attributes of olive oil result from olfactory stimuli triggered by a specific VOCs composition. In this perspective, multidimensional analytical techniques already applied in “omics” approach, have demonstrated to be very effective to study the composition of complex mixtures/fractions. Comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) can be considered the technique of choice for both volatile detailed analysis (profiling) and fingerprinting based classifications and correlations with other samples’ attributes. This technique enables to extend the identification potential of gas chromatography/mass spectrometry to a larger number of compounds, if compared to mono-dimensional techniques, and facilitates the development of functional relationships between chemical composition and other attributes within a set of samples, such as sensory properties.

In the present study olive oils from different geographical regions, produced by fruits harvested at different ripening stages were submitted to VOCs extraction by Head-Space Solid Phase Micro-Extraction (HS-SPME) and separation by GC×GC-MS.

Bi-dimensional data elaboration (run as targeted and untargeted) was carried out by applying the *template matching fingerprinting*, developed by Reichenbach et al. [1] in 2009. This approach adopts the full information from 2D peaks metadata (retention times, MS fragmentation pattern and detector responses) to establish reliable correspondences between the responses of the same chemical entity across multiple chromatograms.

Chemical fingerprinting of set of olive oils produced with olives collected at the optimal harvest time to obtain the best organoleptic quality was correlated to the sensory attributes determined by an official panel.

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POLYPHENOLIC PROFILE IN CIDER AND ANTIOXIDANT POWER

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Cider apple varieties contain relatively large amounts of polyphenolic compounds, which are responsible for the haze and sediment formation because of their interaction with proteins [1, 2]. They are also involved in browning processes because of the effect of polyphenol oxidases [3]. From the organoleptic point of view, polyphenols are related to bitterness and astringency, whose balance defines the overall mouth feel of the beverage [4].

The aim of the work was to find correlations between the individual polyphenolic compounds in Basque ciders and the following physicochemical parameters: *antioxidant activity*, *browning*, *protein precipitating capacity*, *turbidity* and *reduction potential*. These parameters are related mainly with polyphenols and are vital as they affect the taste, the visual aspect and the preservation of the cider. For this purpose we obtained six different musts by using five different varieties of apples of the Basque Country (northern Spain). Five were monovarietal and the sixth one was obtained by mixing the varieties used in the other five musts in equal weight proportions.

Five samples were taken from each of the six musts in different stages of the fermentative process during 5 months. The content of 23 polyphenolic compounds was determined on each sample, as well as the abovementioned five parameters.

The determination of individual polyphenolic compounds was performed by high performance liquid chromatography (HPLC). The method[5] includes a C_{18} column, gradient elution with 2% acetic acid in water and methanol, and direct injection of filtered sample. Standards of pure polyphenolic compounds were injected and their retention times and UV-Visible spectra recorded, in order to identify them in the chromatograms of the samples. Next, the calibration lines were constructed for each compound, measuring at 280, 313 and 350 nm.

Once the phenolic profile of each of the juices and their evolution during fermentation was established, simple correlations between the individual polyphenol content and the five parameters were calculated, as Pearson coefficients at the 0.01 level. Finally, and based on the correlations obtained before, separate multiple linear regression (MLR) models were built for each physicochemical parameter. Those phenols showing the highest individual correlations with the parameter under evaluation were used to perform the MLR analysis.

The most important results achieved from the MLR analysis were the following:

- Procyanidine B1 and procyanidine B2 are the most powerful antioxidants in Basque cider, while p-coumaric acid, (-)-epicatechin and hyperin are those with greatest capacity to precipitate proteins.

- Ciders with higher tyrosol concentration will have less reduction potential and higher antioxidant reservoir.

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DETERMINACIÓN DEL CONTENIDO TOTAL Y/O ESPECIES DE ZN PRESENTES EN LECHE HUMANA A LO LARGO DEL PERIODO DE LACTANCIA Y EN LECHES FÓRMULA

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El Zn es un nutriente esencial para el crecimiento y desarrollo humano tanto prenatal como postnatal. El metal se encuentra prácticamente en la totalidad de las células, pero existe con mayor abundancia en determinados tejidos (el músculo esquelético y el hueso contienen el 90 % del zinc total del organismo). La ubicua distribución del zinc en las células, junto al hecho de que es el oligoelemento intracelular más abundante, indica que sus funciones: catalítica, estructural y de regulación. Así, se conocen más de 300 enzimas cuya función catalítica esta mediada por zinc.

La leche materna es la principal vía de nutrientes para el recién nacido durante los primeros meses de vida y es el alimento ideal para el recién nacido ya que contiene todos los macro- y micro-nutrientes de modo que su composición cambia a lo largo del periodo de lactancia, del calostro a la leche madura, adaptándose a las necesidades nutricionales del recién nacido. Sin embargo, el contenido de zinc en la leche humana puede no ser suficiente para cubrir las necesidades del prematuro, las cuales son mayores que las del recién nacido a término debido a: inmadurez del tracto gastrointestinal, diferencia en la velocidad de crecimiento posnatal y menores depósitos hepáticos. Cuando el niño prematuro se alimenta con fórmulas existe la posibilidad de que otros constituyentes disminuyan la biodisponibilidad del zinc. Además, la biodisponibilidad del Zn desde la leche humana es mayor que en las fórmulas para lactantes (2). La mínima ingesta necesaria para cubrir las pérdidas endógenas de zinc y conseguir una retención suficiente para las necesidades de nuevo tejido se recomienda que sean de 1,1 mg/100 kcal y, como límite superior el de 1,5 mg/100 kcal.

El objetivo de este trabajo es : a) investigar el contenido total de Zn en la leche materna de madres prematuras y a término y sus variaciones a lo largo del periodo de lactancia (calostro, 7, 15 y 30 días tras el parto) y comparar con los de las leches fórmula. b) La especiación cuantitativa del Zn en el suero de la leche humana en los distintos estados del periodo de lactancia y comparar con las especies de Zn presentes en las leches fórmula destinadas a la alimentación del recién nacido prematuro y a término.

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DETERMINACIÓN DE NANOPARTÍCULAS DE PLATA POR CPE-GFAAS CON CORRECTOR ZEEMAN

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A pesar de las numerosas aplicaciones que hoy día tienen las nanopartículas de plata (AgNPs), también pueden ser perjudiciales, por ser contaminantes de las aguas naturales y producir efectos tóxicos tanto en la fauna acuática como en el ser humano. Debido a esto, es útil disponer de métodos de análisis de dichas nanopartículas en las matrices de interés.

Se ha desarrollado un método de extracción en punto de nube (CPE) para separar las AgNPs para, posteriormente, detectarlas por espectrometría de absorción atómica con horno de grafito (GFAAS) y corrector Zeeman. Se utilizó un diseño Plackett-Burman para obtener las condiciones idóneas de extracción: se seleccionó el Triton X-114 como surfactante y la polivinilpolipirrolidona (PVPP) como complejante; se ajustó el pH entre 3,5 y 4,5 y la concentración de sal (tiosulfato sódico, para asegurar la extracción selectiva de las AgNPs) de 10 mM. Tras la separación de fases (la temperatura de nube del Triton X-114 es 23 ºC), una alícuota de extracto se analizó por GFAAS. Las temperaturas de pirólisis y atomización utilizadas fueron 800 y 1300 ºC, respectivamente. Se aplicó el procedimiento a la determinación selectiva de AgNPs en diversas muestras reales de materiales de limpieza (que incorporan AgNPs por sus propiedaes bactericidas). Los límites de detección (LOD) y cuantificación (LOQ) obtenidos fueron, respectivamente, 0,6 y 2,0 g kg^{-1}, referidos a muestra (1,0 y 3,4 µg mL^{-1}, respectivamente, en la porción de ensayo) y la masa característica media obtenida fue 35,6 fg. La precisión del procedimiento total fue del 3,9 %, y 97,8 % fue la recuperación analítica media.

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MONITORIZACIÓN AMPEROMÉTRICA DE ANTIOXIDANTES EN DIFERENTES TIPOS DE CERVEZAS UTILIZANDO ELECTRODOS SERIGRAFIADOS DE CARBONO CON NANOPARTÍCULAS DE ORO MODIFICADOS CON TIROSINASA

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La cerveza es una bebida muy consumida en todo el mundo y su importancia y popularidad se debe, entre otros aspectos, a que es una fuente natural de antioxidantes, fundamentalmente ácidos fenólicos procedentes de la malta y el lúpulo [1], piezas claves en la elaboración de esta bebida. El rol fundamental de estos compuestos fenólicos está relacionado con su participación en la prevención de oxidaciones, ya sea desde el punto de vista industrial en lo que a la elaboración se refiere, o considerando aspectos más relacionados con su demostrada capacidad antioxidante y sus implicaciones en la salud humana.

En la bibliografía se encuentran descritas diversas metodologías analíticas para la determinación de compuestos fenólicos, entre las que cabe mencionar la espectrofotometría UV-Vis, cromatografía de gases, HPLC y electroforesis capilar. En relación a las técnicas electroquímicas, se han desarrollado recientemente metodologías basadas en la utilización de biosensores enzimáticos, especialmente modificados con tirosinasa, como herramientas con un futuro prometedor en la monitorización *in situ* de compuestos fenólicos, considerando aspectos importantes como su elevada selectividad, bajo coste, capacidad de miniaturización, fácil instrumentación y automatización. Asimismo, la inmovilización de estas enzimas sobre electrodos impresos modificados con nanomateriales [3] está abriendo numerosas alternativas muy interesantes específicamente en este campo.

Por todo ello, en este trabajo se ha desarrollado un biosensor de tirosinasa basado en la inmovilización mediante *cross-linking* de dicha enzima sobre electrodos impresos de carbono modificados con nanopartículas de oro, para la monitorización directa de compuestos fenólicos mediante amperometría directa, en 15 muestras de cervezas comerciales. Para ello, se ha llevado a cabo, en primer lugar, la optimización de los parámetros químicos en instrumentales involucrados, como son: la concentración de enzima (250-1000 unidades), el pH (6-8) y el potencial aplicado (entre -0.4V y +0.2V), utilizando un concentración de catecol de $5 \cdot 10^{-5}$ M. Finalmente, la metodología optimizada se aplicó para evaluar la capacidad antioxidante, y su consecuente relación con el contenido en compuestos fenólicos, de 15 cervezas comerciales que fueron convenientemente pretratadas. El contenido en dichos compuestos se determinó en mg/L de tirosol, el compuesto fenólico encontrado en mayor proporción en las cervezas. Muy buena correlación de Pearson (r=0.821, 95%) fue obtenida en la comparación de los resultados obtenidos mediante la aplicación del método propuesto frente al método estándar de Folin-Ciocalteu, lo que demuestra así la utilidad de este biosensor en aplicaciones directas en muestras reales.

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ULTRASENSITIVE DETERMINATION OF CADMIUM BY ETAAS FOLLOWING DISPERSIVE MAGNETIC MICRO-SOLID PHASE EXTRACTION WITH *IN SITU* SYNTHESIS OF Fe_3O_4 NANOPARTICLES

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Cd is considered an important pollutant due to its high toxicity even at very low concentrations. One of the most relevant issues of Cd toxicity is its high bioaccumulation in plants, animals and micro-organisms. Therefore, in order to control the environmental pollution and human exposure, sensitive analytical approaches for determination of Cd are required.

Despite most of conventional analytical techniques provide high sensitivity for trace metal analysis, Cd in environmental samples is usually at very low concentration, so a preconcentration/separation approach for sample pretreatment before analysis is usually required to separate the target analyte and/or to remove matrix effects.

Among the different analytical strategies for sample pretreatment, solid phase extraction (SPE) is one of the most used for preconcentration because of its simplicity, rapidity and ability to attain high enrichment factors [1]. Recent advances in SPE have been focused on the development of new materials as adsorbents. In this way, nanoparticles (NPs) have arisen as new solid phases with improved performance in comparison with conventional solid phases. Compared to micrometer-sized materials, NPs provide higher surface area-to-volume ratio and density of exposed active sites resulting in faster kinetics for adsorption and separation, as well as higher extraction capacity and efficiency [2]. Among the different available nanometer-sized materials, magnetic nanoparticles (MNPs) have been recently used as SPE sorbents. The most used MNPs are based on magnetite nanoparticles (Fe_3O_4 NPs) that can be easy immobilized and/or separated by applying and external magnetic field, thus avoiding problems with high-back pressure in microcolumns (SPE dynamic mode) or a need for filtration/centrifugation (SPE batch mode). The use of Fe_3O_4 NPs for SPE in batch mode usually involves three steps: synthesis of MNPs, sorption of analytes and elution prior measurement. Dispersive micro-solid phase extraction (DM-μ-SPE) with *in situ* synthesis of MNPs allows shortening the operating procedure since Fe_3O_4 NPs and SPE are accomplished simultaneously.

In this work, DM-μ-SPE with *in situ* synthesis of Fe_3O_4 NPs for extraction of Cd and determination by ETAAS is proposed for the ultrasensitive and fast determination of Cd in natural waters [3]. The method is based on the co-precipitation of Cd onto freshly prepared Fe_3O_4 NPs after sonochemical treatment of the aqueous sample containing a Fe(II)+Fe(III) mixture together with the target analyte. The resulting magnetic solid phase (MSP) was easily separated from the aqueous matrix by applying an external magnetic field. After redispersion, a volume of the slurry is injected into the graphite tube for atomization, thus avoiding the elution step. A co-precipitation mechanism for Cd is approached using the Berthelot-Nernst and the Doerner-Hoskins laws. Cd is trapped by occlusion during the formation of Fe_3O_4 NPs. The detection limit was 2.3 ng L^{-1} of Cd. An enrichment factor of 53 is achieved. Finally, a recovery study carried out on spiked natural water samples shows recoveries in the range of 98.2-100%.

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MULTI-ANALYTICAL CHARACTERIZATION OF ENGINEERED CERIUM OXIDE NANOPARTICLES BY SPECTROSCOPIC AND ELECTROCHEMICAL TECHNIQUES

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Nanotechnology is developing rapidly in numerous industries such as chemistry, medicine, energy, computer sciences, or optics, with the consequent increase in the manufacturing and release of synthetic nanoparticles (NPs). Great part of the NPs interest derives from their very small size, which provides them with interesting properties such as high specific surface area, rapid diffusion or high reactivity in liquid and gas phase. It is indeed owed to their small size and the resulting particular properties that questions about their potential toxicity and environmental impact also emerge [1], given the high mobility and rapid transport they exhibit in the environment and inside the body. Full characterization of ENMs is crucial to identify the properties responsible for the ENMs toxic effects.

CeO_2 nanoparticles have interesting mechanical, spectroscopic, catalytic and oxidant/antioxidant properties. These nanoparticles are commercialized as fuel additive for diesel engines to improve burning efficiency of engine carbon deposits. The strong oxidising capacity of CeO_2 makes it an excellent product for reducing the emission of greenhouse gases and particulate number, as well as for diminishing fuel consumption [2]. Beyond the positive effects on air quality, little is known about the intrinsic toxicity of the CeO_2 NPs released to the environment. At the time nanotoxicology deals with the CeO_2 NPs hazards, efforts should be devoted to determine the physicochemical properties responsible for their potential toxic effect.
Here, we propose to apply a platform of analytical techniques to the detection, physical-chemically characterization and quantification of CeO_2 NPs in relation to key properties such as particle size/size distribution, shape, agglomeration/aggregation state, oxidation state or surface chemistry. The analytical platform will combines spectroscopic Raman and UV-VIS Spectroscopy) and microscopic (Transmission Electron Microscopy) techniques to exhaustively characterize the size and stability of CeO_2 NPs [3]. X-ray powder diffraction (XRD) is a non-destructive technique, which can provide with atomic structural information from crystalline samples. This technique can be used to quantify the nanoparticle size of the samples. Electrochemical techniques (Differential pulse voltammetry) will also be employed for detection and quantification purposes, as well as for determining oxidation state of cerium in the CeO_2 NPs, which affects its reactivity and toxicity. A comparative study of bulk CeO_2 NPs and those emitted from the combustion of CeO_2-dopped diesel is envisioned to assess the effect of physical-chemical changes during combustion on the NPs toxicity.

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SIZE CHARACTERIZATION AND QUANTIFICATION OF SYNTHETIC CERIA NANOPARTICLES BY FIELD FLOW FRACTIONATION COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (FFF-ICP-MS)

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Ceria nanoparticles (CeO_2 NPs) have different applications in multiple fields (e.g. polishing, catalysis, UV blocker, electronics, environmental remediation, sensing, biomedicine, etc.) [1], most of them related to their catalytic and sorption properties. For instance, the automotive industry uses the oxygen buffering capacity of the CeO_2 NPs to catalyse the fuel oxidation in diesel engines, where combustion tends to be incomplete. This results into the lower consumption of fuel and lower emission of soot particles and toxic gases. Beyond the positive effects on air quality, little is known about the intrinsic toxicity of the CeO_2 NPs released to the environment. As a consequence, efforts should be devoted to determine the physicochemical properties responsible for their potential toxic effect.

The physicochemical characterization of CeO_2 NPs requires the development of sensitive methods in accordance to the small concentrations expected in environmental samples. The present study proposes the use of Field Flow Fractionation (FFF) coupled to Inductively Coupled Plasma – Mass Spectrometry, (ICP-MS) for the size characterization and quantification of CeO_2 NPs. Despite FFF is a family of powerful techniques for size characterization of NPs of diverse nature [2] there is a lack of studies dealing with the quantitative size-characterization of CeO_2 NPs largely due to the lack of certified size standards of CeO_2 NPs and the poor stability of these NPs in suspension. We have investigated different conditions and separation factors affecting the stability of the CeO_2 NPs suspensions, and discussed different size-calibration strategies. The performance of two FFF separation modes (Asymmetric Flow Field-Flow Fractionation and Hollow Fiber Field-Flow Fractionation) is tested here in the search of the best response in terms of resolution and recovery.

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SÍNTESIS Y CARACTERIZACIÓN DE NANOPARTÍCULAS DE ÓXIDO DE ZINC. EFECTO DE ZnO-NPs FRENTE A LEVADURAS Y BACTERIAS LÁCTICAS

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Según la Unión Europea se define nanomaterial como aquel donde la composición del 50% de sus partículas tiene un diámetro comprendido entre 1-100 nm (EU, 2011). Debido a su pequeño tamaño y en general a su elevada relación área superficial/volumen estos materiales presentan distintas propiedades físico-químicas con respecto al material "bulk" (de mayor masa y volumen) en cuanto a su comportamiento térmico, resistencia material, solubilidad, conductividad, actividad (foto) catalítica y propiedades ópticas [1]. Uno de los nanomateriales más estudiados en la actualidad y sobre los que se desarrollan la mayoría de las aplicaciones son las nanopartículas de metales y óxidos metálicos. En concreto, las nanopartículas de óxido de zinc presentan excelentes propiedades mecánicas, estructura cristalina y un área superficial elevada, lo que hace que sean adecuados para reforzar la matriz de ciertos materiales poliméricos. Por otro lado, debido a sus propiedades, el óxido de zinc posee una elevada actividad antimicrobiana lo que hace que en la industria alimentaria se utilice para evitar la proliferación de microorganismos sobre todo en el empaquetado de alimentos [2].

En este trabajo se ha llevado a cabo la síntesis de ZnONPs mediante la aplicación de un método sol-gel, utilizando diferentes precursores como nitrato y acetato de cinc, 1,3 propanidiol o un biomaterial como es la gelatina [3]. La morfología, tamaño y composición de los nanomateriales resultantes se caracterizó por microscopía de transmisión electrónica (TEM), microscopía electrónica de barrido (SEM) y espectroscopia de infrarrojo con transformada de Fourier. Los resultados obtenidos por TEM pusieron de manifiesto que el mejor procedimiento de síntesis es aquel que emplea como precursor nitrato de zinc y la gelatina como agente director de la formación de estas nanopartículas, seguido de un proceso de calcinación. Con este procedimiento se obtuvieron ZnOPs de tamaños comprendidos entre 5 y 20 nm. Además, el uso de la gelatina tiene la ventaja de que al ser un polímero natural muy utilizado en la industria alimentaria facilita el desarrollo de aplicaciones de las ZnONPs relacionadas con este campo. La eficiencia de formación de ZnOPs fue del 70% utilizando tanto procedimientos de diálisis como ultracentrifugacíon. La estabilidad de las suspensiones de ZNONPs se estudio en presencia de distintos agentes como agua, citrato y ácido ascórbico con y sin el empleo de la sonda de ultrasonidos Una vez caracterizadas las ZnOPS se evaluó su efecto, perjudicial o beneficioso, frente a microorganismos de elevado valor añadido en la industria alimentaria como es el caso de las levaduras del género *Saccharomyces*, o de bacterias del género *Lactobacillus* que son responsables de la producción de alimentos basados en procesos de fermentación. Para ello se llevaron a cabo estudios de viabilidad y de acumulación de Zn.

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SILVER SPECIATION AND CHARACTERIZATION OF SILVER NANOPARTICLES RELEASED FROM PLASTIC FOOD CONTAINERS BY SINGLE PARTICLE ICP-MS

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Plastic food containers incorporating silver nanoparticles (AgNPs) result in longer durability of food due to their antibacterial properties [1]. However, for the assessment of consumer risk, it is necessary not only to quantify the released of silver from the plastic containers into the food [2], but also to identify which species of silver, ionic or NPs, are released to the stored food.

In this study, silver migration from commercial food box and baby feeding bottle made from polycarbonate and polypropylene, respectively, claiming to content nanosilver, into food simulant solutions were evaluated. Following EU regulation 10/20111, the simulant solutions used were water, 3% (v/v) acetic acid, 10% and 90% (v/v) ethanol that represent aqueous, acidic, alcohol-containing and fatty foods, respectively. Silver release to food simulants was investigated at temperatures in the 20-70ºC range and contact time up to 10 days. It was observed that under all assayed conditions, the released of silver from the food box was 2 to 3 orders of magnitude higher than that of the baby bottle, even although the total silver content in the food box was halt to that of the baby bottle. As it was expected, for both food containers, silver migration depended on both the nature of the tested solution and the applied extraction conditions. For both materials, the highest releases were observed with 3% acetic acid at 70ºC for 2h, corresponding to 62 ng dm^2 and 1887 ng dm^{-2} of silver for the baby bottle and the food box, respectively. These amounts represented 0.052% and 4.1% of the total silver content in the raw material, respectively. The continuous release of silver by the extended used of the containers was also evaluated for three consecutive cycles of use.

Single particle-ICP-MS (SP-ICP-MS) analyses confirmed the presence of AgNPs in the water and acidic extracts but also reveal the presence of dissolved silver forms in these extracts. In most cases, AgNPs migrated from the plastic accounted only for a minor fraction of the total silver released from the materials (percentages in the 0.1-8.6%). However, AgNPs migrated into water at 40ºC and 70ºC from the food box accounted for as much as 34% and 69%. SP-ICP-MS results were compared with those obtained with more conventional approaches, such as ultrafiltration through 10 kDa cut-off filter and SEM-EDX analysis.

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CARACTERIZACIÓN DE NANOMATERIALES DE PLATA UTILIZADOS COMO ADITIVOS EN ALIMENTACIÓN ANIMAL: ENSAYOS DE LIXIVIACIÓN

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El interés creciente por los materiales a nanoescala está convirtiendo a las nanopartículas de plata en el nanomaterial más utilizado actualmente, debido a sus propiedades antimicrobianas. El empleo tradicional de plata iónica se ha visto sustituido por el uso de plata metálica en forma de nanopartículas, debido a su mayor estabilidad, mayor actividad antimicrobiana y menor toxicidad para las células eucariotas. Todas estas propiedades, han permitido considerar a estas nanopartículas como alternativa a otros productos prebióticos utilizados actualmente en la alimentación animal. Una de las variables a tener en cuenta en la incorporación de las nanopartículas de plata en la dieta de estos animales es la forma de administración, ya que ha de permitir una dosificación correcta de las mismas. Las arcillas se utilizan en alimentación animal para múltiples aplicaciones tecnológicas, nutricionales, sanitarias y ambientales. En este punto se plantea la utilización de sepiolitas y caolines, recubiertos con nanopartículas de plata como vehículos de administración de plata a los animales.

En primer lugar, los nanomateriales iniciales han sido caracterizados mediante técnicas de microscopía electrónica de barrido (FESEM), con el fin de estudiar la distribución, tamaño y morfología de las nanopartículas de plata presentes. El estudio del comportamiento de estos nanomateriales y la liberación de plata en el ámbito de su utilización, se ha llevado a cabo mediante diversos ensayos de lixiviación y posterior caracterización, detección y cuantificación tanto de nanopartículas de plata como de especies derivadas de plata mediante distintas técnicas analíticas. Se han utilizado diferentes medios de lixiviación (agua ultrapura y HCl 0,01 M), así como ensayos de simulación de procesos digestivos y ensayos de liberación frente al tiempo. El uso combinado de la ultracentrifugación y ultrafiltración junto con la espectrometría de masas con plasma de acoplamiento inductivo (ICPMS) permite la determinación de nanopartículas y de especies disueltas en los lixiviados, mientras que mediante técnicas de dispersión de radiación dinámica (DLS) se pueden obtener las distribuciones de tamaños de las partículas en suspensión en los distintos medios. Por su parte, la técnica de Fraccionamiento en Flujo mediante campos de flujo asimétrico (AsFlFFF) en combinación con sistemas de multidetección (UV-Visible, ICP-MS) ofrece la posibilidad de detectar y caracterizar por tamaño las nanopartículas de plata liberadas así como otras especies macromoleculares presentes en los medios complejos a los que se pueda asociar la plata. En el presente trabajo se presentan los principales resultados obtenidos.

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ANCLAJE DE SONDAS TIOLADAS SOBRE UNA SUPERFICIE PERFLUORADA MEDIANTE REACCIÓN DE "*QUÍMICA CLICK*". OBTENCIÓN DE *MICROARRAYS* CON MEJORES PRESTACIONES

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Actualmente existe la necesidad de desarrollar nuevas herramientas de análisis que permitan una detección rápida y precisa. Se busca asimismo que sean sensibles, de manejo sencillo, multiplexables, de dimensiones reducidas y económicas. Los sistemas de detección en formato *microarray* ofrecen la posibilidad de integrar todas estas cualidades. [1] Para obtener *microarrays* competitivos que presenten alta sensibilidad y selectividad, es crucial disponer de un método idóneo de anclaje de las sondas y, además de reducir al máximo las adsorciones inespecíficas. Las mejores estrategias recurren a la unión covalente, pues permite controlar la orientación de la sonda, y conseguir anclajes más estables. Hay una serie de reacciones que por su ortogonalidad, compatibilidad con disolventes acuosos, rapidez, y altos rendimientos, han emergido como estrategias muy válidas, son las reacciones denominadas de química "*click*". [2] Recientemente nuestro grupo de investigación ha puesto a punto diferentes estrategias de inmovilización covalente y selectiva de biorreceptores mediante reacciones de este tipo, usando acoplamientos tiol-eno, tiol-ino y tiol-epoxy, catalizados todos ellos por luz. [3-5] Los resultados obtenidos destacaron la idoneidad de este tipo de reacciones.

En este trabajo, se avanza un paso más en el desarrollo de *microarrays* de altas prestaciones, y se integra el empleo de la reacciones de acoplamiento tiol-eno con el diseño de una superficie de alta hidrofobicidad. De esta manera se consigue una inmovilización óptima, y se evitan interacciones inespecíficas sin necesidad de bloqueo posterior. Para ello, se plantea una modificación de la superficie que combina dos organosilanos, uno conteniendo un grupo alqueno (viniltrietoxisilano) y otro organosilano que posee una cadena perfluorada, con capacidad de repeler la adsorción inespecífica y aumentar la hidrofobicidad de la superficie.

Las evidencias experimentales llevan a pensar que la acción de la luz sobre el organofluorado provoca una deshidrohalogenación, dando lugar a un doble enlace, que permite la reacción del tiol-eno. A la vez, mantiene un gran número de enlaces C-F que repelen la adsorción no específica. Los ensayos realizados demuestran que las mejores prestaciones se obtienen con una proporción 1:5 de vinil/flúor. Los resultados de hibridación de oligonucleotidos de cadena totalmente complementaria presentan bajo ruido de fondo, además de detectarse concentraciones 5 nM de diana, lo que representa 10 veces más sensibilidad que en si se ancla la sonda empleando sólo vinil organosilano y reacción tiol-eno.

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STUDY OF THE DIRECT INTERACTION BETWEEN GOLD NANOPARTICLES AND CHITOSAN TO FORM NANOCAPSULES.

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A study of the interaction between free anionic gold nanoparticles (diameter: 10 nm) and chitosan (low molecular weight) in a solution is presented as a function of the concentration and pH of the polymer in a solution [1]. Zeta potential measurements, TEM images and UV-Vis spectra indicate the effective aggregation of the nanoparticles (AuNPs) in the presence of chitosan. Furthermore, anionic gold nanoparticles act as crosslink agents to form chitosan nanocapsules with an average molecular size of 260 nm, as it is shown in Figure 1.

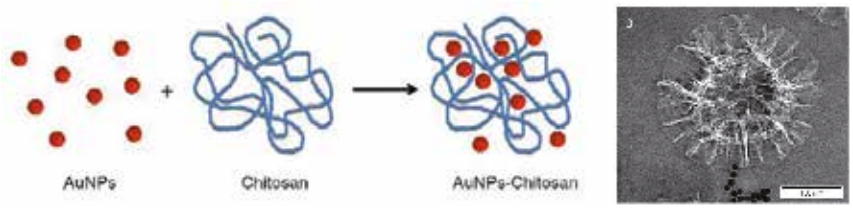

Figure 1. a) Scheme of the direct interaction between AuNPs and Chistosan. B) TEM image showing a chitosan nanocapsule.

The changes of the surface plasmon band (SPB) due to the adsorption of the polymer on the nanoparticle surface allow the usage of the citrate gold nanoparticles as sensors of the biopolymer for analytical purposes, being the limit of detection for chitosan 69 nM.
The optimum pH for the interaction between the biopolymer and the nanoparticles is found at a value of 6.4, obtained from spectrophotometric measurements, applying a deconvolution analysis of the experimental data. A simple model based on molecular surface electrostatic interactions is proposed to understand the pH dependence of the investigated system.

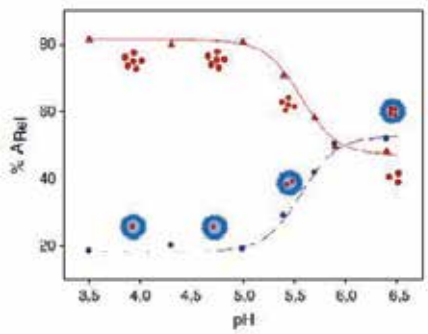

Figure 2. Influence of the solution pH on the interaction of the AuNP–chitosan system.

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EFFICIENT INSULIN SENSING BY NICKEL HYDROXIDE NANOPARTICLES DECORATED MULTIWALLED CARBON NANOTUBES

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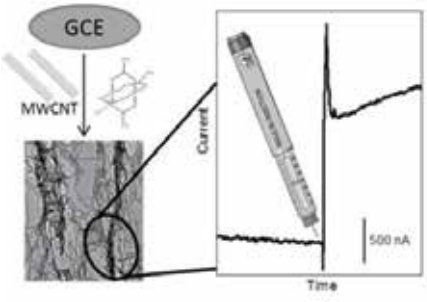
Diabetes is caused by the insufficient release of insulin or loss of insulin action at target tissues, which results in aberrant glucose and lipid metabolism. Insulin is an important polypeptide hormone produced in the pancreas that is used to control the glucose levels in blood within a narrow concentration range [1, 2].

Many different analytical methods have been described for the quantitative analysis of insulin, among them radioimmunoassay [3] MALDI-TOF mass spectrometry [4], capillary electrophoresis [5], HPLC [6], etc. However, most of these methods are time-consuming, cumbersome, and expensive. The use of sensor offers some advantages such as high sensitivity, rapid response, low cost, miniaturization and more important the possibility to construct portable devices easy to use for point of care.

In the present work an insulin sensor based on nickel hydroxide nanoparticles decorated multiwalled carbon nanotubes modified electrode ($Ni(OH)_2$NPs/Nafion-MWCNTs/GC) was prepared, using electrochemical deposition of $Ni(OH)_2$ nanoparticles from dinickel acetate microstructures, previously directly electrogenerated on the electrode surface from a dinuclear paddle-wheel Ni monothiocarboxylate complex. The electrochemical behavior of the modified electrodes in aqueous alkaline solutions of insulin was studied by cyclic voltammetry and chronoamperometry. It was found that the as prepared modified electrode has an excellent electrocatalytic activity towards insulin oxidation, reducing the overpotential and improving the electrochemical behavior, compared to the bare GC electrode. The insulin sensor developed shows excellent analytical features, such as a high sensitivity (5.0 A mol cm^{-2} μM^{-1}), a low detection limit (0.12 μM) and a wide dynamic range (0.12 -10.00 μM). Finally, this sensor have been applied to the determination of insulin in pharmaceuticals and in human plasma. Efficient recoveries for pharmaceuticals and human plasma demonstrate that the proposed methodology can be satisfactorily applied to these types of samples.



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ON-VIAL IMMOBILIZATION OF CdSe QUANTUM DOTS TO ACCOMPLISH A NOVEL SOLID-STATE CHEMILUMINESCENT ASSAY FOR ANTIMONY SPECIATION

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In the last years, a great interest has arisen on new chemiluminescent systems due to the attractive properties of chemiluminescence-based assays such as high sensitivity, wide linear range and simple instrumentation required. In this sense, different nanomaterials have been tried as chemiluminescence (CL) emitters including metal and semiconductor nanoparticles, also known as quantum dots (QDs) [1]. QDs exhibit a great potential to be applied in CL systems, but generally, poor selectivity is obtained and hence, the direct application to real sample is troublesome. In this sense, the implementation of a separation step prior to detection can solve this drawback. To date, several liquid-phase microextraction (LPME) modes have been developed, which allows to achieve analyte preconcentration and sample clean-up [2]. Since CL reactions are performed in aqueous media, a LPME mode providing an aqueous extract is required. Therefore, liquid-liquid-liquid microextraction (LLLME), where target analyte is firstly extracted into an organic phase and then back-extracted into aqueous drop, may be the most appropriate.

So far, QDs-based CL assays involve the use of QDs dispersed in aqueous media, which implies the QDs surface modification in order to impart aqueous solubility. Nevertheless, this process usually causes changes over their optical properties. Therefore, investigation of alternative strategies so as to avoid this drawback should be undertaken.

In this work, on-vial immobilization of QDs allows to build a solid-state CL assay, thus preventing the need for QDs surface modification [3]. To achieve high selectivity, LLLME is performed prior to the chemiluminescent reaction between CdSe QDs and H_2O_2. This approach allows to develop an ultrasensitive and simple assay for detection of Sb(III) and total antimony. Sb(V) was calculated as a difference between the total and Sb(III)concentration.

Experimental parameters affecting the CL system and LLLME process were evaluated. Under optimal conditions, a detection limit of 6 ng/L Sb and an enrichment factor of 95 were obtained. The repeatability and reproducibility of the proposed method, expressed as relative standard deviation (RSD), were 1.3% (n=7) and 2.9% (n=3), respectively. In order to evaluate the applicability of this method for water analysis, several recovery studies were performed in different water samples. Recoveries obtained were in the range 94-105%, which indicates that the proposed assay is suitable for antimony speciation in water samples.

The sensing mechanism whereby Sb inhibits the CL reaction was investigated by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), UV-Vis absorption and fluorescence measurements. The obtained results suggest that the decrease in CL intensity can be ascribed to an inhibition of energy transfer from the capping ligand (HDA) (donor) to CdSe QDs (acceptor) due to the presence of Sb. In the absence of Sb, an oxide layer over QDs surface is formed, which leads to changes in the QDs size.

Acknowledgements
Financial support from the Spanish Ministry of Science and Innovation (project CTQ2012-32788) and the Xunta de Galicia (project 10PXIB 314030 PR) is gratefully acknowledged.

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DETERMINACIÓN DE LOS NIVELES DE FERRITINA Y DE LA RELACIÓN FE:FERRITINA EN LÍNEAS CELULARES DE CÁNCER DE MAMA MEDIANTE ICP-MS

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La ferritina es una proteína globular encargada del almacenamiento del hierro en los seres vivos. Principalmente, se sintetiza y se almacena en el hígado. Los niveles de la ferritina en suero se utilizan como parámetro para el seguimiento de la deficiencia de hierro y otras enfermedades relacionadas con el metabolismo de este elemento en los seres vivos. Además de estos, existen otros factores secundarios tales como infecciones, inflamaciones o presencia de tumores que pueden aumentar valores de la ferritina en suero. En la actualidad, existen estudios que demuestran que un aumento en los niveles de ferritina se asocia con una progresión del cáncer de mama hacia fenotipos malignos más avanzados [1]. El cáncer de mama está reconocido como la neoplasia más abundante en mujeres. El éxito del tratamiento en este tipo de cánceres se basa en la buena comprensión de los mecanismos moleculares implicados en la iniciación y la progresión del tumor. Por lo tanto, existen estudios en los que se sugiere que los cambios en los niveles de ferritina son cruciales y, asociados a esto, la pérdida de regulación de la homeostasis del hierro intracelular.

El objetivo del presente trabajo es la evaluación de la concentración de ferritina en cultivos celulares de cáncer de mama y el seguimiento de la relación Fe:ferritina para entender si el desequilibrio en la regulación del Fe afecta a los niveles de saturación de la ferritina. Para ello, utilizamos un inmunoensayo de tipo sándwich, previamente optimizado, que es específico para la ferritina humana y que, en uno de los anticuerpos, presenta un complejo de rutenio que se utiliza en la monitorización de la ferritina mediante ICP-MS [2]. Este inmunoensayo se aplica a lisados celulares (líneas celulares: MCF-7 y MDA-MB-231) para la determinación tanto de los niveles de ferritina como de las relaciones Fe:ferritina. Para esta última determinación se aplicará un trazador de ferritina con ^{57}Fe isotópicamente enriquecido, recientemente desarrollado, utilizando la dilución isotópica específica.

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ENHANCED DETECTION OF DNA SEQUENCES USING PCR AMPLIFICATION AND ON-LINE GEL ELECTROPHORESIS (GE)-ICP-MS: DETERMINATION OF GENE COPY NUMBER VARIATIONS

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DNA copy number variations (CNVs), which are gains or deletions of genomic segments in some individuals, account for a substantial proportion of human genetic variations. These gene dosage alterations have been shown to be associated with a wide spectrum of human disorders such as autoimmune diseases, autism, schizophrenia and obesity [1]. CNVs can also occur in cancer-related genes, some of which may confer resistance of tumour cells to chemotherapy [2]. Therefore, the design and evaluation of analytical methods that permit the quantification of CNVs in genomic DNA is of special importance for a variety of applications in basic research studies and clinical diagnosis.

In this study, a new analytical strategy for gene quantification has been developed that combines the amplification capacity of end-point polymerase chain reaction (PCR) with the determination of the PCR products by on-column agarose-gel electrophoresis (GE) coupled on-line with inductively coupled plasma mass spectrometry (ICP-MS) for phosphorous detection. The calibration of the GE-ICP-MS system with a DNA ladder permits the direct estimation of the size of the amplified gene after PCR. With this knowledge and considering the compound independent quantification capabilities exhibited by ICP-MS for phosphorous (only dependent on the number of P atoms per molecule), the correlation of the P-peak area of the amplified gene in respect to the gene copy numbers (in the starting DNA) can been established. Such correlation permits the determination of CNVs in genomic DNA using GE-ICP-MS measurements. The suitability of the proposed strategy has been used to address CNVs due to cells exposure to the chemotherapeutic drug cisplatin [3]. Moreover, the multiplex capacity of the developed analytical strategy for the simultaneous quantification of CNVs of different genes has been studied.

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ANÁLISIS METABOLÓMICO EN MUESTRAS DE LAVADO BRONCOALVEOLAR PARA LA IDENTIFICACIÓN DE POSIBLES BIOMARCADORES DEL CÁNCER DE PULMÓN

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El cáncer de pulmón (CP) constituye una de las 10 causas de muerte más comunes por neoplasia en el mundo [1]. La búsqueda de biomarcadores en diferentes fluidos biológicos, para la diagnosis precoz de esta enfermedad, es actualmente un reto de gran interés en medicina. En este sentido, el uso de la metabolómica como técnica de análisis desempeña un papel muy importante, ya que permite analizar el mayor número de metabolitos posibles para obtener la correspondiente "huella dactilar metabolómica" (*fingerprinting*), en la que se pueden identificar moléculas que modifican su concentración en respuesta a una enfermedad. Estas moléculas (biomarcadores) pueden utilizarse para el diagnóstico precoz de esta enfermedad.
El lavado broncoalveolar (LBA) es un fluido que se obtiene durante el estudio exploratorio de los enfermos de pulmón y que permite obtener información sobre constituyentes celulares y bioquímicos de la superficie epitelial del tracto respiratorio inferior, a través de la instilación y posterior aspiración de líquido en uno o varios segmentos pulmonares. Se estima que con el proceso de obtención del LBA se toma muestra en un millón de alvéolos (1% de la superficie pulmonar), obteniéndose aproximadamente 1 ml de secreciones reales pulmonares en el total del líquido recuperado [2]. En general, el LBA permite diagnosticar enfermedades pulmonares [3], como infecciones en personas con problemas del sistema inmunológico, [4] neumonía en personas con respiradores artificiales, la cicatrización del pulmón (enfermedad pulmonar intersticial) e incluso algunos tipos de cáncer de pulmón.

En este trabajo se ha desarrollado un procedimiento metabolómico basado en infusión directa en un espectrómetro de masas de alta resolución (DI-ESI-QTOF-MS) para determinar los perfiles metabolómicos de fluidos LBA procedentes de personas con CP y compararlos con controles que no padecen esta enfermedad (C). Previamente al análisis, las muestras de LBA se trataron mediante el procedimiento descrito por Evans C.R., et al. [5] basado en la adición MeOH:$CHCl_3$ 9:1 para la precipitación de proteínas. Finalmente, los perfiles metabolómicos obtenidos mediante DI-ESI-QTOF-MS tanto en modo de ionización positivo como en negativo, se sometieron a análisis estadístico multivariante (PCA, PLS-DA) con el fin de identificar los metabolitos alterados por el CP.

El análisis estadístico de los resultados mostró una clara diferenciación entre los dos grupos de estudio (C vs CP), y permitió identificar algunos posibles biomarcadores para el diagnóstico del CP, los cuales pueden relacionarse con distintos mecanismos patológicos propios de esta enfermedad.

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ESTUDIO DE BIODISPONIBILIDAD DEL SELENIO A PARTIR DE LA MICROALGA CHLORELLA SOROKINIANA ENRIQUECIDA EN ESTE ELEMENTO UTILIZANDO METODOLOGÍAS -ÓMICAS

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El selenio es un elemento esencial para los seres vivos al formar parte de enzimas con actividad antioxidante [1]; asimismo ciertas formas de selenio se han relacionado con la acción protectora frente a ciertos tipos de cáncer y otras enfermedades [2]. La toxicidad o esencialidad de este elemento no depende solo de la concentración sino de la forma química en que se encuentra, siendo las formas orgánicas (selenoamino ácidos) menos tóxicas que las formas inorgánicas (selenito y seleniato) y nutricionalmente activas.

La biotecnología de microalgas ha ganado relevancia en las últimas dos décadas debido al amplio rango de aplicaciones para la producción de biomasa para alimentación. Las microalgas son un vehículo de alto valor nutricional para suministrar al hombre las formas saludables del selenio, ya que además de producir selenometionina y selenocisteína, son ricas en proteínas y aminoácidos libres, ácidos grasos insaturados y agentes antioxidantes necesarios para el metabolismo de las vitaminas [3].

La biodisponibilidad del selenio se estudió en *Chlorella sorokiniana*, cultivadas en un medio rico en este elemento utilizando 30 ratones separados en grupos con dietas de diferente concentración de selenio: 0.1, 0.5 y 1 mg kg^{-1}. Después de 5 semanas los animales fueron sacrificados y se aplicaron metodologías metalómicas basadas en la cromatografía multidimensional acoplada a ICP-MS a los extractos de órganos de alta actividad metabólica y suero sanguíneo, con el objetivo de establecer las diferencias entre metalobiomoléculas con expresión diferencial (especialmente selenoproteínas) en función de la dieta de selenio consumida. Por otro lado, el empleo de técnicas metabolómicas y procedimientos de extracción en múltiples etapas proporcionó una visión global del metaboloma sanguíneo, que combinado con técnicas de análisis multivariante mostró una clara discriminación entre los grupos de estudio.

El presente trabajo proporciona información sobre la biodisponibilidad del selenio acumulado en esta microalga, indicando que esta biomasa puede constituir alimento alternativo rico ene Se para suplementar la dieta de los seres humanos.

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EVALUACIÓN CUANTITATIVA DE LA INCORPORACIÓN CELULAR Y EN EL ADN DE DIFERENTES FÁRMACOS DE PLATINO EN LÍNEAS CELULARES SENSIBLES Y RESISTENTES A CISPLATINO

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La búsqueda de nuevos fármacos quimioterapéuticos que mejoren las limitaciones asociadas al uso de tratamientos tradicionales, como la resistencia adquirida al cisplatino, es un área en constante investigación. En este sentido, el uso de alternativas al tradicional cisplatino, como el carboplatino y el oxaliplatino, ha sido investigado en numerosos tipos de cáncer, y estas terapias son ampliamente utilizadas en la actualidad. El mecanismo de acción de estos fármacos se cree que comienza con la incorporación de los compuestos en las células, seguida de su interacción con el ADN, formando aductos Pt-ADN con la consecuente inhibición de los procesos de replicación y transcripción y finalmente la muerte celular. En consecuencia, la eficacia de estos tratamientos se considera altamente dependiente del grado de formación de dichos aductos [1, 2].

Para evaluar la eficacia de estos nuevos tratamientos de quimioterapia o para elucidar el origen de la resistencia adquirida a los fármacos, son necesarias metodologías analíticas que permitan trazar de forma cuantitativa las rutas metabólicas que siguen estos compuestos en sistemas celulares. Sin embargo, al tratar de comparar diferentes experimentos, se hacen visibles los problemas metodológicos debidos a la alta variabilidad de parámetros biológicos, como el número de células en cada experimento o la concentración de ADN. En el presente trabajo, se han identificado y corregido estas variaciones para poder comparar el comportamiento biológico de cisplatino, oxaliplatino y pyrodach-2, un nuevo fármaco de platino. Se ha llevado a cabo un estudio detallado de la incorporación celular de cada uno de los compuestos, usando las mismas concentraciones de fármaco en tres líneas celulares diferentes: adenocarcinoma de pulmón (A549), carcinoma de ovario sensible a cisplatino (A2780) y la misma línea resistente a cisplatino (A2780CIS). Para minimizar los errores asociados al conteo de las células, se han normalizado los resultados de platino a masa de células secas después de ser liofilizadas. De forma similar, la acumulación de Pt en el ADN se ha evaluado refiriendo Pt a la concentración de ADN medida por monitorización de ^{31}P usando un sistema de inyección en flujo con detección por ICP-MS.

En conjunto, estas estrategias nos han permitido llevar a cabo un balance de masa de la incorporación celular de Pt que permite la comparación directa de diferentes tratamientos en diferentes tipos de células de cáncer. Además, la incubación de los tres compuestos con un oligonucleótido de ADN sintético, seguida de la digestión enzimática, ha revelado diferentes reactividades entre los fármacos de Pt, así como las diferentes estructuras de los aductos que se forman, utilizando de forma complementaria HPLC-ICP-MS y HPLC-ESI-q-TOF-MS.

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NIVELES DE SELENIO Y ACTIVIDAD DE LA GLUTATION PEROXIDASA EN PLASMA DE PACIENTES CON DIABETES MELLITUS

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La diabetes mellitus (DM) es un trastorno metabólico crónico que se desencadena cuando el organismo pierde su capacidad de producir suficiente insulina o de utilizarla con eficacia para consumir la glucosa, de modo que ésta queda circulando en la sangre (hiperglucemia) y dañando los tejidos con el paso del tiempo. La enfermedad es progresiva y está asociada con alto riesgo de ateroesclerosis, daño renal, neuronal y ceguera [1].

La hiperglucemia en la diabetes causa la glicación no enzimática de grupos amino libres de proteínas y conduce a sus cambios estructurales y funcionales, resultando en complicaciones de la diabetes. La glicación de las proteínas comienza con la formación de la base de Shiff's, seguida por una transposición intermolecular y la conversión en productos de Amadori, formando fracciones proteicas consolidadas, denominada productos de glicación avanzada (AGE) que alteran las funciones de dichas proteínas. Además, en la reacción de glicación no enzimática se generan especies reactivas de oxígeno [2].

El organismo dispone de una serie de sistemas de defensa antioxidante que lo protegen de esas especies: particularmente superóxido dismutasa (SOD), glutatión peroxidasa (GPx) y catalasa (CAT), siendo la GPx una enzima dependiente del selenio (Se) que cataliza la reducción de hidroperóxidos.

La glicación de proteínas no sólo ocurre con la hemoglobina, puede ocurrir en proteínas que participan en el sistema antioxidante (como la GPx) pudiendo afectar a su actividad.
En este trabajo, se llevó a cabo la especiación cuantitativa de selenoproteinas en muestras de plasma humano de sujetos sanos y diabéticos por cromatografía de afinidad, AF-HPLC-ICP-MS, en combinación con dilución isotópica post-columna (IDA) para la cuantificación de selenio y selenoproteinas. Paralelamente, se evaluó también la actividad enzimática de la GPx en diabéticos y personas sanas, observando una diferencia significativa entre los distintos grupos (sanos: 0.61 ± 0,11 U mL^{-1}, diabéticos controlados: 0,40 ± 0,12 U mL^{-1} y pacientes diabéticos no controlados: 0,31 ± 0,09 U mL^{-1}), aunque el nivel de Se total encontrado en diabéticos fue ligeramente inferior al encontrado en los sujetos sanos. Estos datos sugieren que la glicación de la GPx podría llevar a la inactividad de la misma.

Discutiremos las posibilidades de medir dicha actividad de la GPx directamente a través de la oligomerización de la proteína [3] y su relación con la posible glicosilación de la misma.

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STANDARDIZED THAWING PROTOCOL FOR THE EXHAUSTIVE LIPIDOMIC PROFILING OF PLASMA SAMPLES

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Lipid profiling is a promising tool for the discovery and subsequent identification of biomarkers associated with various diseases. However, data quality is quite dependent on the pre-analytical methods employed. To date, potential confounding factors that may affect lipid metabolite levels after the thawing of plasma for biomarker exploration studies have not been thoroughly evaluated. In this study, by means of experimental design methodology, we performed the first in-depth examination of the ways in which thawing conditions affect lipid metabolite levels. After the optimization stage, we concluded that temperature, sample volume and the thawing method were the determining factors that had to be exhaustively controlled in the thawing process to ensure the quality of biomarker discovery. Best thawing conditions were found to be: 4ºC, with 0.25 mL of human plasma and ultrasound (US) thawing. The new US proposed thawing method was quicker than the other methods we studied, allowed more features to be identified and increased the signal of the lipids. In view of its speed, efficiency and detectability, the US thawing method appears to be a simple, economical method for the thawing of plasma samples, which could easily be applied in clinical laboratories before lipid profiling studies.

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A MULTIPLE COMPARISON OF LIPIDOMIC PROFILING OF BLOOD-DERIVED MATRICES IN HIV-INFECTED PATIENTS WITH DIFFERENT SEROLOGICAL EVOLUTION AND CO-INFECTED WITH HC

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Metabolites are involved in most biological processes of human body and can be useful tools for the diagnosis of various types of diseases, as well as the discovery of biomarkers.

The first step for any kind of biological analysis is the choice of biological matrix to be analysed. The choice of the matrix is of great importance to get high quality data sets, reliable results and avoid error prone for the diagnosis of different pathologies.

The main objective of this study was to realize a comparative study of lipid profile obtained through the application of a liquid chromatography-electrospray ionization quadrupole-time-of-flight mass spectrometry (LC-ESIq-ToF-MS) in different blood matrices to evaluate the intrinsic differences that appear in both serum and plasma samples and, consequently, determine whether a single blood-derived biofluid is more informative in the lipid study for the different stages of HIV disease or whether both biofluids are of equal value in terms of discrimination and reproducibility.

The study was performed on plasma and serum samples collected from the same donors individuals with different degrees in the evolution of HIV infection: i) Asymptomatic HIV-infected patients who are seropositive and co-infected with Hepatitis C disease; ii) HIV-infected patients that have developed the acquired immunodeficiency syndrome; iii) HIV-infected patients that have developed the acquired immunodeficiency syndrome and who are co-infected with Hepatitis C disease.

In this case, both matrices are suitable to obtain a lipid fingerprint. Our results showed significant differences in terms of metabolomics features and metabolites concentration, when serum and plasma samples are analysed. Being lower the differences in peak areas and RSD values for plasma analyses than in serum analyses.

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ESTUDIO METABOLÓMICO DE LA RESPUESTA BIOLÓGICA DEL RATÓN *MUS SPRETUS* EN ENSAYOS DE EXPOSICIÓN A DDE. EFECTO PROTECTOR DEL SELENIO

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La presencia de contaminantes orgánicos persistentes, como PCBs y organoclorados, amenaza el entorno del Parque Nacional de Doñana debido al uso de plaguicidas en los cultivos (fresas, cítricos, arroz) y a las actividades industriales colindantes. Entre estos compuestos destaca el DDE (diclorodifenildicloroetileno) por su alta concentración detectada en sangre, hígado y tejido adiposo de diversos carnívoros de dicho entorno [1] . Varios estudios han mostrado el carácter disruptor endocrino del DDE, y aunque hoy se excluye su relación con el cáncer de mama, está probado su carácter antiandrogénico [2], y neurotóxico, relacionándose con enfermedades como el Párkinson y el Alzheimer [3], posiblemente a través de la producción de estrés oxidativo. Por otra parte, el selenio (Se) es bien conocido por su carácter quimiopreventivo contra el cáncer [4] y su efecto protector frente a la toxicidad causada por elementos tóxicos como cadmio, arsénico o mercurio [5, 6]. En este sentido, los ensayos de exposición de organismos modelo a compuestos tóxicos y no tóxicos permitirá obtener información de los cambios metabólicos inducidos por dichos compuestos y para ello, el uso de técnicas ómicas, como la metabolómica, representa una buena aproximación [7, 8].

En este trabajo se ha llevado a cabo ensayos de exposición de *Mus spretus* a DDE en presencia y ausencia de Se con objeto de obtener información de los cambios metabólicos producidos por el DDE así como de la capacidad protectora que ejerce el Se. Por ello se ha desarrollado una doble plataforma metabolómica basada en el uso complementario de DI-ESI-QqQ-TOF-MS y GC-MS para el análisis de los extractos de hígado de *Mus spretus* sometido a los ensayos de exposición. Posteriormente los datos fueron tratados mediante análisis estadístico multivariante para identificar los metabolitos que se alteran en la exposición. Los resultados han mostrado alteraciones en diversas rutas metabólicas como el metabolismo energético (glucolísis, ciclo de Krebs), metabolismo de lípidos (acidos grasos libres), metabolismo de aminoácidos (glutamina, ac. aspártico, ornitina, ect) y estrés oxidativo (inosina). La presencia de selenio muestra un efecto protector en estas alteraciones metabólicas.

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SISTEMAS INTEGRADOS PARA EL DESARROLLO Y SEGUIMIENTO DE REACCIONES BIOANALÍTICAS APLICADAS EN EL ANÁLISIS GENÉTICO

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El análisis de material genético actualmente es casi una rutina en los laboratorios de control de calidad y seguridad alimentaria, monitorización medioambiental y diagnóstico/pronóstico de enfermedades. Para ello, son ampliamente usadas herramientas como la reacción en cadena de la polimerasa (PCR), chips de micromatrices o biosensores. Desarrollar nuevas aproximaciones que mejoren las prestaciones analíticas de los métodos basados en la detección de regiones específicas del genoma en estudio y reduzcan costos, es muy demandado.

En la presente comunicación, se presentan los avances alcanzados en integración, miniaturización y automatización para lograr ensayos rápidos y fiables que puedan ser realizados en instalaciones con bajos recursos y próximos al punto donde dichos análisis son necesarios (*point-of-need*).

Se han puesto a punto reacciones de amplificación isotermas en formatos de ensayo en fase sólida, desarrollando aproximaciones *on-pot* que combinan las etapas de amplificación-hibridación-detección en un mismo espacio físico. Esta aproximación se ha utilizado para la detección de la bacteria *Salmonella spp.*, demostrando su validez como sistema de control microbiológico [1]. También, se han implementado sistemas microfluídicos, tipo lab-on-a-chip, que permiten efectuar ensayos con menos manipulación y mejor reproducibilidad. El método se ha aplicado satisfactoriamente para la discriminación de 5 genes específicos de organismos modificados genéticamente [2]. En esta línea de investigación, se están poniendo a punto nuevas plataformas analíticas que incorporan microrreactores para aumentar el número de analitos que pueden ser estudiados simultáneamente [3]. Estos sistemas integrados permiten el seguimiento de las reacciones bioanalíticas a tiempo real, mostrándose como potenciales sustitutos de métodos altamente extendidos como la qPCR o catálisis enzimática. Esta tecnología de gran capacidad de trabajo y excelentes prestaciones permitirá dar soluciones en diferentes campos, como el genotipado de polimorfismo de único nucleótido (SNPs) de utilidad farmacogenómica y para detectar múltiples amenazas para la seguridad alimentaria tales como presencia de alérgenos, patógenos, etc.

Los métodos propuestos demuestran que los progresos en el conocimiento del genoma pueden ser trasladados al análisis rutinario sin necesidad de personal especializado y equipos sofisticados y caros presentes únicamente en laboratorios centralizados.

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DISEÑO DE CUESTIONARIOS EN EL ENTORNO MOODLE Y SU USO COMO HERRAMIENTA DE AUTOAPRENDIZAJE

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Introducción

Un problema de los procesos de enseñanza-aprendizaje suele ser el bajo rendimiento académico, por absentismo y falta de motivación. Es difícil conseguir que los alumnos lleven las asignaturas al día y que no se "descuelguen" a mitad del semestre. Las clases teóricas proporcionan una cobertura extensa de principios y fundamentos, pero los exámenes se enfocan en muchas ocasiones a la resolución de problemas muy específicos. Es necesario, por lo tanto, que los estudiantes desarrollen de forma independiente las capacidades necesarias para resolver problemas - Competencia específica: Ser capaz de reconocer y analizar un problema y plantear estrategias para su resolución -. Deben entender los conceptos y los principios, pero también es importante que empiecen a pensar como químicos, es decir, aprender a ser competentes de forma metodológica ante nuevas situaciones - Competencia general: Poseer los hábitos, capacidad de aprendizaje y autonomía necesarios para proseguir su formación posterior -. Para abordar estas desconexiones, un grupo de profesores nos planteamos la elaboración de "Cuestionarios" en el entorno Moodle [1, 2]. Su diseño responde más a una herramienta de aprendizaje autónomo que de evaluación. Se trata de actividades no presenciales y deben ser resueltos en un tiempo determinado, una vez completado el estudio de cada tema [3, 4].

Objetivo

Diseñar bancos de preguntas y cuestionarios en el entorno Moodle para asignaturas de la titulación de Grado en Química.

Metodología

- ✓ Elaboración de bancos de preguntas de diversa índole: respuesta múltiple, verdadero o falso, emparejamientos y respuestas cortas.
- ✓ Diseño de los cuestionarios, que normalmente constan de 10-12 preguntas y para cuya cumplimentación disponen de un único intento y un tiempo limitado.
- ✓ Estudio de los resultados y comparación con los obtenidos sin estas herramientas

Resultados

Se presentan los resultados de algunas asignaturas de diversos cursos. Como resumen puede indicarse que aquellos alumnos que realizan los cuestionarios periódicamente superan los cursos en porcentajes muy superiores a los que no siguen la dinámica propuesta. Una buena retroalimentación de las preguntas elaboradas ha sido evaluada por los alumnos como muy positiva y útil para el aprendizaje y comprensión de conceptos de difícil entendimiento.

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TRABAJO DE FIN DE GRADO MULTIDISCIPLINAR A TRAVÉS DEL ESTUDIO DE VARIABLES FÍSICO-QUÍMICAS DE UN ACUARIO

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De acuerdo con el R.D. 1393/2007, en su artículo 12.3 sobre las enseñanzas oficiales de grado, se establece que estas enseñanzas concluirán con la elaboración y defensa de un Trabajo de Fin de Grado (TFG). En el Grado en Química de la Universidade da Coruña esta asignatura obligatoria tiene asignados 15 créditos ECTS [1]. Su objetivo es ofrecer a los estudiantes la oportunidad realizar un trabajo técnico y una memoria, relacionados con los distintos campos del desempeño profesional propio de la titulación. Por lo que El TFG debe constituir un proyecto integral en el ámbito de la Química, a través del cual el alumno deberá demostrar que ha adquirido las competencias planteadas para el Grado que lo capacitan para el desempeño profesional y que está en condiciones de obtener el título de Graduado en Química.

En esta comunicación se presenta un TFG que se ha diseñado combinando técnicas de diversas ramas del conocimiento, como son la química inorgánica, la química analítica y la biología, con el objeto de realizar un trabajo experimental en el que el alumno pueda integrar diferentes conocimientos adquiridos durante sus estudios. Y se plantea con el enfoque de que pueda relacionar dichos conocimientos con actividades que pueden ser parte de nuestra vida cotidiana o que puedan ser objeto de actividades profesionales así como de investigación avanzada.

Para ello se ha llevado a cabo el estudio de las diferentes especies de compuestos nitrogenados que se pueden generar en un medio acuoso, como consecuencia diferentes niveles de aporte de CO_2 y modificación del pH. El sistema considerado es un acuario plantado de agua dulce (80 L), con aporte de CO_2 controlado. Se llevan a cabo diversos ensayos aportando al sistema cantidades variables de compuestos nitrogenados y de otros nutrientes inorgánicos (KNO_3, KH_2PO_4). Desde el punto de vista analítico, se aplicaron los métodos y técnicas instrumentales adecuadas para la determinación de diferentes especies de nitrógeno en el agua del sistema (NO_3^-, NO_2^-, NH_4^+), así como de otros parámetros analíticos que permiten caracterizar dichas muestras: alcalinidad, pH, dureza, conductividad, y también se estudió la biomasa generada. Se relacionaron los resultados obtenidos con la actividad biológica del sistema y con la evolución del ciclo del nitrógeno que se establece en un acuario.

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EVALUACIÓN AUTOMÁTICA DE COMPETENCIAS EN UN EJERCICIO DE INTERCOMPARACIÓN MEDIANTE ANOVA

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La herramienta telemática experimental Goodle GMS (Grading Management System), fue desarrollada recientemente en el Departamento de Ingeniería de Sistemas y Automática de la Universidad de Sevilla, para la recogida y calificación automática de prácticas de asignaturas con una fuerte componente matemática [1]. Esta herramienta proporciona importantes ventajas en la evaluación automática de ejercicios experimentales, en particular en aquellas áreas con contenido científico y/o experimental, en las cuales se evalúen resultados numéricos, ya que es capaz de parametrizar los ejercicios individualizándolos y permitiendo al profesor implementar técnicas innovadoras de auto-aprendizaje que, a su vez, ayudan al estudiante a medir continuamente su conocimiento a lo largo del curso. Así, Goodle GMS ha sido aplicada satisfactoriamente en nuestro Departamento para la evaluación de prácticas de laboratorio de Química Analítica [2, 3], así como para la resolución de ejercicios numéricos con contenido instrumental [4]. Esta metodología permite a los alumnos practicar las competencias de análisis de datos, complementando las prácticas de laboratorio. El procedimiento consiste en que a cada alumno se le proporcionan ejercicios personalizados generados de forma aleatoria a partir de su DNI, lo que permite a Goodle GMS analizar la respuesta del alumno a partir de los mismos datos.

El objetivo del presente trabajo ha consistido en la aplicación del sistema Goodle GMS a la evaluación de un ejercicio de intercomparación, utilizando para ello los datos que los alumnos han obtenido en una práctica realizada en la asignatura optativa Control de Calidad en los Laboratorios Analíticos, de 4º curso del Grado en Química, durante el curso académico 2014/2015. Dicha práctica consiste en el análisis de dos analgésicos, paracetamol y ácido acetilsalicílico, en un preparado farmacéutico, mediante cromatografía líquida de alta resolución con detección UV-Visible.

La evaluación de los resultados se ha realizado mediante un test *t* del dato medio obtenido por cada alumno con el considerado como verdadero. Además, la comparación entre los resultados obtenidos por todos los alumnos se ha realizado mediante un test ANOVA, el cual compara los resultados medios obtenidos realizando un análisis de la varianza para detectar si alguno de los resultados aportado por cada alumno difiere estadísticamente de los demás. Los criterios de evaluación para la autocorrección incluyen tanto el error cometido en la realización experimental de la práctica, como el resultado obtenido en el test *t*.

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DESARROLLO DE INSTRUMENTACIÓN ELECTRÓNICA BASADA EN ARDUINO Y SU USO EN LA EXPERIMENTACIÓN EN QUÍMICA BÁSICA

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En los últimos años, se ha popularizado el interés por desarrollar herramientas creativas y económicas para la enseñanza de la Química, tanto a nivel universitario como preuniversitario [1]. Una de las áreas más interesantes y de utilidad en Química General es el desarrollo de instrumentos electrónicos, sobre todo teniendo en cuenta el impacto que tiene la electrónica en el alumnado actual. Los estudiantes usan continuamente diversas herramientas, juguetes y *gadgets* electrónicos y están familiarizados con el uso de videojuegos, computadoras y *smartphones* [2]. Los circuitos integrados programables, conocidos como microcontroladores, ofrecen numerosas y muy variadas posibilidades para la automatización del laboratorio y su uso merece ser estudiado en el currículo de Química [3].

En el presente proyecto, se construyó un instrumento electrónico portátil basado en el microcontrolador Arduino. Este instrumento tiene la utilidad de mezclar líquidos y de controlar la temperatura de la mezcla a lo largo del tiempo. El proyecto se basa en un conjunto de módulos Arduino, tales como: un sensor de temperatura sumergible (que permite leer la temperatura con precisión decimal), una reconstrucción de un vaporizador de leche (del cual se extrajo el motor/agitador, de 4.5 volt) que permite la correcta agitación de los líquidos dentro de un recipiente aislado térmicamente y una pantalla de cristal líquido (LCD), usada como interfaz de usuario para recoger de forma sencilla los resultados de los experimentos y para controlar la activación del motor. Cabe destacar que todas las partes del instrumento construido fueron baratas y de fácil obtención.

Una vez construido el instrumento electrónico, que actúa de forma similar a un calorímetro, este fue utilizado para llevar a cabo algunos experimentos de calorimetría, prácticas habituales en los laboratorios universitarios y preuniversitarios de Química Básica. En concreto, se pudieron obtener: a) la curva de enfriamiento de una mezcla de agua caliente; b) la constante calorimétrica del instrumento construido; c) diversas entalpías de reacciones ácido-base; d) la estequiometría de la neutralización de ácido cítrico e hidróxido de sodio y e) la entalpía de descomposición del agua oxigenada.

Este proyecto es un claro ejemplo de como construir un instrumento electrónico utilizando herramientas relativamente baratas y accesibles. Además este instrumento portátil puede ser utilizado para cuantificar parámetros químicos de interés, así como para tratar temas científicos de cierta complejidad. También resulta interesante destacar que a través de este tipo de proyectos el alumnado puede tener un primer contacto con el desarrollo de equipos electrónicos.

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LA MOTIVACIÓN EN EL AULA, ELEMENTO IMPRESCINDIBLE PARA LA ADQUISICIÓN DE COMPETENCIAS NECESARIAS PARA EL MUNDO LABORAL

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El Grado en Enología fue implantado en la Universidad de Extremadura (UEx) en el curso 2010/2011. En su Plan de Estudios se encuentra la asignatura optativa "Vino y Salud", ofertada en el octavo semestre [1].

La docencia de la asignatura está dividida en clases magistrales y seminarios. El seguimiento de los alumnos en las clases magistrales se lleva a cabo desde la plataforma Moodle [2], que permite un control de asistencia y la posibilidad de realizar pruebas de evaluación simultáneamente por todos los alumnos desde un ordenador identificándose con sus claves personales.

Por otro lado, con objeto de hacer más atractiva la asignatura, y conseguir la motivación de los alumnos, las actividades de seminario se diseñaron de forma dinámica y cooperativa. Antes de su puesta en marcha se realizó un análisis DAFO [3] para comprobar la viabilidad del mismo y prever soluciones a los problemas que pudiesen aparecer. En los seminarios, se abordaron en profundidad las competencias transversales, sobre todo aquellas más demandadas actualmente en el mercado laboral y que mejoran la empleabilidad de los estudiantes. Entre estas destacaron CT1, CT2, CT4, CT9 y CT10 [1]. El programa que se desarrolló se resume en la Figura 1.

Figura 1. *Esquema del desarrollo de los seminarios.*

Por último, la evaluación de la asignatura tuvo en cuenta tanto las clases magistrales (60% de la nota) como los seminarios (40% de la nota, en la que se tiene en cuenta tanto al profesor como al resto de compañeros). La experiencia ha sido muy positiva ya que el grado de implicación de los alumnos ha sido bastante elevado.

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TWITTER COMO HERRAMIENTA DOCENTE EN LAS CLASES PRÁCTICAS DE LABORATORIO DE UNA MATERIA DEL GRADO EN QUÍMICA

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Según el último estudio sobre redes sociales del Interactive Advertising Bureau (IAB), Twitter es, después de Facebook, la red social con mayor frecuencia de uso, y una de la que más ha subido en número usuarios en el último año [1]. La sencillez de Twitter como aplicación y que Facebook presenta una identidad digital más bien centrada en contactos cercanos [2-3], fueron los principales motivos para elegir Twitter como herramienta docente. En esta comunicación se da a conocer el uso de Twitter como un espacio para el apoyo interactivo al proceso enseñanza-aprendizaje de las prácticas de laboratorio de la materia Química General III perteneciente al Grado en Química de la Universidad de Santiago de Compostela durante el curso 2014-15. El programa de prácticas de la materia es el siguiente:

Práctica nº1.- Estudio de reacciones en disolución acuosa.
Práctica nº2.- Hidrólisis de sales. Disoluciones reguladoras. Medida del pH.
Práctica nº3.- Ejemplo práctico de un proceso de neutralización: valoración ácido-base.
Práctica nº4.- Separación e identificación de metales pesados.
Práctica nº5.- Aplicaciones prácticas de reacciones en disolución acuosa: Separación e identificación de cationes.
Práctica nº6.- Reacciones de oxidación-reducción. Pila galvánica. Célula electrolítica.

Los objetivos de esta experiencia docente innovadora fueron proporcionar al alumnado, fuera del aula/laboratorio, la información esencial de cada una de las prácticas que se desarrollan en el laboratorio, resaltando los puntos más importantes de las mismas. Esto es muy importante, ya que lo primero que tiene que hacer el alumnado es contestar a un cuestionario sobre la práctica correspondiente antes de entrar en el laboratorio. Aunque se informa al estudiantado con tiempo suficiente de que tiene que leer en el manual de prácticas de la materia la práctica que va a realizar, se ha observado que la mayoría no dedica el tiempo suficiente a esta tarea y además, la dejan para última hora. Utilizando Twitter, como la mayoría de los/as estudiantes dispone de un smartphone y/o tableta, pudieron leer la información suministrada por la docente a tiempo real, y dosificada en las publicaciones efectuadas, según las características de cada práctica, antes de acudir al laboratorio. La metodología seguida fue la siguiente:

1) Creación de una cuenta en Twitter: QX3 Lab (@carmen_yebra).
2) Informar al alumnado de la existencia de esta cuenta, mostrando los tuits publicados relativos a la Práctica 1.
3) Invitar al alumnado para que se hagan seguidores de la cuenta de Twitter QX3 Lab.
4) Publicación de tuits correspondientes a cada una de las prácticas de la materia durante la semana anterior a la realización de las mismas con el hashtag #QX3USC15.
5) Creación de una cuenta en Storify (https://storify.com/carmen_yebra) para poder acceder a toda la información publicada asociada al hashtag #QX3USC15 sin la necesidad de tener cuenta en Twitter.

La limitación de caracteres en Twitter ha permitido sintetizar las ideas más importantes y recurrir a gráficos y enlaces que conducen a simulaciones, imágenes, vídeos, etc. Como conclusión, se puede decir que Twitter ha hecho posible dinamizar las clases prácticas, motivar, llamar la atención, implicar al estudiante aumentando su participación y mejorando las calificaciones obtenidas en los cuestionarios. La experiencia está siendo valorada por el alumnado como de gran utilidad para su aprendizaje.

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Comunicaciones Pósters

Bioanálisis y Análisis Forenses (BF), Instrumental y Métodos (IM), Quimiometría y Cualimetría (QC), Medioambiente (MA)

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DETERMINACIÓN DE LEVETIRACETAM EN MUESTRAS CADAVÉRICAS MEDIANTE CROMATOGRAFÍA DE GASES ACOPLADA A ESPECTROMETRÍA DE MASAS

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La epilepsia es un importante problema de salud, siendo la enfermedad crónica incapacitante más común del sistema nervioso. Se estima que afecta a unos 50 millones de personas en todo el mundo. Aproximadamente un 30% de los pacientes afectados se tratan con fármacos antiepilépticos y es frecuente el uso conjunto de varios como tratamiento [1]. Entre estos fármacos, los más empleados son la gabapentina, la pregabalina, la carbamacepina, el ácido valproico y el levetiracetam.

El Levetiracetam, (S)-α-etil-2-oxo-1-pirrolidina acetamida, es un anticonvulsivante utilizado como tratamiento para tipos específicos de epilepsia. Al contrario que los fármacos antiepilépticos tradicionales, el Levetiracetam posee menores efectos secundarios, una mayor eficacia y una farmacocinética más predecible [2]. En los últimos meses se ha detectado un aumento de este medicamento en las muestras, procedentes de cadáveres, recibidas en el Laboratorio de Toxicología Forense de la Universidad de Santiago de Compostela. Debido a ello se ha puesto a punto una metodología de extracción con detección por Cromatografía de Gases acoplada a Espectrometría de Masas (CG/EM), para la determinación del mismo.

Se han probado diferentes métodos de extracción, incluyendo la Extracción Líquido-Líquido con terbutilmetiléter y la Extracción en Fase Sólida (SPE), resultando la SPE con cartuchos Strata C-18-T (55 µm, 140 A), la técnica con la que se han obtenido mejores resultados.

La determinación se ha realizado por CG/EM en modalidad SIM, utilizando como patrón interno Levetiracetam-D_6. Los iones seleccionados fueron 112, 126 y 227 para el Levetiracetam y 115, 132 y 233 para su análogo deuterado (los iones subrayados fueron utilizados como iones cuantificadores). El método analítico incluye las siguientes condiciones cromatográficas: temperatura del inyector 250 ºC, tiempo de purga de 2 minutos, inyección en modo *splitless* (sin división de flujo), columna capilar HP-5 de 30 m x 250 µm, con un programa de temperatura que va desde 90ºC a 250ºC con una rampa de 20ºC min^{-1}, la fuente de iones a 230ºC, el detector en impacto a electrónico a 70 eV y empleándose como gas portador el Helio con un flujo de 1.0 mL min^{-1}. Los tiempos de retención así obtenidos fueron 9,70 min para el Levetiracetam y 9,66 min para el Levetiracetam-D_6.

Se ha escogido un rango de concentraciones dentro del rango terapéutico, que va desde 2,5 a 25 µg mL^{-1}, (incluyendo un punto 0), para realizar las curvas de calibrado. El método ha sido validado siguiendo las guías de la FDA [3], estudiando linealidad, selectividad, límites de cuantificación inferior y superior (ULOQ y LLOQ) y detección (LOD), exactitud, precisión y recuperación, cumpliendo los requerimientos allí establecidos.

El método desarrollado, ha sido aplicado a muestras reales de sangre cadavérica, procedente de autopsias, en las cuales el médico forense tiene sospecha de un consumo, bien sea porque existe una prescripción previa del fármaco, o bien por sospecha de una posible intoxicación.

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REUSABILITY OF µPAD FOR GLUCOSE BASED ON MULTI-ENZYME CO-EMBEDDED ORGANIC–INORGANIC HYBRID NANOFLOWERS

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A reusable paper-based microfluidic analytical device for detection of glucose is presented. A facile synthesis for creating hybrid organic–inorganic nanoflowers using $Cu_3(PO_4)_2 \cdot 3H_2O$ as the inorganic component and two enzymes: horseradish peroxidase (HRP) and glucose oxidase (GOx) directly onto a cellulosic support is reported. A colorimetric enzymatic cascade reaction occurs upon the oxidation of the glucose and catalysed by the GOx to form hydrogen peroxide which oxidizes the chromogenic reagent TMB aided by HRP generating a colour change in the detection zone. The high surface area and functionality of bi-enzymatic system due to the presence of both enzymes confined in the nanoflowers structure permits the reuse of the µPAD. A cellulosic paper was selected as the optimum substrate to immobilize the particles and carry the analyte through the paper channel from the sampling until the detection zone as is shown in the figure 1. The µPAD was designed in two parts overlapped each other. The first part consists of two zones, one where the analyte is deposited and other where the chromogenic reagent is immobilized and the colour change is detected. The second part is formed by the reaction zone with the cellulosic paper where the nanoflowers are immobilized. A colour coordinate, S (saturation), of HSV colour space was used as analytical parameter to determine glucose using a digital camera. This system offers a great potential for a variety of applications, such as biotechnology and biomedical chemistry.

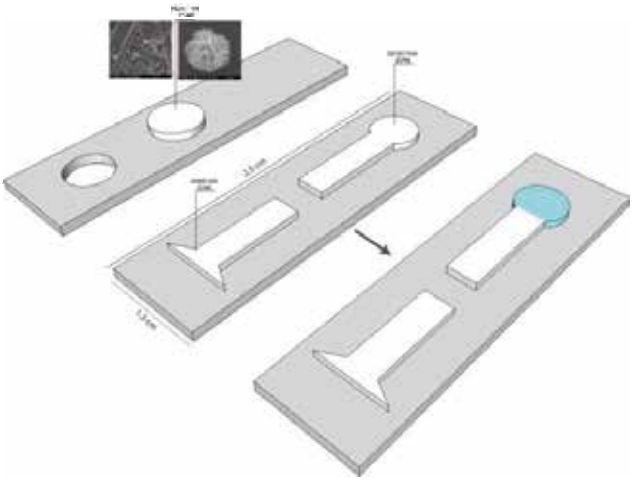

Figure 1

Acknowledgments
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DETECTION OF TETRAHYDROCANNABINOL RESIDUES ON HANDS BY ION MOBILITY SPECTROMETRY. CORRELATION WITH SALIVA ANALYSIS

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Ion mobility spectrometry (IMS), an analytical technique for the determination of volatile and semivolatile compounds based on the gas-phase separation of the resulting ions under a weak electric field at ambient pressure, reached its maturity between the late XX century and early XXI century [1]. Since then, IMS has been primarily used for the analysis of explosives, illicit drugs and chemical warfare agents with dedicated commercially available equipment.

IMS measurement of complex biological samples, such as blood, urine or saliva, produces a mixture of reagent and analyte product ions [2], which could complicate the interpretation of IMS spectra of drug metabolites and interfere in analyte determination. The incomplete resolution of peaks is a common situation in the analysis of complex biological samples by IMS, being necessary the application of a sample pretreatment step or the search of less complex alternative biological fluids such as nasal fluids [3] or fingerprint residues.

The aim of this study is to evaluate IMS as a high-throughput, cheap and efficient analytical tool for detecting residues of tetrahydrocannabinol (THC) on hands. The usefulness of the hand residues as potential samples for the determination of THC handling and abuse has been studied and the correlation between data obtained from cannabis consumers, whom were classified as positives after saliva analysis and from those who were classified as positive samples taking into consideration the information from hand residue analysis has been evaluated. Sampling consisted of wiping the hands with borosilicate glass microfiber filters and direct introduction of them into the IMS after thermal desorption. The possibility of false positive response, due to the presence of other compounds with similar drift time than THC, has been evaluated and minimized by application of the truncated negative second derivative algorithm. In addition the possibility of false negative responses, mainly by competitive ionization due to nicotine has also been studied.

Acknowledgements
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DETERMINATION OF COCAINE IN SEIZED SAMPLES BY VIBRATIONAL SPECTROMETRY

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The diversity of methods used in drug trafficking include, additionally than packed pure compounds, the use of bottled liquids, impregnated wax and paper, polymeric materials and impregnated clothes. So, nowadays official laboratories involved in abuse drug analysis have a tremendous problem to quantify cocaine these samples, especially in impregnated supports, because the recommended method by the United Nations Office of Drug and Crime (UNODC) requires a preliminary extraction of adsorbed cocaine followed by its determination by gas chromatography with flame ionization detection and it consumes more than twenty four hours for extraction and the handling of a big amount of samples. So, for screening purposes it becomes necessary to develop fast direct analytical procedures, suitable to provide quantitative data on the amount of cocaine present in liquids and impregnated materials without the need of a previous leaching of the drug and/or its preconcentration.

Vibrational spectroscopy measurements by mean of diffuse reflectance in the near infrared region or attenuated total reflectance in the middle one offers a fast way for direct sample analysis without any sample treatment and avoiding its destruction.
In this communication we have shown different examples of application for the quantification of cocaine in falsified bottled vinegars, impregnated clothes, wax and cardboards using ATR or DR techniques.

Partial least squares models were built for the direct determination of cocaine in seized impregnated smuggled materials based on the attenuated total reflectance middle infrared spectra (ATR-MIR) and diffuse reflectance spectra in the near range (DR-NIR) obtained directly from the surface of the impregnated materials. ATR-FTIR using an accessory for liquids was also applied to quantification of dissolved cocaine in simulated vinegars, being employed only one drop of the liquid. The aforementioned procedures offered fast, cheap and environmentally friendly green alternatives to the reference method based on the extraction of the drug and its quantification by gas chromatography.

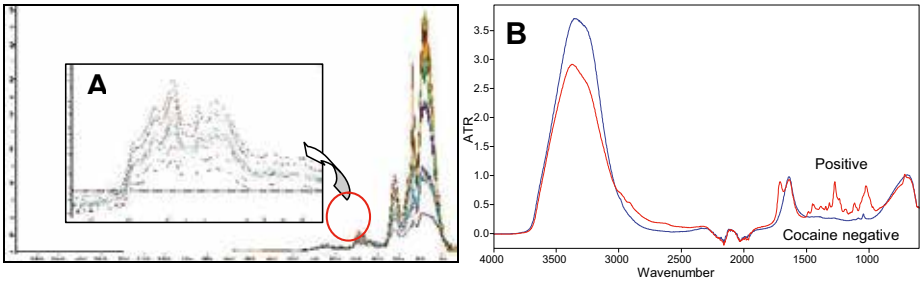

Figure A. Reflectance NIR spectra of an impregnated wax sample at sampling points.
Figure B. ATR-FTIR spectra positive and negative of cocaine containing vinegar samples.

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DATACIÓN DE TINTAS MEDIANTE MODELIZACIÓN MULTIVARIANTE DE MEDIDAS ESPECTROSCÓPICAS (UV-vis-NIR)

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El análisis de tintas de bolígrafo es una herramienta importante en el estudio forense de determinados documentos cuestionables. La mayoría de estos estudios están relacionados con la confirmación y detección de alteraciones en testamentos, reclamaciones a seguros, contratos o declaraciones de impuestos [1]. Algunas de estas modificaciones pueden ser confinadas si se estima el tiempo en que diferentes secciones del mismo documento fueron escritas. Aunque los últimos avances en datación de tintas se han realizado mediante el análisis destructivo de la muestra para la medición de los compuestos volátiles mediante cromatografía de gases acoplada a espectrometría de masas (GC/MS) [2], debido a la importancia de los documentos, la utilización de una metodología de análisis no-destructiva cobra especial relevancia.

En este estudio se pretende estudiar la viabilidad de la datación de tintas a través de la modelización multivariante de medidas espectroscópicas UV-vis-NIR en reflectancia difusa. Para ello se realizo un experimento de calibración con los espectros recogidos de una tinta negra comercial (Inoxcrom®). Este modelo de calibración constó de 48 muestras con diferentes intervalos de envejecimiento artificial que variaron entre 1 y 256 horas. A todos los espectros obtenidos se les realizó secuencialmente y de forma separada, 4 tratamientos matemáticos diferentes (*Norris*, *Savitzky-Goley*, *Standard Normal Variate*, SNV y *Extended Multiplicative Stantard Correction*, EMSC). Los parámetros característicos de los modelos de calibración como pendiente, ordenada, coeficiente de regresión y error de predicción en las medidas (RMSEC) fueron comparados y discutidos. A su vez, 5 tintas correspondientes a diferentes bolígrafos (4 negros y uno azul) fueron interpolados a modo de muestras externas de validación en los diferentes modelos hallando el tiempo estimado de deposición de las tintas sobre el papel.

De los 4 modelos matemáticos de tratamiento aplicados, los mejores resultados se obtuvieron con el tratamiento mediante la primera derivada (*Norris*) y el autoescalado *SNV*. Se obtuvieron resultados de datación prometedores para algunas de las tintas estudiadas en términos de exactitud y precisión mientras que, para otras, se observó como el modelo no funcionaba probablemente debido a las diferencias entre la composición de la tinta usada como estándar (Inoxcrom®) y la tinta analizada. Aún así, la metodología propuesta muestra una gran potencialidad para su aplicación futura en la datación de tintas en documentos.

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CONTINUOUS INJECTION IN CAPILLARY ELECTROPHORESIS FOR THE COMPARISON OF QUESTIONED DOCUMENTS

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The analysis of questioned documents presents a real interest in forensic casework, as counterfeiting cases are common crimes where the evidence (questioned document) is subjected to analysis to determine the ink composition, the origin of the sample, or carry out comparative analysis between two samples. Focusing on the technique, a wide variety of instrumental and methodologies have been proposed in this field [1].

The most appropriate analysis are those who minimise sample destruction and provide fast, reliable, and easy-to-interpret results, which are desirable in court. This work presents the use of microstructured capillaries (MSCs) in a CE equipment for the continuous injection of two ink samples from questioned documents in a CE-DAD equipment, in order to obtain fast and reliable comparisons between these samples. For this purpose, a customized 6-hole capillary, previously employed in CE-LIF [2], was employed to optimize a method previously developed by our research group to differentiate blue inks from pens by CE-DAD [3]. Once the method was optimized to use this MSC for a valid continuous injection, it was tested on different samples present on questioned documents (blue and black pens, and black printings from ink-jet printers), to check the potential of the method proposed. Finally, a blind test, including 20 pairwise comparisons of signatures, made with different blue pens, was performed to establish the discrimination power of the method. This work presents an original strategy where MSCs are used to allow the injection of large sample volumes preserving separation performance and enabling a rapid and reliable comparison between samples from questioned documents. Also, this strategy could be modified to test its potential for other analyses where comparison between samples is required.

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APLICACIÓN DE QUANTUM DOTS DE Mn-ZnS MODIFICADOS CON POLÍMEROS DE IMPRONTA MOLECULAR EN LA DETECCIÓN FLUORESCENTE DE COCAÍNA EN SALIVA

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Se ha desarrollado un método rápido de determinación de cocaína en saliva basado en la disminución de la señal de fluorescencia de una dispersión de quantum-dots (QDs) de Mn-ZnS recubiertos con un polímero de impronta molecular selectivo a cocaína y a sus principales metabolitos (benzoilecgonina, BEC, y ecgonina metil-ester, EME). Las condiciones operacionales de medida implican una longitud de onda de excitación de 297 nm, una longitud de onda de emisión de 596,5 nm, 100 mg de QD-PEG-MIP, un pH de 5,5 (uso de dihidrógeno fosfato de sodio / hidrógeno tampón de fosfato disódico), y 15 min como tiempo de retardo antes medición de fluorescencia.

Durante la validación del método se llevó se estudió el efecto matriz de la muestra de saliva mediante la comparación de las rectas de calibrado (decaimiento de la señal fluorescente al incrementar la concentración de cocaína) preparadas en medio tamponado, en saliva (dilución 1:1), en saliva tras ultracentrifugación (10000 rpm, 4°C, 10 minutos), y en saliva muestreada con los sistema comerciales *Salivettte*. Se encontró ausencia de efecto matriz cuando se somete la muestra de saliva a ultracentrifugación, obteniendo idénticos resultados tras fortificar las alícuotas de saliva antes o después del proceso de centrifugación. Por el contrario, el efecto matriz es importante al emplear saliva diluida (1:1) y también tras el muestreo con los dispositivos *Salivette*. Al ser el sistema de muestreo con *Salivette* el procedimiento más higiénico de recogida de saliva (las muestras de saliva bajo control toxicológico llegan al laboratorio en este formato), se optó por validar la metodología analítica con rectas de calibrado a través de estos dispositivos. El límite de detección (LOD) y el límite de cuantificación (LOQ) del método se ha establecido en 0,118 y 0,392 mg L^{-1}, respectivamente. El método fue validado en conformidad con la orientación de la FDA. Por otra parte, tanto la precisión intra-día como la precisión interdía ofrecieron valores de coeficientes de variación inferiores al 10 y al 15 %, respectivamente. La exactitud del método a través de la evaluación de la recuperación analítica (tres niveles de concentración distintos) fue igualmente aceptable (recuperaciones analíticas cercanas al 100% en todos los casos).

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DESARROLLO DE METODOLOGÍA ANALÍTICA BASADA EN CROMATOGRAFÍA LÍQUIDA Y ESPECTROMETRÍA DE MASAS DE ALTA RESOLUCIÓN Y MASA EXACTA (LC-HRMS) PARA EVALUACIÓN DE LA FUNCIÓN TIROIDEA

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Actualmente un 35% de la población mundial tiene problemas de salud relacionados con una ingesta nutricional de yodo insuficiente, y la consiguiente alteración de la función tiroidea. En la práctica clínica habitual, su diagnóstico se lleva a cabo mediante el análisis de los niveles de hormonas tiroideas ***triyodotironina*** **(T3)** y ***tiroxina*** **(T4)** en suero, entre otros.

Las hormonas tiroideas son esenciales en la regulación de numerosos procesos biológicos y metabólicos; por tanto, niveles incorrectos de estas hormonas pueden tener consecuencias negativas para la salud. En particular, las mujeres embarazadas sufren importantes cambios hormonales durante la gestación, que unido a un aumento de las exigencias metabólicas del feto, puede conllevar cambios significativos en la función tiroidea materna. Esto puede tener consecuencias negativas, tanto para la madre como para el feto.

La metodología analítica que emplean actualmente los laboratorios clínicos para el análisis de T3 y T4 en suero está basada en inmunoensayos enzimáticos. Sin embargo, los resultados de estos test clínicos muestran una elevada variabilidad dependiendo de la especificidad de los anticuerpos [1] y los errores asociados a la determinación son elevados (≥10%), lo cual ha generado cierta controversia acerca de su utilidad clínica [2, 3]. Por otra parte, algunos estudios confirman que los test de inmunoensayo no proporcionan resultados fiables en el caso de mujeres gestantes, debido a los importantes cambios fisiológicos que se producen durante el embarazo [4].

Entre los métodos analíticos recomendados por su mayor capacidad de detección y especificidad frente a los inmunoensayos, están aquellos que emplean técnicas acopladas de cromatografía líquida con espectrometría de masas [1] o con plasma de acoplamiento inductivo-espectrometría de masas [5]. Pero aún, no se validado ninguno de ellos como método de referencia a nivel internacional para el análisis de rutina de T3 y T4.

Bajo este contexto, se ha trabajado en el desarrollo de metodología analítica para la determinación de hormonas tiroideas en suero, empleando técnicas analíticas acopladas LC-HRMS, que mejoran la capacidad de detección y cuantificación debido a su elevado poder de resolución, exactitud de masa y posibilidad de realizar análisis retrospectivo.

A este respecto, la etapa de tratamiento de muestra es crucial para obtener unos resultados exitosos en la determinación final y ha de ser razonablemente simple para facilitar su posterior implantación en los análisis clínicos de rutina. Debido a los bajos niveles de detección exigidos para las hormonas, se han aplicado procedimientos de tratamiento de muestra dirigidos a la eliminación de los fosfolípidos presentes en la matriz de suero, que habitualmente ocasionan supresión de la ionización de los analitos de interés en la fuente de electrospray, disminuyen la reproducibilidad y reducen el tiempo de vida de la columna analítica.

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ISOLATION, CHARACTERIZATION AND COMPREHENSIVE PROTEIN PROFILING OF MEMBRANE VESICLES SECRETED BY FUNGAL PATHOGENS

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Fungi produce membrane vesicles containing a variety of cargo to transport complex molecules across their cell walls. This cargo usually contains lipids, proteins and polysaccharides, which might be associated with virulence factors. Although these membrane-derived vesicles are gaining clinical and scientific interest, very little is still known about vesicle production, size, morphology and biochemical composition.

In this work, we provide methodological details for isolation, characterization and identification of the protein cargo in different vesicles from fungus pathogens. The proposed method involves three main steps: (1) isolation and purification of membrane-derived vesicles from the culture media; (2) size and morphological characterization by optical methods and EM images; and (3) protein purification and in-solution trypsin digestion followed by mass spectrometry analysis of the resulting peptides.

(1) We have studied *Alternaria infectoria*, *Cryptococcus neoformans*, and *Candida albicans.* All strains were propagated in a defined minimal media and the membrane-derived vesicles secreted by each pathogen were isolated by ultracentrifugation and sucrose density gradient.
(2) Optical methods including dynamic light scattering (DLS) and Zeta Potential were used to study the size and heterogeneity of the vesicles population and to determine their surface net charge. Electron microscopy (TEM and SEM) was also used to visualize vesicles isolated from supernatants and those that were associated to fungal cells.
(3) Protein cargo in vesicle suspensions was determined by mass spectrometry. Proteins from isolated vesicles were purified by acetone precipitation and digested with trypsin. The digestion extracts were cleaned-up by solid phase extraction and the resulting peptides were analyzed using NanoLC coupled to an LTQ XL linear ion trap mass spectrometer.

Our results have shown that *A. infectoria* seems to contain a large number of nuclear-related proteins, which are involved in DNA repair and replication. Additionally, we have identified other proteins involved in polysaccharide metabolism, which is related with the biosynthesis of glycogen and the synthesis of secreted polysaccharides that can be used as immunomodulators or adhesion factors. For *C. neoformans* we have observed a high number of ribosomal and translation-related proteins. This fact suggests a mechanism to influence host cell translation. Finally, we have found a few potential virulence-related proteins in *C. albicans,* which are involved in the biological activity of these extracellular vesicles.

In conclusions, our study has allowed to get a deeper knowledge of the pathogenic mechanisms related to these fungal cells.

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VALIDACIÓN DEL ENSAYO DE POTENCIA DE ANTICUERPOS FRENTE AL ANTÍGENO DE SUPERFICIE DEL VIRUS DE LA HEPATITIS B EN INMUNOGLOBULINAS HUMANAS

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Se desarrolla y valida un procedimiento para determinar la potencia de anticuerpos frente al antígeno de superficie del virus de la Hepatitis B (HBsAg) en muestras de inmunoglobulinas humanas intramusculares en el laboratorio de Virología y Biología Molecular de la AEMPS. El ensayo se realiza siguiendo la normativa establecida ICH *guideline* (Topic Q 2 (R1)): "*Validation of AnalyticalProcedures: Text and Methodology*, (CPMP/ICH/381/95) y en la Directriz Europea "*Oficial Control Authority Batch Release of Human Immunoglobulin".* Los documentos: Monografía 0722: *Human Hepatitis B Immunoglobulin* y Monografía 1016: *Human Hepatitis B Immunoglobulin for Intravenous Administration*, de la Farmacopea Europea, Ed. 7, establecen tolerancias para a las potencias declarada y estimada. El ensayo se realiza con el kit comercial "Biolelisa anti-HBs: Test de ELISA para la detección y cuantificación de anticuerpos contra el antígeno de superficie de la hepatitis B (anti-HBs) en suero humano" (Biokit).Se evaluaron los siguientes parámetros de validación:

1. Especificidad.La solución de especificidad se ensayó diluida 1/10 con el control negativo del kit por duplicado. También se analizó el blanco y el control negativo del kit, ambos por duplicado en tres ensayos independientes. Los resultados obtenidos indican que la especificidad para la IGIM líquida es correcta, ya que la solución de excipientes ha dado una absorbancia muy similar a la obtenida para las muestras blanco del ensayo y para el control negativo del kit, lo que indica que los excipientes no interfieren con el ensayo de detección de anticuerpos anti-HBs.
2. Linealidad. Se utilizan 6 concentraciones del estándar internacional del NIBSC (WHO-IS:HBs) de forma que incluya las concentraciones de trabajo que se van a utilizar en el ensayo (1.000; 0.500; 0.250; 0.125 UI mL^{-1}) y una concentración por encima (1.429 UI mL^{-1}) y otra por debajo (0.0625 UI mL^{-1}). Recta de regresión estimada: log Absb= 0.1470+0.9289log UI mL^{-1}; (r^2=0.9987).
3. Límite de detección. Se considera como límite de detección para este ensayo el valor de 0.01 UI mL^{-1}, que es el establecido por el fabricante.
4. Precisión.

4.1 Repetibilidad. Se realizan 6 repeticiones independientes en las 4 concentraciones de trabajo. El CV es 5.0 %, que cumple con el criterio de aceptación de repetibilidad establecido para el ensayo (CV≤20%).

4.2 Precisión intermedia. Se realizan 6 ensayos independientes con el mismo Kit de reactivos, por el mismo analista en dos días diferentes. El CV es 6.3 %. Adicionalmente se hicieron dos ensayos con el mismo diseño por el mismo analista empleando dos kits de reactivos diferentes. El CV es 9.1 %. Ambos CVs cumplen con el criterio de aceptación de precisión establecido para el ensayo (CV≤20%).

5. Exactitud. Se utiliza el procedimiento de adición de cantidades variables de patrón a una muestra. A un fondo de muestra de IGIM preparada a una concentración determinada (IGIM fondo = 0.325 UI mL^{-1}), se añade el estándar a 3 niveles de concentración (P1=2 UI mL^{-1}, P2=0.8 UI mL^{-1} y P3= 0.250 UI mL^{-1}). Se preparan dos series por duplicado. Los porcentajes de recuperación obtenidos son: 97, 103 y 104% para los tres niveles de concentración, respectivamente. El intervalo al 95% de confianza para la recuperación es 92% -110%.

Se concluye que el procediendo desarrollado para de determinar la potencia de anticuerpos frente al antígeno de superficie del virus de la Hepatitis B en inmunoglobulinas humanas, queda validado para su utilización en las condiciones del ensayo.

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DESARROLLO DE UNA PLATAFORMA METALO-METABOLÓMICA BIDIMENSIONAL PARA EL ESTUDIO DEL METABOLISMO DE FOSFOLÍPIDOS EN LA ENFERMEDAD DE ALZHEIMER

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La enfermedad de Alzheimer (EA) es la forma de demencia más común entre la población de edad avanzada, la cual se caracteriza por una etiología compleja y multifactorial. Uno de los rasgos patológicos característicos de este desorden neurodegenerativo son las anormalidades en el metabolismo de fosfolípidos y otros lípidos de membrana, tradicionalmente relacionado con la sobre-expresión de distintas fosfolipasas, que puede conducir a la ruptura de membranas celulares y provocar la muerte neuronal. En este contexto, estudios metabolómicos recientes han demostrado que esta homeostasis anómala de fosfolípidos provoca alteraciones significativas en los niveles de estos lípidos en muestras de suero sanguíneo [1-3], por lo que estos compuestos podrían ser de gran interés para investigar los mecanismos patológicos asociados al desarrollo de la EA, y así descubrir potenciales biomarcadores de diagnóstico.

Con el objetivo de estudiar de forma directa las alteraciones en el metabolismo de fosfolípidos asociadas a la EA, se optimizó un procedimiento metalo-metabolómico bidimensional basado en la separación de las distintas especies en suero mediante cromatografía líquida de ultra alta resolución en fase reversa (RP-UHPLC) y posterior detección complementaria mediante espectrometría de masas atómica (ICP-Q-MS) y molecular (ESI-QTOF-MS) [4]. En primer lugar, se realizó el acoplamiento con ICP-MS para detectar de forma selectiva las hetero-biomoléculas presentes en la muestra mediante la monitorización elemental de m/z = $^{47}PO^{+}$, permitiendo así simplificar la búsqueda de fosfolípidos en los perfiles cromatográficos. Posteriormente, la identificación de las distintas especies individuales se llevó a cabo mediante espectrometría de masas de alta resolución y experimentos de fragmentación MS/MS.

La aplicación de esta plataforma bidimensional en muestras de suero de controles sanos y pacientes de EA, y posterior análisis multivariante de los resultados (PLS-DA), reveló cambios significativos en los niveles de múltiples fosfolípidos, incluyendo, fosfatidil-colinas, fosfatidil-etanolaminas, plasmenil-colinas, plasmenil-etanolaminas, y distintas clases de liso-fosfolípidos. Estos hallazgos podrían sugerir que las anormalidades en el metabolismo de lípidos de membrana en la EA tienen un origen multifactorial, donde confluyen distintos procesos patológicos como la sobre-activación de fosfolipasas, disfunción la peroxisómica, el estrés oxidativo, alteraciones en la composición de ácidos grasos y el catabolismo acelerado de liso-fosfolípidos.

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A SENSING PLATFORM FOR THE DETECTION OF NEURO-ACTIVE TRYPTOPHAN METABOLITES AS PUTATIVE BIOMARKERS OF NEUROLOGICAL AND NEURODEGENERATIVE DISORDERS. POINT-OF-CARE TESTING.

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The main objective of this work is the development of an immunosensing platform for the analysis of tryptophan metabolites as putative biomarkers of neurological and neurodegenerative disorders. The rationale for this work relies on extending the number of circulating biomarkers in search of a signature or pattern profile for the diagnostics of neurodegenerative diseases and, particularly, of Alzheimer disease. In addition to the diagnostic capacity, the development of new AD drugs and related clinical trials also require cost-effective tools for treatment monitoring and drug evaluation. Other approaches focus on protein signatures that are dissimilarly expressed in cases compared to controls, our searched signature has a quantitative component corresponding to the levels of the targeted markers in different biofluids, preferably non-invasive samples.

The analytical technology supporting our vision consists of an electrochemical immunosensor platform targeting neuro-active tryptophan metabolites related with the kynurenine-quinolinic pathway. The presented work will describe the configuration of the immunosensing nanoassemblies as well as the transduction chemistry. A careful selection of immunizing and reporting chemistry has resulted onto the required relative sensitivity-selectivity of the immunoassays; this immunochemistry has been produced within a collaborative project. The results presented here will describe the analytical parameters obtained with the electrochemical sensing platform including the analysis of controlled samples.

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MICROEXTRACCIÓN EN FASE LÍQUIDA UTILIZANDO FIBRAS HUECAS (HF-LPME) DE 2,4, 2,5 Y 2,6-DINITROFENOL EN ORINA Y SALIVA Y POSTERIOR DETERMINACIÓN MEDIANTE UPLC-Q-TOF-MS

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Los dinitrofenoles son compuestos considerados altamente tóxicos y han sido descritos en bibliografía como disruptores endocrinos, capaces de provocar efectos adversos sobre la salud humana, animales y microorganismos, al alterar los sistemas hormonales y reproductores. Es por ello, que resulta importante desarrollar procedimientos de análisis para su detección en matrices alimentarias, ambientales y fluidos biológicos. Su problemática radica en que ejercen sus efectos a concentraciones del orden de ng L^{-1} y se metabolizan rápidamente [1] por lo que es importante el desarrollo de procedimientos analíticos de alta sensibilidad y selectividad para la determinación de los mismos a bajas concentraciones. Se trata de compuestos que requieren precauciones extremas en su manipulación para evitar la contaminación y transferencia. Son compuestos tóxicos por inhalación, ingestión y contacto con la piel, que además, presentan efectos acumulativos por lo que una exposición continuada puede desembocar en alteraciones del sistema endocrino y reproductor de forma irreversible.

El objetivo del presente trabajo consiste en el desarrollo de un procedimiento de análisis de alta sensibilidad utilizando para ello una técnica de tratamiento de muestra de alta preconcentración, como es, la microextracción en fase líquida utilizando fibras huecas (HF-LPME) en configuración de tres fases y posterior determinación mediante UHPLC-Q-TOF para determinar 2.4-dinitrofenol (2,4-DNP), 2.5-dinitrofenol (2,5-DNP) y 2.6-dinitrofenol (2,6-DNP) en muestras de orina y saliva. Este procedimiento se aplicó posteriormente a muestras recogidas entre el personal que trabajaba en el laboratorio de improviso, con el fin de detectar posibles transferencias inesperadas y evitar episodios que pongan en riesgo la salud del personal que trabaja con estas sustancias.

La separación cromatográfica se llevó a cabo en una columna Acquity BEH C_{18} (50mm × 2,1 mm I.D., tamaño partícula 1,7 μm) termostatizando a 25ºC y una fase móvil constituida por 80% agua (A) y 20% acetonitrilo (0,1% Acido fórmico) (B) en modo isocrático durante 5 min, a flujo de 0,5 mL min^{-1}. Los analítos fueron extraídos mediante microextracción en fase líquida siguiendo un procedimiento previamente descrito [2]. Para ello, se emplearon fibras huecas de polipropileno (Accurel® Q3/2) de 13 cm usando como membrana líquida dihexiléter, y pH 2 y 13 como fases donadoras y aceptoras, respectivamente. En estas condiciones, se obtuvieron los siguientes límites de detección (LOD) y cuantificación (LOQ) para 2.4-DNP, 2.5-DNP y 2.6-DNP respectivamente, LODs: 4,2 μg L^{-1}, 11,62 μg L^{-1}, 1,42μg L^{-1} y LOQs: 13,92 μg L^{-1}, 38,62 μg L^{-1}, 4,72 μg L^{-1}.

Los resultados obtenidos fueron negativos en la mayoría de los casos excepto una muestra de orina y saliva en la que se detectó la presencia de 2,4-DNP. Adicionalmente, se investigó la presencia de metabolitos de todos los compuestos determinados, detectándose el glucurónido del 2,4-dinitrofenol y el glucurónido de 2-amino-4-nitrofenol en la muestra de orina. Este hallazgo pone de manifiesto la necesidad de extremar las precauciones en cuanto al uso de equipos de protección individual (EPIs) para el trabajo con este tipo de compuestos que a pesar de no presentar una elevada volatilidad si son altamente transferibles por inhalación. Asimismo, se pone de manifiesto la posible validez del procedimiento para la extracción y detección de metabolitos de dichos compuestos.

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MÉTODO PARA LA BIOMONITORIZACIÓN DE BISFENOL A EN CABELLO HUMANO MEDIANTE CROMATOGRAFÍA LÍQUIDA-ESPECTROMETRÍA DE MASAS

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El bisfenol A (*bisphenol A*, BPA) es un producto químico que se utiliza desde hace muchos años como componente para la fabricación de policarbonato y resinas epoxi-fenólicas. Esta sustancia está autorizada actualmente para la fabricación de materiales plásticos mediante el Reglamento (UE) 10/2011 de la Comisión, de 14 de enero de 2011, sobre materiales y objetos plásticos destinados a entrar en contacto con alimentos. El policarbonato es un tipo de plástico rígido transparente que se usa tanto para hacer envases de alimentos como otros muchos objetos no relacionados con la alimentación con los que día a día estamos en contacto, como pueden ser los recibos de caja registradora fabricados en papel térmico, CDs o DVDs, juguetes, cosméticos, etc. Por su parte, las resinas epoxi-fenólicas se utilizan en recubrimientos y revestimientos de conservas y depósitos de alimentos y bebidas.

La exposición humana a este contaminante se ha evaluado fundamentalmente mediante su determinación en matrices biológicas como plasma, suero y orina [1]. También se ha evaluado la posible exposición de bebés y fetos a través de la biomonitorización de BPA en leche materna [2] y placenta, respectivamente. Actualmente, los estudios de biomonitorización tienden hacia el uso de matrices no invasivas como pueden ser el cabello, uñas, orina o saliva. De entre las citadas matrices, el cabello parece ser una de las matrices de mayor interés ya que posee una gran estabilidad, proporciona información sobre exposición a corto y a largo plazo, desde semanas, meses o años en función de la longitud del cabello. Además, su contenido lipídico (2-4%) la convierte en una matriz de interés para la biomonitorización de compuestos lipofílicos [3].

En este trabajo se presenta el desarrollo de un método analítico para la extracción y determinación de BPA de cabello humano. El tratamiento de la muestra consistió en tres etapas: lavado, hidrólisis y extracción. Se ensayaron diferentes reactivos en cada una de dichas etapas con el fin de eliminar en lo posible impurezas presentes en la muestra, sin pérdida de BPA, y extraer con el mejor rendimiento posible el BPA presente en las muestras de cabello. Las mejores condiciones resultaron ser lavado de la muestra con agua y una disolución acuosa de dodecilsulfato sódico, hidrólisis con una disolución metanol-ácido trifluoroacético y extracción sólido-líquido con acetona en baño de ultrasonidos. El extracto se evaporó a sequedad y reconstituyó con metanol. La determinación se realizó mediante cromatografía líquida de alta resolución acoplada a espectrometría de masas de triple cuadrupolo. Se empleó BPA d_{14} como patrón interno.

El método se validó en términos de recuperación, precisión, linealidad y límites de detección y cuantificación. Se obtuvo una recuperación del 82% y una precisión, expresada como desviación estándar relativa, del 4%. Los límites de detección y cuantificación fueron 1.8 ng g^{-1} y 6.1 ng g^{-1}, respectivamente. La aplicabilidad del método quedó comprobada tras su aplicación a muestras de cabello de seis voluntarios. Se detectó la presencia de BPA en cinco de las seis muestras analizadas en un rango de concentración comprendido entre 24 ng g^{-1} y 158 ng g^{-1}.

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DETERMINACIÓN DE MEFEDRONA EN ORINA MEDIANTE CROMATOGRAFÍA DE GASES ACOPLADA A ESPECTROMETRÍA DE MASAS

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En los últimos años se ha observado un incremento importante del consumo de nuevas sustancias psicoactivas conocidas como "drogas de diseño", siendo las más consumidas los cannabinoides sintéticos, feniletilaminas y catinonas sintéticas. Las catinonas sintéticas son derivados sintéticos de la catinona, un alcaloide natural presente en la planta *Catha edulis* [1]. Dentro de este grupo, una de las más consumidas es la Mefedrona, que se comercializa principalmente como "sales de baño" o "fertilizante para plantas" a través de Internet para intentar eludir así las responsabilidades legales.

Estructuralmente similar a las anfetaminas, la Mefedrona es un estimulante del SNC que presenta efectos análogos a los de sustancias como la Metilendioximetilanfetamina (MDMA, más conocida como éxtasis), Metanfetamina y Cocaína. La Mefedrona se puede encontrar en forma de polvo, cristales, pastillas o formas similares a éstas (como pequeñas bolas). Su consumo puede ser por vía oral, parenteral, por inhalación o por inserción rectal [2].

Este trabajo desarrolla un método analítico para la determinación de Mefedrona en muestras de orina mediante Cromatografía de Gases acoplada a Espectrometría de Masas (GC-MS) empleando como patrón interno la Metanfetamina-d^5, eligiendo como rango de concentraciones 0,1-20 μg mL^{-1}. En primer lugar se ha optimizado un procedimiento de extracción, demostrando la extracción líquido-líquido (LLE) un buen rendimiento de extracción, siendo además una técnica sencilla, rápida y económica. Se utilizó acetato de etilo como disolvente de extracción, siendo necesaria la derivatización de la muestra para mejorar la volatilidad de los analitos, utilizando como derivatizante una disolución de PFPA y acetato de etilo (1:1). Posteriormente, se ha puesto a punto el método analítico con las siguientes condiciones cromatográficas: temperatura del inyector a 250 ºC, tiempo de purga de 2 minutos, inyección en modo *splitless* (sin división de flujo), columna capilar HP-5 de 30 m x 250 μm, empleándose como gas portador el Helio con un flujo de 1.0 mL min^{-1}. Tras inyección en modalidad SCAN de una disolución de patrones, los tiempos de retención obtenidos fueron de 9,2 minutos para la Metanfetamina-d5 y de 11,3 minutos para la Mefedrona. Posteriormente, se eligieron los iones cualificadores y cuantificadores de ambos compuestos (Metanfetamina-d5: *208*, 163 y Mefedrona: *91*, 58, 65 y 160) para realizar el estudio en modalidad SIM.

El método analítico fue validado siguiendo los parámetros de la FDA [3], estudiando linealidad, selectividad, límites de cuantificación (LOQ) y detección (LOD), exactitud, precisión y recuperación. Se obtuvieron unos coeficientes de correlación de 0,999 para la Mefedrona, siendo el LOD de 0,03 μg mL^{-1} y el LOQ de 0,1 μg mL^{-1}. Además, se observó una buena precisión y exactitud del método, siendo la recuperación satisfactoria. El método validado puede ser aplicado a muestras reales de posibles consumidores de drogas de abuso, sobre todo cuando se sospecha un consumo de drogas de diseño o compuestos anfetamínicos, lo que permitirá conocer el estado real de consumo de esta sustancia en nuestro medio.

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ANTIBODY FUNCTIONALIZED NANOPARTICLES FOR MULTIPLEX DETERMINATION OF FOOD ALLERGENS

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Around 2% of the population and up to 8% of children suffer from food allergy, with symptoms ranging from relatively mild to severe or sometimes even fatal consequences [1]. In commercial processed food products, allergenic foods are used as ingredients in many food products for their nutritional value. European Directive 2003/89/EC reinforces the general rule that all substances that have been intentionally introduced in food stuff should be indicated under their specific name in the list of ingredients [2]. For that, an effective and cost-effective analytical technique is necessary for ensuring food safety for allergic people. We present a multiplex competitive micro-immunoassay on a compact disc for testing food allergens under the labeling regulations. The assay is developed for the simultaneous determination of wheat (gluten), milk (casein and β-Lactoglobulin) and egg (ovalbumin) proteins using specific antibody functionalized gold nanoparticles. In applying the assay for screening purposes a new method with good recovery yields is developed for the simultaneous extraction of the four allergens. The assay was also evaluated by the analysis of both liquid and solid food samples, reaching recovery values ranging from 72-112%, demonstrating its suitability for the simultaneous determination (extraction and quantification) of allergens in less than 60 minutes. The detection limit achieved was 0.18, 0.03, 0.03 and 0.09 mg/L for gluten, casein, β-Lactoglobulin and ovalbumin respectively. This figures being down to the alarm levels ruled by the European Union.

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SCREENING MULTI-CLASE DE DROGAS DE ABUSO EN ORINA MEDIANTE HPLC-MS/MS Y MICROEXTRACCIÓN LÍQUIDO-LÍQUIDO DISPERSIVA

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Se ha desarrollado un procedimiento rápido de detección por cromatografía líquida de alta resolución y espectrometría de masas en tándem para el *screening* multi-clase de drogas de abuso (cocaína, opiáceos, anfetaminas y drogas de diseño) en muestras de orina. Como paso previo, se ha explorado las posibilidades de la microextracción líquido-líquido dispersiva como procedimiento rápido de aislamientos de las drogas y/o metabolitos de interés. De las distintas combinaciones dispersante-extractante se ha seleccionado el par acetonitrilo-diclorometano, el cual ofreció las mejores recuperaciones analíticas de compromiso para todos los compuestos estudiados. El procedimiento optimizado consistió finalmente en ajustar el pH de la muestra de orina (1,0 mL) a 9,0, y llevar a cabo la microextracción con 4 mL de la mezcla acetonitrilo-diclorometano (3,76 mL de acetonitrilo y 0,24 mL de diclorometano) con agitación magnética durante 2 minutos. Se obtuvo un mejor rendimiento del proceso extractivo diluyendo la muestra de orina (1:1) con una disolución acuosa de NaCl al 10 %(m/v). Tras centrifugación (4000 rpm, 4°C, 15 minutos), evaporación a sequedad en corriente de N_2 de la fase orgánica y redisolución en 50 µL (2 mM acetato amónico en metanol), los extractos se analizan por HPLC-MS/MS (columna Kinetex 5µ C18 100 Å) para cocaína y metabolitos (benzoilecgonina, ecgonina metil éster, y cocaetileno); morfina, 6-monoacetilmorfina y codeína (abuso de opiáceos); anfetamina y metanfetamina; y buprenorfina y norefedrina (nuevas drogas diseño).

La metodología previamente descrita ha sido validada de acuerdo a las directrices propuestas por la *Food and Drug Administration* (FDA), y ha sido aplica con éxito al *screening* multi-clase de drogas de abuso en muestras de orina.

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MICROSOLID PHASE EXTRACTION BASED ON POROUS MEMBRANE PROTECTED MOLECULARLY IMPRINTED POLYMER FOR COCAINE AND METABOLITES ASSESSMENT IN HUMAN URINE BY HPLC-MS/MS

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Molecularly imprinted polymers (MIP) for selective cocaine recognition were packed inside a polypropylene membrane, and the protected MIP was then used for pre-concentrating cocaine and metabolites (benzoylecgonine, ecgonine methyl ester, and cocaethylene) from human urine before high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS).

MIP synthesis was performed by the precipitation method (N_2 atmosphere, constant stirring at 40 rpm, 60°C for 24 hours) using cocaine as a template, ethylene dimethacrylate (EDMA) as a monomer, divynilbenzene (DVB) as a cross-linker, and 2-2'-azoisobutyronitrile (AIBN) as an initiator. Variables affecting the MIP-MIMSPE (batch mode, 50 mg MIP) process were fully studied. Optimum loading (retention) conditions were: urine (5 mL) pH adjustment at 5.5 (sodium dihydrogen phosphate/sodium hydroxide buffer), and mechanical stirring at 200 rpm at 30°C for 15 minutes. Target elution was performed with 5 mL of hexane/2-propanol/ammonium hydroxide 72:20:8 under ultrasounds irradiation for 8 minutes. The eluates were further N_2 evaporated to dryness, and the residue re-dissolved in 100 µL of mobile phase (2 mM ammonium acetate methanol). A pre-concentration factor of 50 was achieved.

HPLC-MS/MS targets separation/detection was achieved under a gradient elution which involves two mobile phases: aqueous 2 mM ammonium acetate, pH 7.5 (A) and 2 mM ammonium acetate methanol (B). The flow rate (Phenomenex Kinetex C18 column) was set at 0.20 mL min^{-1}, and the gradient program consisted of 0% A for 0.5 minutes, followed by a 1 minute ramp until 30% A, 2.5 minutes ramp until 0% A, and 1 minute hold at 0% A. The developed method was fully validated acoording to the FDA guidance and applied to several urine samples.

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DISPOSABLE ELECTROCHEMICAL DNA SENSORS BASED ON DUAL FUNCTIONAL GRAPHENE DERIVATIVE PLATFORMS APPLIED TO THE DETECTION OF *TP53* GENE IN BIOLOGICAL SAMPLES

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Novel effective in practice disposable electrochemical DNA sensors are described for the detection of a target DNA sequence on p53 tumor suppressor (*TP53*) gene, one of the most popular genes in cancer research. The electrochemical platform consists of screen-printed carbon electrodes (SPCEs) functionalized with a water-soluble reduced graphene oxide-carboxymethylcellulose (rGO-CMC) hybrid nanomaterial. This functional hybrid nanomaterial, which has demonstrated to provide excellent transduction systems for the preparation of enzymatic biosensors [1], greatly improved the performance of the developed electrochemical DNA biosensor, offering important advantages over the use of the single nanomaterial (rGO) or other common nanomaterials such as multi-walled carbon nanotubes (MWCNTs). Two different configurations involving hairpin specific capture probes of different length covalently immobilized through carbodiimide chemistry on the surface of rGO-CMC-modified SPCEs have been implemented and compared. Upon hybridization, a streptavidin-peroxidase (Strep-HRP) conjugate was employed as electrochemical indicator. Hybridization was monitored by recording the amperometric responses measured at −0.10 V (vs an Ag pseudoreference electrode) upon addition of 3,3′,5,5′-tetramethylbenzidine (TMB) as redox mediator and H_2O_2 as the enzyme substrate. The new bioplatforms, with 15-days storage stability, gave limits of detection of around 3 nM without any target or signal amplification. Moreover, the use of a 33 nts DNA hairpin beacon sequence provided increased selectivity for SNP detection, strongly required in cancer diagnostic assays, allowing complete discrimination between the fully matched and SNP-containing DNAs in only 30 min. The implemented DNA platforms permit the direct target DNA quantification, as well as single nucleotide polymorphism (SNP) discrimination in undiluted human serum and saliva samples, and in cDNAs from human breast cancer cell lines. Such attractive features emphasize the crucial role of the rGO-CMC hybrid in minimizing background contributions and measuring low levels of the target DNA, thus offering great promise for implementation of platforms which allow straightforward and fast mutation screening related to most human cancer types, which makes such platforms excellent as new diagnosis tools in clinical analysis.

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DIFFERENTIATION OF TEXTILE POLYMERS BY NIR-HIPERSPECTROSCOPY AS A PROMISING TECHNIQUE TO DETECT COUNTERFEIT CLOTHES

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In many situations of legal significance, differentiation between textile materials is of high importance. In this sense, having the availability of testing and non-destructive techniques to distinguish allegedly counterfeit clothes would be very helpful. Near infrared hyperspectral imaging (NIR-HSI) is a technique to explore surfaces while providing more detailed information than single point spectroscopy. A complete spectrum along the desired wavelength range is measured for each spatial pixel. The resulting HS images have a three-dimensional arrange, and are referred to as hypercubes. This work used NIR-HSI for analysing clothing fragments made by different textile polymers such as cotton and polyester with similar colour. Working images were registered between 1000 and 1700 cm^{-1} employing a NIR-HS camera. Then, these images were subjected to mathematical treatment commonly used in spectroscopic analyses for standardising and preparing the data. All the data processing was carried out using Matlab. The applied pre-treatments consisted of tools such as standard normal variate (SNV) normalization, smoothing with Savitzky-Golay filtered, or first derivative, mean-centring, and Fourier transforming. After the mathematical data processing, a masking procedure and reference classes' creation were carried out. Afterwards, a test set was predicted against the PLS-DA model in order to validate the proposed method.

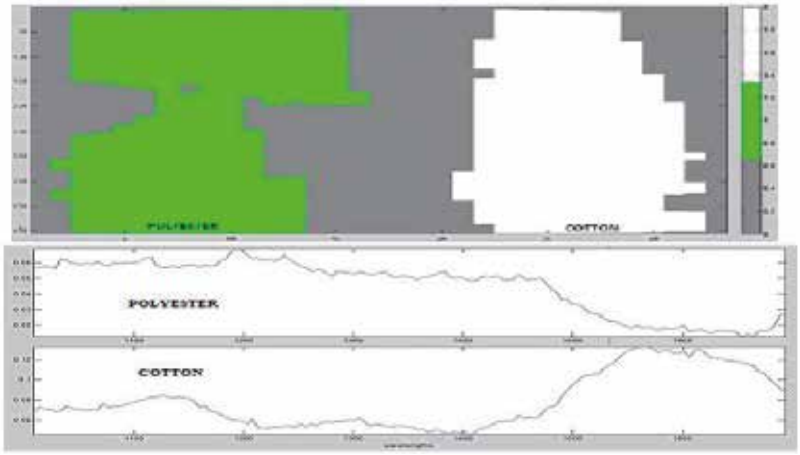

Figure 1. Descriptive map of the analysed textile samples according to the type of textile polymer. Green and white coloured areas represent the polyester and cotton classes, respectively. Their spectra are also shown.

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MICROEXTRACCIÓN CON ADSORBENTES EMPAQUETADOS (MEPS) PARA LA DETERMINACIÓN DE BENZODIAZEPINAS EN PLASMA

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Las benzodiacepinas son los hiposedantes más útiles y versátiles, y el porcentaje de población que consume estos medicamentos es muy alta, por esto las benzodiacepinas están involucradas en numerosos casos de intoxicaciones medicamentosas. Se desarrolla un método de análisis basado en la microextracción con adsorbentes empaquetados (MEPS) [1] para la determinación de alprazolam, bromazepam, clonacepam, diazepam, loracepam, lormetacepam y tetracepam en plasma y la posterior determinación mediante cromatografía líquida de ultra eficacia con detector de serie de diodos (UPLC-DAD) [2]. El método analítico fue validado para determinar la linealidad, sensibilidad, límites de detección y cuantificación, selectividad, precisión y exactitud [3].

Se usa el diseño de experimentos [4] como herramienta de ayuda para la optimización del proceso de extracción obteniéndose como condiciones óptimas 300µL de muestra, fase C18, la muestra se cargó 8 veces, 50 µL de metanol como eluyente, pH=7 y tiempo de secado de 0,5 min.

La cuantificación se lleva a cabo con el UPLC-DAD usando ACQUITY UPLC® BEH Shield RP18 (100 x 2,1 mm, 1,7 µm tamaño de partícula) y una fase móvil compuesta por acetonitrilo tampón fosfato 1mM pH=6 con un flujo de 0.4ml min^{-1}, a 30ºC. El detector ofrece una respuesta lineal que cubre un rango de concentración de 0.01-10 µg mL^{-1} en plasma; el rango de los límites de detección va de 0.5-4.7 ng mL^{-1} y los límites de cuantificación de 1.3- 9.3 ng mL^{-1}; los coeficientes de variación fueron menores del 14 %; y las recuperaciones calculadas abarcan el rango 94,0-125,2 %. El método ha sido aplicado a muestras reales, obteniendo 5 positivos para alguna de las benzodiacepinas estudiadas.

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ESTRATEGÍAS ANALÍTICAS PARA EL ESTUDIO DE AGREGACIÓN DE PROTEINAS EN PRESENCIA DE Sb(III) – Sb(V)

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El empleo masivo de antimonio en la fabricación de diodos, municiones, catalizadores y principalmente sistemas de freno vehicular, ha generado la contaminación de centro urbanos, presentándose generalmente en material particulado. Este material puede entrar fácilmente por las vías respiratorias, ingresando Sb a la sangre, donde luego puede ser alojado en órganos vitales, generando desde irritación cutánea, ulceras estomacales hasta cáncer de hígado, pulmón y riñones, como ha sido encontrado en humanos debido a exposición laboral. Además se ha observado daño al ADN en humanos expuestos a Sb [1]. Los mecanismos de citotoxicidad pueden abarcar procesos como generación de estrés oxidativo, aberraciones al ADN y agregación proteica, en que metales pesados y metaloides presentan un rol fundamental en la etiología de enfermedades. En estos mecanismos, los principales factores a estudiar son las rutas de ingreso, vías metabólicas e interacción del agente contaminante con macromoléculas (por ejemplo ADN y proteínas).

En el medio celular Sb(III) y As(III) se encuentran como $Sb(OH)_3$ y $As(OH)_3$, respectivamente. Estas especies pueden ingresar a células por medio del canal AQP9 en humanos y mamíferos, la función de este canal es permitir el ingreso a la célula de glicerol, urea y agua, lo que también puede ocurrir con partículas de similar tamaño y características químicas, como lo son las especies trivalentes de estos metaloides. Por otro lado se encuentran Sb(V) y As(V), que presentan mayores diferencias a pH fisiológico. As(V) se encuentra como $HAsO_4^{2-}$ y puede ingresar por transportadores de ión fosfato Pho84p y Pho98 (HPO_4^{2-} a pH fisiológico); no es el caso de Sb(V), el que se encuentra como $[Sb(OH)_6]^-$ dentro de la célula. Además existen diferencias metabólicas como es la biometilación, proceso que ha sido evidenciado para As en humanos y no para Sb. En relación a efectos sobre proteínas, se conoce que As(III) y As(V) genera agregación de proteínas in vivo e in vitro, no así Sb [2].

Pese a las diferencias entre estos 2 metaloides y a la poca información existente para Sb, muchos grupos de investigación [3] proponen que sus mecanismos de toxicidad son similares. Es por esto la relevancia de este estudio, que esperamos nos acerque a conocer las vías en que Sb puede generar toxicidad celular y finalmente, los efectos ya mencionados en humanos.

En este estudio se investiga el seguimiento de agregación de albúmina de suero bovino (proteína mayoritaria y principal agente reductor en la sangre), en presencia de Sb(III) y Sb(V); y el efecto protector de glutatión (GSH), analizado por medio de espectrometría de fluorescencia molecular y espectrometría de dicroísmo circular. Además, se estudian los posibles cambios redox generados en dicho proceso de agregación proteico, mediante análisis de especiación de Sb vía HPLC-ICP-MS.

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FIA-HRMS: AN EFFECTIVE STRATEGY FOR THE SCREENING OF PSYCHOACTIVE SUBSTANCES

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Recreational drugs (illicit drugs, human and veterinary medicines, legal highs, etc.) often contain lacing agents and adulterants which are not related to the main active ingredient. Serious side effects and even the death of the consumer have been related with the consumption of mixtures of psychoactive substances and/or adulterants [1], so it is important to know the actual composition of recreational drugs. Moreover, the introduction of new "legal" substances (Legal Highs), which are sold as "bath salts" or "plant food", has also raised concern due to the misinformation showed in its packaging, where the actual composition is not indicated. These new products often contain synthetic drugs such as synthetic cathinones [2] and β-cannabinoids [3]. The high number of substances marketed as legal highs and their fast development and modification by drug designers challenges analytical tasks. For this reason, it is important to develop selective non-target analytical methods that are able not only to provide a reliable identification of the psychoactive compounds and the lacing agents present in the sample, but also to adapt to the constant introduction of new substances in the market.

In this work a method based on Flow Injection Analysis coupled to High Resolution Mass Spectrometry (FIA-HRMS) is proposed for the wide-range screening of recreational drugs and Legal Highs. The samples of recreational drugs and Legal Highs were dissolved in methanol:acetonitrile 1:1 and directly injected into the FIA-HRMS system. As most of the psychoactive substances are acid-base compounds, a 1:1 mixture of methanol: 0.1% aqueous formic acid as carrier flow, and electrospray ionization in both positive and negative polarities were used. Mass spectrometry was performed in a Q-Exactive mass spectrometer in two data acquisition modes, full scan at high mass resolution (HRMS) and data dependent tandem mass spectrometry (ddMS/HRMS). This data contained enough spectral information to achieve high-confidence identification of the substances present in the samples by searching in both a custom-made database and on-line spectral databases. Identification was based on the accurate mass and the isotopic pattern of the ions observed in full scan mode and the main product ions observed in the product ion scan (MS/HRMS). The results of the screening showed that most of the recreational drugs supposed to be cocaine only contained the NSAID metamizole, and that most of the legal highs marketed as herbal blends were actually mixtures of mostly pure compounds (e.g. butylone, caffeine and 2-aminoindan). Also, the presence of adulterants such as phenacetin, caffeine and levamisole was quite common.

Acknowledgements
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PHOTODEGRADATION OF COSMETIC PRESERTVATIVES IN ARTIFICIAL SKIN: IDENTIFICATION OF 2,8-DICHLORODIBENZO-P-DIOXIN AND OTHER UNWANTED PHOTOPRODUCTS

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Personal care products (PCPs) such as moisturizing creams, body lotions or sunscreens, amongst others, are daily used topical products intended to be in prolonged contact with the skin. The antioxidant BHT or the antimicrobials triclosan (TCS) and phenyl benzoate (PhBz) are frequently found ingredients in PCPs, which exhibit chemical structures capable of absorbing UV-light, thereby undergoing photochemical reactions [1-3]. Upon UV exposure, reactive intermediates of photounstable ingredients may behave as photo-oxidants, and even promote phototoxic or photoallergic contact dermatitis. The interaction of these photoproducts with cosmetic excipients or skin components, like sebum, may lead to the formation of new molecules with unknown toxicological properties, which can be even more toxic than the parent compound [4, 5]. However, the photochemical stability and further phototransformations of cosmetic preservatives in topical applications exposed to UV-light are largely unknown.

In this study, the photochemical behavior of BHT, TCS and PhBz in an artificial skin model was investigated through two sets of photodegradation experiments: (i) UV-Irradiation (8W, 254 nm) of artificial skin directly spiked with the target preservatives, (ii) UV-irradiation of artificial skin after the application of a cosmetic cream fortified with the target compounds. Subsequently, pressurized liquid extraction (PLE) was used to isolate the target preservatives and their transformation products. The follow-up of the photodegradation kinetics of the parent preservatives, the identification of the arising by-products (TPs), and the monitorization of their kinetic profile was performed by gas chromatography-mass spectrometry (GC-MS).

The photochemical transformation of triclosan into 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD) and other dioxin-like photoproducts has been demonstrated in this work. Furthermore, seven BHT photoproducts, and three benzophenones as PhBz by-products, most of them with unknown toxicological properties, have also been identified. It is worthy to notice that this is the first time that the phototransformation of cosmetic ingredients applied onto an artificial skin model has been investigated, which represents a valuable contribution to the requirements of public institutions like the Federal Food and Drug Administration (FDA) in the field of cosmetics safety evaluation [6].

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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND OTHER HAZARDOUS SUBSTANCES IN FOOTBALL PITCHES

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In last years exists a growing interest in the transformation of used tires in recycled materials such as playgrounds, animal flooring, sport fields, due to the resistance of these surfaces. Although in Spain recycled tires are considered safe waste, several studies have demonstrated the presence of metals and other hazardous substances in recycled tire playground surfaces [1].

In the case of the outdoor surfaces such as football pitches which are exposed to different weather conditions, rainwater can accumulate and hazardous substances can be carried by runoff water and leached through the soil.

The aim of this work is to study the presence of polycyclic aromatic hydrocarbons (PAHs), considered as ubiquitous contaminants in tire recycled football pitches surfaces. The United States Environmental Protection Agency (EPA) classified 16 of them as priority-pollutants based on their toxicity, potential for human exposure and frequency occurrence at hazardous wastes. The presence of other harmful compounds including plasticizers, antioxidants and antiozonants was also analyzed. This study demonstrates a partial transfer of the contaminants from the recycled tire surface to the water put in contact with the sample.

Different football pitch samples were analyzed with UAE and SPME. For the analysis of the water put in contact with the surfaces, HS-SPME was employed as extraction technique followed in both cases by GC-MS and GC-MS/MS. The tandem mode MS/MS was employed in order to identify and quantify trace levels of these priority pollutants.

Acknowledgments
This research was supported by FEDER funds and projects GPC2014/035 (Consolidated Research Groups Program, Xunta de Galicia) and UNST10-1E-491 (Infrastructure Program, Ministry of Science and Innovation, Spain).

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ELECTROCHEMICAL BEHAVIOUR OF Ag (I) AT Pt ELECTRODEIN1-BUTHYL-3-METHYL-IMIDAZOLIUM CHLORIDE (BMIMCl)AT 343-363 K

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The electrochemical reduction of Ag (I) on a platinum electrode, has been studied in the ionic liquid 1-buthyl-3-methylimidazolium chloride (BMIMCl) at 343-363 K, by square wave voltammetry (SWV), cyclic voltammetry (CV), convolutive potential sweep voltammetry (CPSV), chronoamperometry (CA), and chronopotentiometry (CP).

It has been found that during cathodic polarization, deposition of metallic Ag from the BMIMCl onto the platinum surface proceeds in a single step:

$$Ag(I) + 1e \rightarrow Ag(0)$$

which has been found reversible or quasi-reversible depending on the experimental conditions (i.e scan rate).

The diffusion coefficient of Ag(I) (D) has been determined by different techniques and compared with thosereported in the literature in another similar media [1].The validity of the Arrhenius law was also verified.

Electro-crystallization of silver plays an important role in the whole electrodeposition process [2]. Experimental current–time transients followed the theoretical models based on instantaneous nucleation with three-dimensional growth of the nuclei at the studied temperatures.

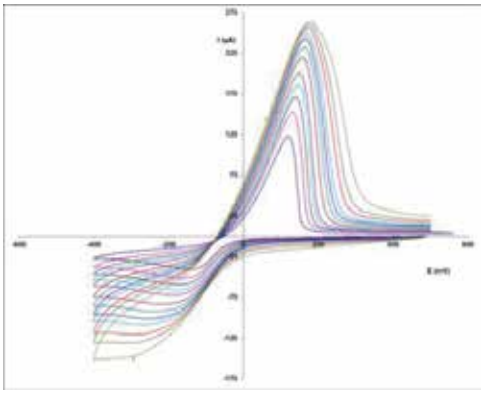

Figure 1: Cyclic Voltammograms obtained with anAg(I) solution (Co= 2.60.10^{-5}mol cm^{-3}, T=343 K) on a Pt electrode (S = 0.165 cm^{2}). Scan rates ranging from 20 to 700 mV s^{-1}Pseudoreference: Ag.

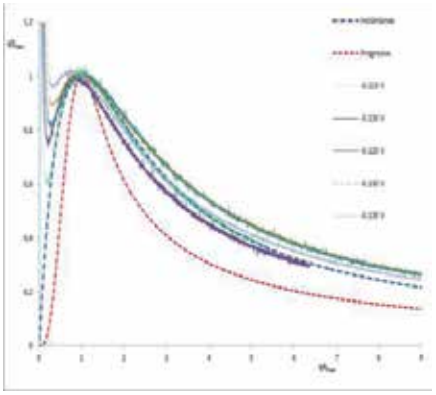

Figure 2.-Comparison of the dimensionless experimental data derived from the current–time transients with the theoretical model for instantaneous and progressive nucleation at different working potentials (from -0.115 to -0.135 V vs Ag) at 363 K

Acknowledgements
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THE USE OF AAg PSEUDOREFERENCE ELECTRODE FOR ELECTROCHEMICALSTUDIES OF COPPER IN 1-BUTHYL-3-METHYL-IMIDAZOLIUM CHLORIDE (BMIMCl)

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In electrochemical studies, a three-electrode cell incorporating a stable reference electrode is essential to avoid uncertainty of the electrode reactions. However, a universal reference electrode has not so far been reported in the literature for electrochemical studies in ionic liquids. Instead, the use of pseudoreferenceelectrodes (e.g. silver, gold or platinum wires immerseddirectly into the solution) is very common, but their potentials could be unstable and vary with many factors, making their use unappropriated in electrochemical studies.

The stability of the Ag pseudo-reference electrode was checked by studying the electrochemical behavior of Cu(I) and Cu(II) solutions in the 1-buthyl-3-methyl-imidazolium chloride ionic liquid.

For solutions containing Cu(I), a Ag wire can be used as a pseudo-reference electrode. Notwithstanding, for the studies with Cu(II) solutions this material must be avoided in order to prevent the chemical reaction:

$$Cu(II) + Ag(0) \rightleftharpoons Cu(I) + Ag(I).$$

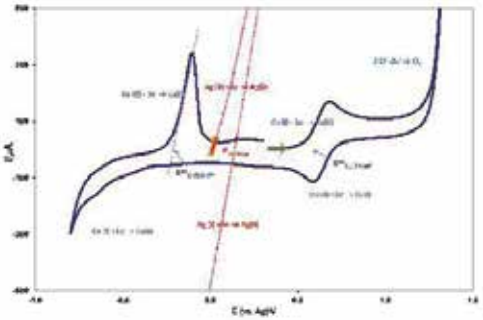

Fig.1.- Cyclic voltammograms obtained with: (red) purified BMINCl on a Ag electrode, (blue) a solution of Cu(I) in BMIMCl on a Pt electrode ($C_0 = 2.35 \times 10^{-5}$ mol cm^{-3})

Figures 2(a) and (b) show the electrodeposits obtained with either Cu(I) or Cu(II) solutions using a Ag reference electrode. Pure Cu electrodeposits were obtained from a Cu(I) solution, whereas a Cu-Ag intermetallic compound was obtained from a Cu(II) solution.

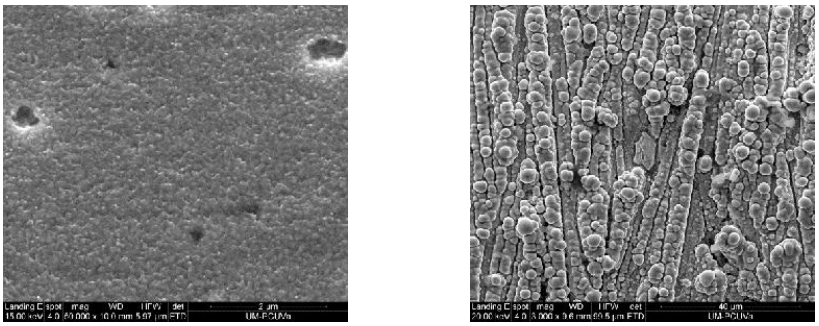

Figure 2.- Electrochemical deposits obtained using the Ag pseudoreference electrode in BMIMCL containing (a) Cu(I) and (b) Cu(II).

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EVALUATION OF THE THERMOCHEMICAL PROPERTIES OF THE $HoCd_x$ INTERMETALLIC COMPOUNDS USING ELECTROCHEMICAL TECHNIQUES

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The electrode reaction of Ho(III)/Ho couple in the eutectic LiCl-KCl, at Cd liquid electrodes (i.e a Cd pool and a Cd coated W electrodes) was investigated in the temperature range of 673-823K. In both electrodes, the electrochemical reduction of Ho(III) was observed at less cathodic potential values than at the surface of an inert W electrode, due to the decrease of Ho activity in the metal phase.

The formation of intermetallic compounds was studied. Electromotive force, *emf,* measurements for five intermetallic compounds in two-phase coexisting states were carried out using a Cd coated tungsten electrode. The activities and relative partial molar Gibbs energies of Howere obtained for $HoCd_6$, $HoCd_{45/11}$, $HoCd_3$, $HoCd_2$ and HoCd. The formation energy of each intermetallic compound, and the global formation constants were also calculated. The linear dependence of the Gibbs free energies with temperature yields to the enthalpies and entropies of formation of the five intemetallic compounds.

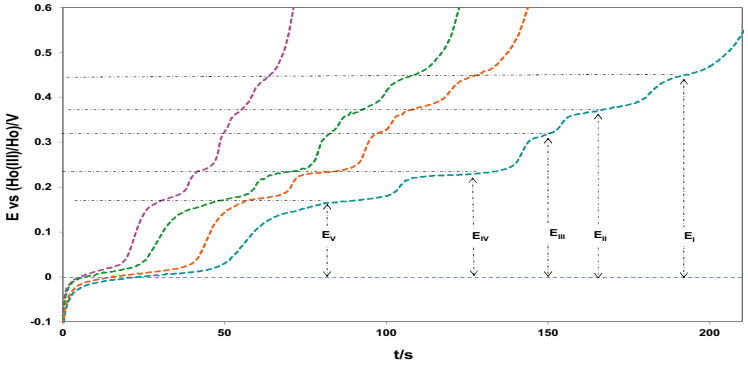

Figure 1.- OCP obtained with a CdFE.Experimental conditions: Ed =-2.25 V, td = 20, 40, 60, 100 s

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MULTIVARIATE OPTIMIZATION OF METHOD FOR SB DETERMINATION BY HG-AFS IN HAIR SAMPLES OF PATIENTS UNDERGOING CHEMOTHERAPY AGAINST LEISHMANIASIS

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A method was development for determination of total antimony in hair samples from patients undergoing chemotherapy against *Leishmaniasis* based on the administration of pentavalent antimonial drugs. The method is based on assisted microwave digestion of the samples in a pressurized system, reduction of Sb^{5+} to Sb^{3+} with KI solution (10% w/v) in ascorbic acid (2 %, w/v) and its subsequent determination by hydride generation atomic fluorescence spectrometry (HG-AFS). The proportions of each component (HCl, HNO_3 and water) used in the digestion were studied using a constrained mixtures design. The optimal proportions found were 50% water, 25% HNO_3 and 25% HCl. Variables involved in the generation of antimony hydride were optimized using a Doehlert design revealing that good sensitivity is found when using 2.0% w/v $NaBH_4$ and 4.4 mol L^{-1} HCl. Under the optimum experimental conditions, the method allows the determination of antimony in hair samples with detection and quantification limits of 1.4 and 4.6 ng g^{-1}, respectively, and precision expressed as relative standard deviation (RSD) of 2.8% (n = 10 to 10.0 mg L^{-1}). The developed method was applied in the analysis of hair samples from patients who take medication against *Leishmaniasis*.

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APPLICATION OF A NOVEL SORBENT FUNCTIONALIZED WITH 2-(5-BROMO-2-PYRIDYLAZO)-5-DIETHYLAMINOPHENOL IN THE ONLINE PRECONCENTRATION OF CADMIUM AND ZINC

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An online preconcentration system for Cd and Zn determination was developed. This system involves solid-phase extraction of metals using a minicolumn filled with Amberlite XAD-2 modified with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP)and detection by flame atomic absorption spectrometry (FAAS). After the preconcentration step, hydrochloric acid was used in the elution process, in order to transport the analytes directly to the spectrometer flame. A Doehlert desing was used to optimize the variables involved in the preconcentration performance. The developed system provides enrichment factors of 30 and 88 fold, limit of detection of 0.63 and 0.38 μg L^{-1} and precision (RSD, 20.0 μg L^{-1}, N = 10) of 4.3 and 5.4% for Cd and Zn, respectively. The optimized procedures were applied in the determination of Cd in fertilizer samples and Zn in drinking water. Accuracy was accessed by Cd determination in a certified reference material (NIST SRM 1573a, tomato leaves) through spike test for zinc in water samples. A t test was applied to compare results obtained in this study with the values of the certified reference materials and results are not significantly different, which confirms the accuracy of developed procedures.

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DISEÑO Y CONSTRUCCION DE SISTEMAS MICROFUÍDICOS AUTOMÁTICOS DE ANÁLISIS

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El desarrollo de nuevos métodos automáticos de análisis en flujo puede ser potenciado extraordinariamente mediante la construcción de nuevos dispositivos que permitan la integración de los distintos componentes dentro de un chip polimérico.

En esta contribución se describen distintos métodos y herramientas para la construcción de este tipo de dispositivos, tales como los tornos y fresadoras controlados por ordenador, y las impresoras 3D que permiten la construcción de estos dispositivos de forma monolítica.
En todos los casos se mencionarán los programas de ordenador que pueden ser utilizados, tanto para el diseño, como para el control de las máquinas herramienta utilizadas en la fabricación de los distintos componentes.

A título de ejemplo, se incluirán gráficas, dibujos y fotografías de elementos utilizados en las técnicas de flujo, y se describirán algunas aplicaciones de los sistemas desarrollados a la determinación de parámetros de interés ambiental

Agradecimientos
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VALIDATION OF A MODIFIED QUECHERS METHOD FOR PESTICIDES IN GRAPES AND WINE USING GC/MS. COMPARISON WITH UPLC/MS

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The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation method was modified to accommodate wine and grapes matrices, and provide good analytical results (recoveries in the range of 70-120% and RSDs <20%) for the target pesticides [1].

The method consisted of 10 mL sample in 10 mL of dichloromethane to carry out the analytical extraction. The $MgSO_4$/NaCl salt mixture (4:1, w/w) was added to the extract to induce phase separation and force the pesticides into the dichloromethane layer. Subsequently, a 5 mL aliquot was cleaned up using 125 mg of PSA and 750 mg of $MgSO_4$.

Ultra performance liquid chromatography combined with time-of-flight mass spectrometry (UPLC-MS) and gas chromatography (GC-MS) methods for determination of these pesticides after the QuEChERS extraction were compared. The methods were validated in terms of linearity, matrix effects, limits of detection (LOD) and quantification (LOQ), recovery and repeatability [2].

These methods could be useful in the routine qualitative and quantitative analysis of pesticides in wine and grapes. However, UPLC method is faster and consumes less eluent while GC is more sensitive.

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DETERMINACIÓN ENZIMÁTICA DE AMINAS BIOGÉNICAS COMO PASO PREVIO AL DESARROLLO DE UN BIOSENSOR ÓPTICO

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Este trabajo presenta el desarrollo de una nueva metodología para la determinación enzimática de aminas biogénicas, especialmente putrescina, cadaverina e histamina, basado en los cambios producidos en el espectro de absorción molecular de la peroxidasa (HRP) al adicionar los analitos, sobre una mezcla de HRP-DAO (diamino oxidasa). El objetivo final es la implementación de la metodología analítica para el desarrollo de un biosensor óptico.
En el sistema enzimático desarrollado, la DAO reacciona con las aminas biogénicas produciendo H_2O_2, el cual reacciona de forma reversible con la HRP, de acuerdo al modelo matemático desarrollado por el grupo de investigación [1].

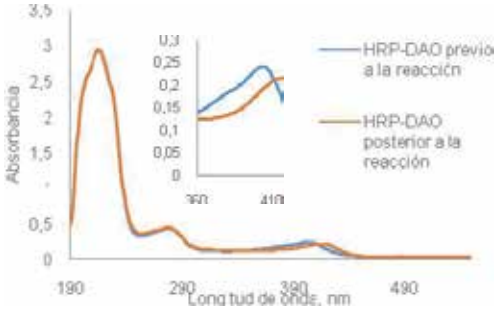

Figura 1: Espectro de Absorción Molecular HRP-DAO antes y después de la reacción con putrescina ($2{,}61 \cdot 10^{-6}$ M)

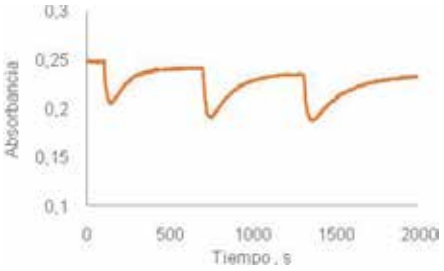

Figura 2: Variación de Abs a 400 nm tras la adición de alícuotas sucesivas de putrescina ($2{,}61 \cdot 10^{-6}$ M)

En la figura 2 se observan los cambios de absorbancia de la mezcla HRP-DAO a 400 nm tras la adición de alícuotas sucesivas de putrescina. Esta longitud de onda es un máximo característico de la HRP (Figura 1) que tras la adición de putrescina reacciona con el H_2O_2 generado en la reacción DAO/putrescina, provocando la transformación a HRPII (Abs_{max}=420nm). De acuerdo con el mecanismo descrito [1], HRPII se reduce nuevamente a HRP lo que permite la determinación en continuo de putrescina con una misma alícuota de HRP-DAO.

A partir de los estudios obtenidos en disolución (rango de respuesta lineal entre $2{,}6 \cdot 10^{-6}$ M – $2{,}6 \cdot 10^{-5}$ M) se va a desarrollar una lámina sensora de poliacrilamida en la que queden entrampadas las dos enzimas utilizadas (HRP y DAO) y que pueda ser utilizada como base de un biosensor óptico para la determinación en continuo de aminas biogénicas.

Agradecimientos
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DETERMINATION OF LOW B/Ca RATIOS IN CARBONATES USING ICP-QQQ

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The very low B/Ca ratios characteristic of some natural biogenic carbonates, are of interest for research in ocean acidification but represent an analytical challenge [1, 2]. We describe a method using a novel instrument configuration (ICP-QQQ), for which we are not aware of any previously published geological applications, and for coccoliths, a sample type unique in its low B content and organic phases. Detection limits as low as 0.41 µmol mol^{-1} were achieved.

Isobaric interferences, out of the reach even for SF-ICP-MS, can be solved using this instrument, which permits the safe measurement of the lowest abundance Ca isotope (^{46}Ca). This allows maximizing the B concentration measured while maintaining both B and Ca signals in counting mode. More significantly for low B samples, the ICP-QQQ is also able to overcome the interference of the ubiquitous ^{12}C tail on the ^{11}B mass, which otherwise leads to significant overestimates at very low B concentrations. This could be the reason for the significantly lower B/Ca ratios observed for the low B content interlaboratory calibration standards (Carrara and OKA), while matching for the high B content standards was good. Finally, results obtained in the analysis of coccoliths grown in laboratory culture seems to corroborate that SIMS analysis of the samples mounted in Indium leads also to B/Ca overestimates due to porosity effects, as previously observed using LA-ICP-MS. This approach also permits the interference-free measurement of P/Ca and S/Ca ratios, which could be used as indicators of the complete removal of the organic matter from the samples.

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COLORIMETRIC μ-PAD FOR ALKALINE AND ALKALINE-EARTH IONS BASED ON IONOPHORE-CHROMOIONOPHORE CHEMISTRY

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Solid state ionophore-chromoionophore optical sensors are based on a well-known chemistry of recognition to determine the concentration of different cationic species, obtaining accurate and precise results in a simple way through an ion exchange reaction [1]. The reagents included in sensing membrane are lipophilic-derivatives of pH indicators, ionophores and chromoionophores, contained in a polymeric membrane and the main disadvantage of this type of sensors are their usual long equilibration time with analyte due to the hydrophobic nature of the membrane. Consequently, this drawback make difficult to transfer of this recognition chemistry to microfluidic paper-based devices (μ-PAD), because the aqueous sample cannot get wet the recognition areas properly and, for that reason, the analyte is prevented from producing the optical change.

We have followed the strategy to develop a μ-PAD based on ionophore-chromoionophore mechanism, consists on including chemicals needed in micelles, using a non-ionic copolymer surfactant as Pluronic F-127 [2].

In this work, we present μ-PAD for alkaline (Na(I) and K(I)) and alkaline-earth (Ca(II) and Mg(II)) ions determination based on ionophore-chromoionophore chemistry contained in micelles [3]. The micelles are immobilized on positively-charged nylon support that allows the flow of the sample through them by capillarity.

The prepared μ-PAD contains one reception area and four different recognition areas, containing the different ionophore-chromoionophore chemistries. Once the recognition areas of the μ-PAD reacts with the sample, changing its color, it is digitalized by a scanner, using the H colour coordinate obtained from the image as analytical parameter to build the calibration.
In summary, we have transferred the ionophore-chromoionophore chemistry to a μ-PAD that permits the determination of alkaline and alkaline-earth analyzing the color of the sensing areas.

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MICROFLUIDIC THREAD-BASED DEVICE FOR POTASSIUM DETERMINATION

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In the last years, the development of solid state colorimetric sensors has been focused on the miniaturization of the sensing area that allows the determination of multiple analytes through multisensory and the use of new supports that makes the sensor cheaper and smaller. This miniaturization also makes possible the use of a very low volume of samples to perform the determination.

In this work we suggest the use of thread [1] as a novel support for an ionophore-chromoionophore optical sensor for potassium determination [2]. The thread used as support is immersed, for a short time in a THF solution that contains all the reagents necessaries to react with potassium. The thread, 0.5 cm long, is included in a microfluidic thread-based device as recognition element. The sample is added at the paper-made reception area and the sample flows through the thread by capillarity, changing its color depending on the potassium concentration of the sample.

The microfluidic thread-based device was digitalized using a flatbed scanner, and the mode of the H parameter of the around 2200 pixels, that defines the sensor in the digital image, was used as analytical parameter to relate the color change with the concentration of potassium in the sample. The volume necessary to perform the determination was around 2 μL.
In this way, we have developed a very small microfluidic thread-based device (7.5 mm long) and cheap device for potassium determination.

Acknowledgments
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ANALYSIS OF UV FILTERS IN COSMETICS BY PRESSURIZED LIQUID EXTRACTION-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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UV filters are substances that are exclusively or mainly intended to protect the skin against certain UV radiation by absorbing, reflecting or scattering this radiation. However, in spite of being required for this reason, they can cause some adverse effects. Thus, the EU established a specific Regulation (EC) Nº 1223/2009 laying down the rules that must follow all marketed cosmetic products in order to ensure the protection of human health.

An analytical method based on pressurized liquid extraction (PLE) followed by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) has been developed for the simultaneous analysis of 16 different organic UV filters, both water and fat-soluble,in cosmetic products. The extractions were carried out in 1 mL extraction cells and the amount of sample required was only 100 mg. The experimental conditions were optimized by means of experimental design tools. Main factors affecting the PLE procedure such as solvent type, and extraction temperature were evaluated. The validated methodology was successfully applied to the analysis of different types of cosmetic formulations including sunscreens, hair products, lipsticks, facial creams, amongst others.

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DEVELOPMENT OF A METHOD BASED ON ULTRASOUND-ASSISTED EMULSIFICATION MICROEXTRACTION -GAS CHROMATOGRAPHY MASS SPECTROMETRY FOR THE ANALYSIS OF UV FILTERS IN WATER SAMPLES

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UV filters are substances that are exclusively or mainly intended to protect the skin against certain UV radiations by absorbing, reflecting or scattering them. However, in spite of being required for this reason, they can cause some adverse effects. Therefore, the EU established a specific Regulation (EC) Nº1223/2009 laying down the rules that must follow all marketed cosmetic products in order to ensure a high level of protection of human health. In addition, it is important to monitor the filters not only in personal care products but also in waters, rivers, lakes, pools and wastewater, among others. This is because these compounds may enter the environment through the bath, swimming, shower, etc. In fact, UV filters are classified as emerging contaminants. Moreover, these compounds are lipophilic and therefore can bioaccumulate and biomagnify through the food chain.

For these reasons, in the present work, a methodology based on ultrasound-assisted emulsification microextraction (USAEME) followed by gas chromatography–mass spectrometry (GC–MS) has been developed for the simultaneous analysis of different classes of UV filters including methoxycinnamates, salicylates, p-aminobenzoic acid derivatives, and others in different water samples. The extraction parameters such as the extraction solvent, the temperature and the time of extraction, and the addition of salt were optimized by means of experimental design tools. Good linearity (R^2> 0.997), quantitative recoveries (>85% for most of compounds) and satisfactory precision (RSD < 10% in most cases) were achieved under the optimal conditions. The validated methodology was successfully applied to the analysis of different types of water samples including seawater, spas, swimming pools and aquaparks.

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ANALYSIS OF UV FILTERS IN WATER SAMPLES BY SOLID-PHASE MICROEXTRACTION - GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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UV filters are substances widely used in the manufacture of cosmetics and personal care products. These compounds are designed to absorb, reflect or scatter the radiation in the UV region, protecting human skin against direct exposure to deleterious wavelengths of sunlight. Due to awareness of the harmful effects of UV radiation and the possible occurrence of cancer, the consumption of sunscreens has increased in recent years. These compounds may enter the environment through the bath, swimming, domestic wastewater discharges, etc. In fact, UV filters are considered as emerging environmental pollutants. Some of them have adverse health effects like estrogenic activity. Moreover, these compounds are lipophilic and therefore can bioaccumulate and biomagnify through the food chain. For these reasons, it is important to monitor the filters not only in personal care products but also in waters, rivers, lakes, pools and wastewater, among others. In addition, they are frequently present at trace levels in water samples, so sensitive analytical methods to determine UV filters in this kind of matrices are needed.

In this work, a methodology based on solid phase-microextraction (SPME) followed by gas chromatography–tandem mass spectrometry (GC–MS/MS) has been developed for the simultaneous analysis of different classes of UV filters including methoxycinnamates, salicylates, benzophenones, p-aminobenzoic acid derivatives, and others in different water samples. This extraction technique has some advantages like the absence of organic solvents, which makes it an environmentally friendly technology; the speed of the technique, which increases the capacity of sample processing; the small size of the required device, which facilitates its use in fieldwork; and the sensitivity. In situ derivatization with K_2CO_3 and acetic anhydride was carried out to improve chromatographic performance, above all polar compounds like benzophenones. The extraction parameters such as the fiber coating, the extraction mode and the addition of salt were optimized by means of experimental design tools. The validated methodology was successfully applied to the analysis of different types of water samples including seawater, spas, swimming pools and aquaparks.

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LIQUID CHROMATOGRAPHY/DIELECTRIC BARRIER DISCHARGE (TANDEM) MASS SPECTROMETRY FOR THE DETERMINATION OF MULTICLASS CONTAMINANTS IN FOOD AND ENVIRONMENT

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Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) associated to electron impact (EI) and electrospray ionization (ESI) sources respectively, are the most widely used techniques in laboratories dealing with advanced trace analyses for different purposes. The tendency towards research dealing with water-soluble components from biological samples (proteins, peptides, metabolites or lipids) has fostered the impressive growth of LC-MS market in the last 10 years. With the aim of extending the applicability of LC-MS coupling to a wider range of compounds with different physicochemical properties, new ionization sources have been investigated. Amongst them, Dielectric Barrier Discharge Ionization (DBDI) LC-MS interface is based on the use of a low-temperature helium plasma, which features the possibility of simultaneous ionization of species with a wide variety of physicochemical properties. In this work, we comment on the current research we are addressing in our laboratory related to the application of LC-DBDI-MS(/MS) for trace analysis of different analytes including multiclass priority organic contaminants and residues such as pesticides, polycyclic aromatic hydrocarbons, organochlorine species, pharmaceuticals, personal care products, and drugs of abuse in diverse samples such as urine, food and water. LC-DBDI-MS performance for this application was assessed and compared with standard LC-MS sources (electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The methodology was found to be effective to detect a wide array of organic compounds at concentration levels in the low ng L^{-1} - μg kg^{-1} range in wastewater and food matrices, respectively.

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POSSIBILITIES OF HIGH-RESOLUTION CONTINUUM SOURCE FLAME ATOMIC ABSORPTION SPECTROMETRY FOR DETERMINATION OF ESSENTIAL ELEMENTS IN COMMERCIAL DRINKS AND DIETARY SUPPLEMENT PRODUCTS

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Natural fruit juices, mineral waters, teas and soft drinks, as well as multimineral dietary supplements are widely consumed in the world and constitute a rich source of minerals. A quality control of trace, minor and major elements in these samples is required in order to ensure the purity and content of these minerals or to detect possible contamination processes. In this context, there is an increasing demand for multi-elemental methods to quickly characterize samples, reducing time and analytical cost. Analytical methodologies based on direct analysis of the sample, without previous treatment, also present important advantages as quality control tools because they are simple, increase the speed of analysis and reduce the risk of contamination and losses of analytes. In addition, these methods allow a cost reduction and they are environmental-friendly as the use of toxic and corrosive reagents is reduced.

High-resolution continuum source atomic absorption spectrometry with flame atomizer (HR-CS FAAS), has further improved the potential of AAS techniques for direct and fast sequential multi-element determination. This approach allows the determination of a high number of elements with different concentration levels in a fast sequential mode due to the possibility of using main and secondary analytical lines and the use of the line wings to enhance the sensitivity to the required level and, therefore, extend the linear working range [1-2]. In this way, all elements can be determined in a single run using the same sample without the need of additional measurements or different dilutions of sample solution [3]. In addition, possibilities to perform direct analysis of sample are increased due to the simultaneous monitoring of a narrow spectral environment around an analytical line make easy the detection and correction of spectral interferences from matrix sample [4], reducing or avoiding sample pretreatments.

The aim of this work is the development of a direct, fast and reliable methodology for the fast sequential determination of Cu, Zn, Mn, Mg and Si in different types of beverages and dietary supplement products by HR-CS FAAS, with minimal sample consumption. To achieve this purpose, absorption lines were selected, and burner height and flame composition were optimized for each element. A flow injection valve was used to obtain a transient analytical signal and reduce the sample consumption up to only some microliters per element. The use of the side pixel registration approach was investigated to extend the linear working range. The principal analytical parameters were calculated and the proposed analytical approach was applied with successful results to determine the metal contents in 17 different samples of commercial beverages and dietary supplement products.

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THE INFLUENCE OF SAMPLE PARTICLE SIZE IN RAMAN SPECTRA: EFFECT ON THE ANALYSIS OF PURE COMPOUNDS AND MIXTURES USING SAMPLING OPTICS OF DIFFERENT SPOT SIZE.

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Raman spectroscopy is becoming a popular technique for the development of analytical methods applicable to the pharmaceutical and food industry. When considering to analyze a sample using this technique, the spectrum obtained is a combination of the chemical and physical properties of the object being measured: the first probably represents the main contribution to the appearance of the spectrum, which allows for example, the building of spectral libraries; but the latter is equally important and it has been less studied.

The relationship between Raman signal and powder properties was described by Schrader et al. [1], as an extension of the Kubelka-Munk theory of the optical properties of crystal powders [2]. Research relating particle size and Raman spectra have been performed both at nano- and micro-scale; in the case of nanoparticles, as particle size reduces, the system enters a regime where quantum mechanical effect becomes very important, which results in the form of confinement of phonons that is very effectively traced in Raman scattering studies [3]. Considering materials in the micro-scale, some studies have been performed using different inorganic salts, with crystal size ranging from 75 µm to 600 µm; the experimental finding related to the intensity of the signal was that signal increases as particle size decreases, however this observation is contrary to the theoretical predictions. The optical system used for excitation and collection of Raman scattering was a fiber probe, and over it is the sample container that has a glass window on the bottom facing the probe [4, 5]. Another study using flufenamic acid in the range of 65 µm - 215 µm found the same tendency contrary to the theoretical predictions, in this case an immersion optic with a flat sapphire window attached to a probe was used [6].

In our experiments we are investigating the influence of sample particle size in Raman spectra using another optical system: a non-contact optic sampling device, in which the sample is placed on a software-controlled x-y-z mapping stage, and illuminated via a parabolic mirror objective that includes a video camera, to additionally collect visual images of the sampling positions. The samples analyzed are organic compounds, or a salt with an organic moiety in the range of 10 µm - 100 µm; smaller sizes where chosen given that, prior to or during the formulation process of a pharmaceutical product, the active pharmaceutical ingredient (API) is often micronized and the resulting particle sizes are less than 100 µm [7, 8]. Two instruments were used for the measurement of the samples: a conventional macro-Raman system (500 µm spot diameter) and a Raman microscope (50 µm spot diameter). Having two different laser spot size we are able to apply the sampling theory in our mixtures: if the goal is to quantify a component of the sample, it is necessary to be able to measure the bulk composition of the product; this is related to the diameter of the laser as the resulting spectrum reflects the volume sampled along the acquisition time of the spectra [9].

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DETERMINACIÓN DE POSIBLES BIOMARCADORES DE CÁNCER DE PRÓSTRATA Y VEJIGA MEDIANTE HS-PTV-GC-MS

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En el trabajo realizado se pone a punto un método analítico para la determinación de alanina, sarcosina, etilglicina, valina, leucina y prolina en muestras de orina mediante su derivatización con etilcloroformiato y su posterior análisis con el sistema *HS-PTV-GC-MS*. Este tipo de compuestos se han propuesto como posibles biomarcadores metabólicos de cáncer de próstata y vejiga [1-4].

Debido a la presencia de grupos altamente polares (-NH_2, -COOH), el análisis de estos compuestos mediante cromatografía de gases requiere una derivatización previa de los mismos para la que se ha utilizado como reactivo derivatizante el etilcloroformiato [5, 6]. En este trabajo, con el fin de hallar las condiciones óptimas de la reacción de derivatización, se han realizado estudios de pH, volumen de piridina y volumen de etanol y etilcloroformiato. La mayor eficiencia de derivatización se consigue a pH 9 y añadiendo 120, 100 y 120 µL de etanol, piridina y etilcloroformiato, respectivamente.

El uso de generación de espacio de cabeza evita la presencia de interferencias de compuestos no volátiles presentes en la matriz, lo que hace de esta técnica una excelente opción para matrices complejas como son las muestras de orina. Las variables que afectan al espacio de cabeza como volumen de muestra, temperatura de equilibrio y tiempo de equilibrio, han sido evaluadas, encontrándose los mejores resultados para 3 mL, 90 °C y 30 min, respectivamente. Además, se ha optimizado el modo de inyección *solvent vent* utilizado en el inyector de temperatura programada, así como las rampas cromatográficas y variables del espectrómetro de masas utilizado como detector.

En las condiciones experimentales optimizadas, se realizaron los calibrados de todos los compuestos, mostrando un comportamiento lineal y sin fallo de ajuste. Se ha comprobado la existencia de efecto de matriz en muestras de orina, y se ha propuesto el método de adición estándar para la cuantificación de las muestras.

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QUANTIFICATION OF TRACE ELEMENTS IN ROCKS BY LASER ABLATION ICP-OES

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Introduction: Chemical composition (especially trace element concentration and isotope ratios) in archaeological artifacts provides highly valuable information for research on provenance and trade in prehistory contexts [1, 2]. The most commonly available analytical techniques used for these purposes [3] – texture, mineralogical and chemical composition - are generally of destructive nature; thus their use on unique artifacts and those of high archaeological value should be considered cautiously.

Laser Ablation systems (LA) coupled to instruments such as ICP-MS or ICP-OES are widely recognized due to the combination of the pseudo-non-destructive character of LA and the high sensibility and multi-elemental power analysis of the ICP. [4]. LA-ICP-MS allows high spatial resolution, single point and surface analysis. On the other hand it highlights some limitations given by the ICP chosen option, MS or OES. The main weak point is consequence of its matrix dependent character due to the facts that (i) the laser beam causes selective mass fractionation during the ablation and could alter the accuracy of the measurement and (ii) sample heterogeneity can lead to misinterpretation of results.

Igneous rocks constitute a family of materials with some properties determined by their volcanic or plutonic origin and the presence of some main components (SiO_2, alkaline feldspar, plagioclase…) [5]. This family of rocks has been extensively used to make artifacts and is consequently the commonly found at archaeological sites.

Scope: The aim of this work is to set up a methodology for the quantification of elemental composition in igneous rocks by LA-ICP-OES as an alternative to the traditional (destructive) acid digestion of samples and at the same time minimize the minimum sample size.

Method: We designed a methodology with the purpose of minimizing sample damage by the ablations and keeping the detection limits as low as possible. We prepared and analyzed by LA-ICP-OES some pellets made of several certified reference materials (CRM) of different igneous origin. Sample preparation stage and instrumental measurement conditions were optimized: pellet physical preparation (pressure, time) and fixation (selection and amount of binding substances), laser conditions (fluency, frequency, spot size, and ablation sampling mode) and ICP OES parameters (nebulizer flow, RF power, and acquisition, integration time).

Discussion and conclusion: After optimization, we are able to build acceptable calibration lines using the CRMs and taking into account their geological classification. We stress the importance of a well-considered sampling strategy for this kind of samples considering their intrinsic heterogeneity. Our next step will be to apply this methodology for the study of archaeological artifacts made of this kind of material.

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MILD PYROLYSIS OF LOW-QUALITY COALS AS A RELIABLE ALTERNATIVE TO PRODUCE LIQUID FUELS FOR VEHICLES AND VALUABLE CHEMICALS

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The high demand of global energy and scarcity of new crude oil reservoirs fostered interest in alternative liquid fuels, creating a favorable opportunity for coal-to-liquids technologies. The primary aim of the research is to characterize chemically low-temperature tars and other products obtained by mild pyrolysis in order to lay the fundamentals for the possible production of synthetic fuels. Two high-volatile bituminous coals from the Novo-Grodovskay (NG) and Yuzhno-Donbassay (YD) mines of Donetsk basin were pyrolyzed at 520 °C. Pyrolysis gases were studied using a VTI-II gas analyzer. Primary tars were characterized by Column Liquid Chromatography, Infrared spectrometry and ^{1}H Nuclear Magnetic Resonance.

NG-coal yielded more gaseous products (12.8 wt. %), although less carbon residue and pyrogenetic water than YG-coal. The maximum yield for the liquid products (16.92 wt. %) was achieved for NG-coal. The main components of gases were methane (44.5–43.5 vol. %), hydrogen (38–39 vol. %) and alkenes (3.2 vol. %). Gas from NG-coal had the highest amount of H_2S (7.5 vol. %), indicating a semicoke desulfurization during the mild pyrolysis. The high content of CO and CO_2 (12.5 vol. %) in the gases indicates intensive breakdown of the oxygenated functionalities of the parent coals during the mild pyrolysis. The liquid products from YD-coal yielded 10 % more malthenes than that from NG-coal. The primary tar from NG-coal had the highest content on asphaltenes and carboides plus free carbon (16.4 and 16.6 wt. %, respectively). The quantity of asphaltenes of the liquid part of NG-coal increased approximately by 50 % compared to YD-coal.

Primary tars are composed of variety families of organic hydrocarbons. Their abundance was found to be: aromatic > polar > aliphatic compounds. It was observed that mild pyrolysis of NG-coal lead to a higher yield of primary tar (16.92 wt. %), which, in turns, contains less impurities, like sulfur (0.93 wt. %), organic bases (1.7 wt. %), carboxylic acids (0.8 wt. %) and phenols (8.7 wt. %). The pyrolysis gas from NG-coal constitutes a high-quality gaseous fuel because of its comparatively high calorific value 23-24 MJ/m^3. In addition, it can be used as feedstock for producing elemental sulfur. After removal of sulfur, the carbon-rich semicoke (89.8 wt. %) produced form NG-coal can be applied to produce metallurgical coke, graphites and activated carbons. On the other hand, this solid product can be gasified to hydrogen sulfide and a subsequent conversion to sulfur using the Claus method. Thus, mild pyrolysis of bituminous coal from Novo-Grodovskay mine yields more valuable chemical feedstocks for possible commercial production of high-quality fuels.

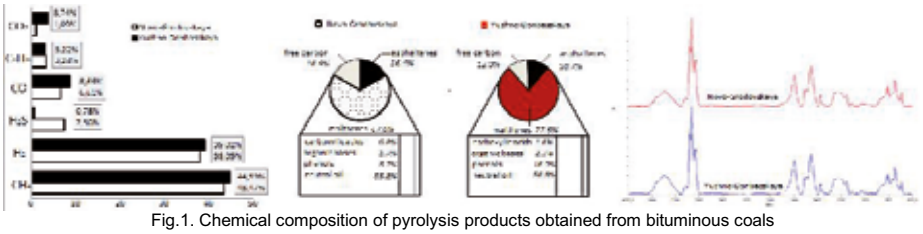

Fig.1. Chemical composition of pyrolysis products obtained from bituminous coals

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DIGITAL IMAGES FOR THE DETERMINATION OF pH WITH TEST PAPER

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Experimental analytical measurements are frequently acquired in order to take rapid decisions (screening, etc.) and many of them deal with the evaluation of colours. Quite a lot of times, the comparison of the obtained colours with standards is needed. However, sometimes no standards are available and, in any case, the task turns into a very subjective decision. This has been the matter of a recent and probably first world-wide trending topic (Figure 1).

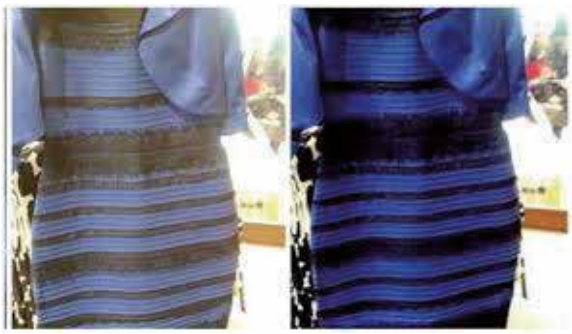

Figure 1. "The dress" became a world-wide trending topic some few months ago (February-March, 2015) because 69% population sees the colours at the left, whereas 31% population sees the colours at the right. The "*true*" colours are those at the right. Photograph taken from: http://losmormones.org/2212/elder-holland-declara-el-color-del-vestido

In the last few years a lot of very rapid instrumental systems have been developed to acquire digital images (scanners, cameras, smartphones, etc.) and most of the times they are simple and cheap. The digital images obtained can rapidly be handled by dedicated algorithms or computational programs. Pre-determined information can be easily obtained straightforward and objectively interpreted.

In this work, pH test paper strips have been used and digital images have been obtained from them with both a flatbed scanner and a digital camera. Experimental data from the images have been finally handled using multivariate calibration methods such as Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS) to evaluate pH. Samples of beer and different eye-drops were tested. Results are compared with those found at a glance and those with a pH-meter, which was used as a reference (Table 1). The scanner performed better than the human eye, but it was under commercial pH-meters.

Table 1. pH values as measured with pH-meter and with pH test paper; in the last case the colour was evaluated both visually and with a flatbed scanner-multivariate calibration. pH plastic strips from Panreac. Three replicates. Standard deviation is given in parenthesis.

Sample	pH-meter	Scanner (pH test paper)		Visual (pH test paper)
		Found	RSD (%)	
Beer	4.20 (0.08)	4.4 (0.3)	7	4-5
Eye-drops 1	6.63 (0.02)	7.0 (0.2)	3	7
Eye-drops 2	6.01 (0.04)	5.4 (0.2)	4	5
Eye-drops 3	7.27 (0.05)	8.1 (0.2)	2	8
Eye-drops 4	7.14 (-)	6.8 (0.1)	1	7

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ISOTOPE RATIOS DETERMINATION IN SAMPLES WITH LOW ANALYTE CONCENTRATION VIA MULTICOLLECTOR-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY USING A NON-ENRICHED STANDARD

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Nowadays, there is an increasing interest in the use of isotopic analysis for clinical purposes. Most recent research focuses on diseases related with the uptake or excretion of essential elements such as Fe, Cu and Zn. Isotopic analysis using MC-ICP-MS at low analyte concentrations is not an easy task and so, applications are typically restricted to the ppm or high ppb level. Most of the strategies suggested to overcome this issue are focused on an improvement of the instrumental sensitivity, e.g., the use of an aerosol desolvating system, of a high-efficiency interface or a combination of both. Also the use of Faraday cups with $10^{12}\Omega$ resistors, instead of the conventional $10^{11}\Omega$ resistors or of ion-counting devices allows the instrumental sensitivity to be increased. Nevertheless, simple methods for isotopic analysis at low concentrations of the target element and/or small amounts of sample are still of high interest to extend the applicability of MC-ICP-MS to biosamples characterized by low analyte concentrations and/or limited availability.

Therefore, a simple, reliable and versatile approach for isotopic analysis by MC-ICP-MS at lower analyte concentrations has been developed and validated. For such purpose, a non-enriched in-house standard that was previously characterized for its isotopic composition was used to dope the analyte fraction, as obtained after sample digestion and isolation. The suitability of this methodology has been investigated for Cu and Fe in whole blood samples.

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PULSED GLOW DISCHARGE TIME-OF-FLIGHT MASS SPECTROMETRY FOR ELEMENTAL DEPTH PROFILING AND MOLECULAR FINGERPRINTING. APPLICATION TO THE ANALYSIS OF POLYMERS,CERAMICS AND THIN SOLAR CELLS.

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Glow discharges (GDs) either with optical emission or mass spectrometric detection are well recognized atomization/excitation/ionization sources for the analysis of solid materials (conductive and insulating) with depth resolution capabilities in the nanometric range.

During the last years, the combination of a pulsed GD (PGD) source with a time-of-flight mass spectrometer (TOFMS) has received much attention and nowadays it is commercially available. PGD-TOFMS offers features of particular analytical interest, [1] including:

i) The high acquisition rates (a full spectrum up to m/z 210 is acquired in 29 s) make this instrumentation ideal for the analysis of fast transient signals.
ii) The sensitivity and mass resolution is independent of the number of selected isotopes.
iii) The temporal distribution of the applied power enables to obtain not only elemental but also molecular information from analyzed materials. In fact, it is possible to differentiate three time regimes along the GD pulse duration: prepeak, plateau and afterglow, each of them characterized by different predominant ionization processes. The last region (afterglow) is the most investigated so far because it shows enhanced sensitivity compared to prepeak and plateau for both elemental and polyatomics.
iv) Moreover, the very recent possibility of obtaining information in negative mode is particularly interesting when dealing with analysis of polymer based materials.

The analytical potential of PGD-TOFMS to provide elemental and molecular specific information will be here demonstrated through three selected groups of applications: ceramics, photovoltaic materials based on thin film solar cells and polymeric materials deposited on silicon wafers. Moreover, the nature of the information achievable in negative mode in polymers will be thoroughly investigated and compared with positive detection mode.

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CHEMICAL FINGERPRINTS IN AN UNDERWATER ARCHAEOLOGICAL SHIPWRECK USING A REMOTE LASER-INDUCED BREAKDOWN SPECTROSCOPY SYSTEM

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Nowadays, one of the most important areas of interest in archeology is the characterization of the submersed cultural heritage. Mediterranean Sea is rich in archaeological findings due to storms, accidents and naval battles since prehistoric times. Chemical analysis of submerged materials is an extremely valuable source of information on the origin and precedence of the wrecks, and also the raw materials employed during the manufacturing of the objects found in these sites. Sometimes extracting the archeological material from the marine environment is not practical due to the size of the sample, or is not permitted by the legislation or preservation practices. In these cases, the in-situ analysis turns into the only alternative.

The versatility of laser-induced breakdown spectroscopy (LIBS) has been successfully tested in oceanography [1]. Advantages such as rapid and in situ analysis with no sample preparation make LIBS a suitable alternative for field measurements. A fiber-optics-based remote instrument has been designed for the recognition and identification of artworks in underwater archaeological shipwrecks. The LIBS prototype featured both single-pulse (SP-LIBS) and multi-pulse excitation (MP-LIBS). The use of multi-pulse excitation allowed an increased laser beam energy (up to 95 mJ) transmitted through the optical fiber. This excitation mode results in an improved performance of the equipment in terms of extended range of analysis (to a depth of 50 m) and a broader variety of samples to be analyzed (i.e., rocks, marble, ceramics and concrete). In this work, parametric studies in the laboratory such as gas flow pressure, beam focal conditions and angle of incidence, among others, were performed to optimize the best conditions for field analysis. Finally, results obtained in these field trials confirmed the capability of remote LIBS for in-situ analysis of underwater archeological samples.

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DETERMINACIÓN SIMULTÁNEA DE CONSERVANTES Y FILTROS ULTRAVIOLETA EN PRODUCTOS PARA EL CUIDADO PERSONAL Y DEL HOGAR MEDIANTE CROMATOGRAFÍA ELECTROCINÉTICA MICELAR Y DAD

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Los consumidores están expuestos a una amplia gama de cosméticos y de productos para el cuidado personal y del hogar, así como a los ingredientes que los componen. Con el fin de garantizar la seguridad, eficacia y calidad de los productos cosméticos, la normativa europea en vigor sobre dichos productos reglamenta una serie de sustancias sujetas a restricciones y/o prohibiciones [1]. En el anexo V de la normativa, se presenta un listado de los conservantes autorizados para su uso en productos cosméticos y sus condiciones de uso. En el anexo VI, se presenta el listado de los filtros ultravioleta admitidos y las restricciones pertinentes. Debido al extendido uso de los cosméticos y a la creciente preocupación por los posibles efectos perjudiciales para la salud que sus ingredientes pudieran tener, es imprescindible que los productos cosméticos sean sometidos a estrictos controles de calidad. Para ello, resultan necesarios el desarrollo de metodologías analíticas que permitan conocer y controlar la composición de los productos comerciales [2].

El objetivo de este estudio fue desarrollar y optimizar un método para cuantificar de forma simultánea 14 conservantes (incluyendo parabenos e isotiazolinonas) y 2 filtros ultravioleta derivados de la benzofenona, seleccionados de entre los ingredientes mencionados en el Reglamento 1223/2009. La cuantificación se llevó a cabo mediante la técnica denominada cromatografía electrocinética micelar (MEKC) y se utilizó un detector ultravioleta de diodos en hilera (DAD). La etapa previa de optimización de las variables significativas que afectaban al método se basó en el uso del diseño experimental y en la aplicación de una función de respuesta cromatográfica modificada (MCRF) [3].

La cuantificación de los 16 analitos se llevó a cabo utilizando el método del patrón interno. Las características analíticas estudiadas incluyeron límites de detección (0.91 a 2.80 μg mL^{-1}) y de cuantificación (2,7 a 8,4 μg mL^{-1}), rangos lineales (hasta 65 μg mL^{-1} aprox.), repetibilidad (2,0 a 8,8 %DER), precisión intermedia (2,2 a 14,2 %DER) y ensayos de recuperación (90,4% a 114,8%). Por tanto, y en base a los criterios utilizados habitualmente en la validación de métodos analíticos, las características analíticas del método se consideraron satisfactorias.

Finalmente, el método MEKC optimizado y validado se utilizó para cuantificar los compuestos seleccionados en distintos productos comerciales para el cuidado personal y del hogar, incluyendo geles, perfumes, champús, cremas para las manos, ambientadores para el hogar, detergentes, pastas dentífricas y cremas solares.

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MINIATURIZED AND DIRECT SPECTROPHOTOMETRIC MULTI-SAMPLE ANALYSIS OF TRACE METALS IN NATURAL WATERS

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Trends in the analysis of trace metals in natural waters are mainly based on the development of sample treatment methods to isolate and pre-concentrate the metal from the matrix in a simpler extract for further instrumental analysis. However, direct analysis is often possible using more accessible techniques such as spectrophotometry. In this case a proper ligand is required to form a complex that absorbs radiation in the UV-Vis spectrum. In this sense, the hydrazone derivative, di-2-pyridylketone benzoylhydrazone (dPKBH) forms complexes with Cu and V that absorb light at 370 nm and 395 nm respectively. Although spectrophotometric methods are considered as time and reagents consuming, this work is focused on its miniaturization, by reducing the volume of sample, as well as time and cost of analysis.

In both methods, a micro-amount of sample is placed into a microplate reader with capacity for 96 samples, which can be analyzed in times ranging from 5 to 10 minutes. The proposed methods have been optimized using a Box-Behnken design of experiments. For Cu determination, concentration of phosphate buffer solution at pH 8.33, masking agents (ammonium fluoride, and sodium citrate) and dPKBH were optimized. For V analysis, sample pH=4.5 was obtained using acetic acid/sodium acetate buffer, and masking agents were ammonium fluoride and 1,2-cyclohexanediaminetetraacetic acid.

Under optimum conditions both methods were applied to the analysis of certified reference materials TMDA-62 (lake water), LGC-6016 (estuarine water) and LGC-6019 (river water). In all cases results proved the accuracy of the method.

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APLICACIÓN DE LA MICROEXTRACCIÓN LÍQUIDO-LÍQUIDO DISPERSIVA BASADA EN LA SOLIDIFICACIÓN DE LA GOTA FLOTANTE AL ANÁLISIS MULTIRESIDUO DE CONTAMINANTES EN AGUAS

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En los últimos años se ha ido incrementando el interés por desarrollar métodos analíticos rápidos, sencillos, de bajo coste y menos contaminantes que los métodos de extracción líquido-líquido y de extracción en fase sólida convencionales. De entre estos métodos novedosos, la microextracción líquido-líquido dispersiva (*dispersive liquid-liquid microextraction*, DLLME) ha despertado un especial interés ya que es poco costosa, fácil y rápida de aplicar, requiere bajos volúmenes de muestra y de disolventes orgánicos y puede permitir elevados factores de preconcentración. Las ventajas operativas y analíticas de DLLME han hecho que varios autores hayan descrito ya su aplicabilidad a la determinación de contaminantes orgánicos en muestras acuosas [1]. DLLME se basa en la adición a unos pocos mililitros de muestra acuosa de unos pocos microlitros de una mezcla de disolvente extractante y dispersivo. El disolvente extractante suele ser un disolvente clorado de mayor densidad que el agua. El disolvente dispersivo es un disolvente polar y soluble en agua, que facilita el contacto entre el disolvente extractante y la muestra favoreciendo de esta manera la extracción. La extracción se realiza en unos pocos minutos y el disolvente extractante se separa de la emulsión generada mediante centrifugación. Los principales inconvenientes de DLLME son el uso de disolventes clorados, aunque en volúmenes del orden de unos 100 µL y la recolección de dicho disolvente tras la extracción, ya que queda como una pequeña gota en el fondo del tubo de centrífuga donde se ha realizado la extracción. Para poder aprovechar las ventajas de DLLME, y minimizar sus inconvenientes, se ha propuesto el uso de disolventes extractantes con menor densidad que el agua, con lo que quedarán en la parte superior de la disolución tras la centrifugación, y con puntos de fusión de entre 10-30ºC con lo que podrán solidificarse fácilmente en un baño de hielo, facilitándose así u recolección tras la extracción, y volver a estado líquido a temperatura ambiente. Esta variante recibe el nombre de microextracción líquido-líquido dispersiva basada en la solidificación de la gota flotante (*dispersive liquid-liquid microextraction based on the solidification of the floating organic drop,* DLLME-SFO).

En este trabajo se presenta el desarrollo de un método DLLME-SFO para la determinación de dieciséis contaminantes emergentes y prioritarios entre los que se encuentran cinco compuestos fenólicos (nonilfenol, bisfenol A y metil, etil y propilparabenos), cuatro estrógenos (17α-etinilestradiol, 17β-estradiol, estriol y estrona), seis compuestos perfluorados (sulfonato deperfluorooctano y cinco ácidos carboxílicos perfluorados), y el retardante de llama hexabromociclododecano. El posterior análisis se llevó a cabo mediante cromatografía líquida acoplada a espectrometría de masas. Se optimizaron diversas variables como el tipo y volumen de los disolventes extractante y dispersivo, tiempo de extracción, influencia de la fuerza iónica y pH de la muestra. Las condiciones optimas resultaron ser acidificación de 10 ml de la muestra acuosa a pH = 1, empleo de 80 µL de 1-undecanol como disolvente extractante y 500 µL de metanol como disolvente dispersivo y tiempo de extracción de 5 minutos. El método se validó en términos de recuperación, precisión, linealidad, y límites de detección y cuantificación. La precisión del método, expresada como desviación estándar relativa, se encontró en el rango del 1-16%. Los límites de detección del método estuvieron comprendidos entre 0.001-1.126 µg L^{-1} en agua superficial. La aplicabilidad del método se evaluó mediante análisis de tres muestras de agua superficial. En dichas muestras se detectaron los cuatro compuestos perfluorados analizados de mayor longitud de cadena alquílica.

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PESTICIDES IDENTIFICATION AND QUANTIFICATION IN *GINKGO BILOBA* NUTRACEUTICAL PRODUCTS BY GC-QqQ-MS/MS

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Nutraceutical products based on botanical ingredients are often perceived as being safe because of their "natural" origin, traditional use, historical evidence of safety, and over-the counter availability. Despite the impression that these botanicals or herbals are cultivated in the wild, many of these products are farmed using conventional agricultural practices, including pesticide application to control insects, molds and other pests. Regulation EC 396/2005 [1] defines maximum residues limits (MRLs) for pesticides in every food and feeding but only in the raw material, so products like nutraceuticals are not included. Consequently, analyses of pesticide residues not only in raw agricultural crops, but also in nutraceutical products are one of the principal preventive measures employed to ensure public health and safety. Therefore, it is necessary to develop a method for the rapid and sensitive determination of multiple pesticide residues.

In this work, gas chromatography coupled to triple quadrupole mass spectrometry (GC-QqQ-MS/MS) was used for pesticides identification and quantification in *Ginkgo Biloba* nutraceutical products. *Ginkgo biloba* leaf extract is well recognized as ingredient in various pharmaceutical and nutraceutical products all over the world due to the many types of bioactive constituents that contains. Flavonol glycosides, terpenetrilactones, gingkolides and proanthocyanidins are included in the extract and they contribute to improve cognition and memory [2]. For the extraction, a QuEChERS like procedure was applied followed by a clean-up step with a mixture of sorbents (primary secondary amine, graphitized black carbon, C_{18} and zirconium oxide) due to the matrix complexity, decreasing matrix effect.

The method was validated for more than 150 pesticides, and recoveries were evaluated at 10, 50 and 100 μg kg^{-1}, ranging between 73 and 107 %. The relative standard deviation for intra-day precision was always lower than 20 % and for inter-day precision lower than 25 %. Limits of detection ranged from 0.1 to 10.0 μg kg^{-1}, while limits of quantification ranged from 0.5 to 20.0 μg kg^{-1}. The validated method was successfully applied to the analysis of *Ginkgo Biloba* nutraceutical samples. Nine samples were analysed and four of them contained pesticide residues (deltamethrin, kresoxym-methyl, myclobutanyl and procymidone). The highest concentration was found for deltamethrin at 10.1 μg kg^{-1}.

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CARACTERIZACIÓN SUBMICROMÉTRICA DE MATERIALES LAMINADOS MEDIANTE IONIZACIÓN LÁSER DE FEMTOSEGUNDOS Y ESPECTROMETRÍA DE MASAS DE TIEMPO DE VUELO EN CONFIGURACIÓN EXCITACIÓN/COLECCIÓN COLINEAR

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La industria de los materiales con aplicaciones tecnológicas emplea técnicas cada vez más finas a la hora de preparar dispositivos de mayor eficiencia y menores tamaños cuyas propiedades dependen en gran medida de una correcta distribución de sus componentes químicos. Lo que conlleva necesariamente un ajuste de las técnicas empleadas para su caracterización con el objeto de responder a estas necesidades de la industria. En este sentido, los análisis en profundidad constituyen una herramienta necesaria para delimitar con precisión las distintas láminas que pueden conformar estos materiales y que juegan un papel fundamental en su funcionamiento.

Algunas de las propiedades que se piden a las técnicas analíticas apropiadas para este tipo de caracterización incluyen la no destrucción de las muestras, la rapidez y la fiabilidad de los resultados. La espectrometría de masas con ionización láser (LIMS) es una de las técnicas más prometedoras que permiten llevar a cabo estudios directos sobre el sólido provocando un mínimo daño al mismo. Acoplada a un analizador de tiempo de vuelo (TOF), LIMS permite la monitorización simultánea de especies, tanto atómicas como moleculares presentes en la muestra, y buena precisión para delimitar capas, característica que se espera mejorar gracias al uso de láseres de pulso ultracorto ya que la tasa de ablación media debe ser menor que en los habituales láseres pulsados de nanosegundos, aumentando la resolución de lo análisis. Además, los menores efectos térmicos asociados al uso de estos láseres, ayudarían en gran medida a evitar la reposición de material desbastado sobre la superficie analizada, permitiendo estudios más fieles de las interfaces entre estratos.

La presenta comunicación presenta las primeras aplicaciones de la caracterización de materiales laminados de interés tecnológico mediante el uso de un láser de pulsos ultracortos que se enfoca mediante un sistema óptico completamente reflexivo (cassegrain), que actúa a su vez como lente iónica. De este modo, la geometría de excitación y colección es colinear, lo que mejora la resolución espacial significativamente (< 5 micrómetros).

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EVALUATION OF DIFFERENT HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY, MIXED-MODE AND OTHER AQUEOUS NORMAL-PHASE APPROACHES FOR THE HPLC/MS-BASED DETERMINATION OF CHALLENGING POLAR PESTICIDES

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The aim of the study was to evaluate the performance of different chromatographic approaches for the liquid chromatography/mass spectrometry (LC-MS(/MS)) determination of 24 selected highly polar pesticides and residues, namely aminomethylphosphonic acid, amitrol, chlormequat, cyromazine, daminozide, diethanolamine, difenzoquat, diquat, ethephon, glufosinate-ammonium, glufosinate-N-acetyl, glyphosate, 2-imidazolidinethione, maleic hydrazide, mepiquat, 3-(methylphosphinico)propionic acid, morpholine, nereistoxin, paraquat, phosphorous acid, propylene thiourea, streptomycin, triethanolamine, and trimethylsulfonium iodide. The studied compounds, which are in most cases unsuitable for conventional LC-MS(/MS) multiresidue methods, were tested on nine different chromatographic columns, including two different hydrophilic interaction liquid chromatography (HILIC) columns, two mixed-mode columns (Sielc Technologies Obelisc N and Obelisc R), three normal phase columns operated in HILIC-mode (bare silica and two silica-based chemically bonded columns (Spherisorb cyano and amino)), and two standard reversed-phase C_{18} columns. Different sets of chromatographic parameters in positive (for 17 analytes) and negative ion mass spectrometric detection modes (for nine analytes) were examined. In order to compare and contrast the different approaches, a semi-quantitative classification was proposed, calculated as the percentage of an empirical performance value, which consisted of three main features: (i) the capacity factor *(k)* to measure analyte separation from the void, (ii) the relative response factor (sensitivity) and (iii) peak shape based on analytes' peak width. No single method was able to provide appropriate detection of all the 24 studied species in a single run. The best suited approach for the compounds ionized in positive mode was that based on a UHPLC HILIC column with 1.8 µm particle size, which provided appropriate results for 22 out of the 24 species tested. In contrast, the detection of glyphosate and aminomethylphosphonic acid could only be achieved with a zwitterionic-type mixed-mode column (Obelisc N), although this column proved to be suitable only for the pesticides detected in negative ion mode. Finally, the selected approach (UHPLC HILIC) was found to be useful for the determination of pesticides in orange matrix using HILIC-QTOFMS, with limits of quantitation in the range from 0.02 to 0.56 mg Kg^{-1}.

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OPTIMIZATION OF A DERIVATIZATION REACTION FOR THE DETERMINATION OF INTERMEDIATE PRODUCTS OF MAILLARD REACTION

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Glyoxal (GL) and methylglyoxal (MGL) are considered as glucose degradation and oxidation products and are known as reactive carbonyl species [1]. Both are the two major α-dicarbonyl compounds found in human body and have attracted much attention because of their possible clinical significance in chronic and age-related diseases. Thus, diabetic patients have higher levels of GL and MGL in their plasma than healthy people [2, 3].

GL and MGL are frequently detected in fermented foods and beverages due to microbial activity. For example, they have been found in different brands of beer, wine, vinegar and other beverages, such as tea, coffee and some sodas. Furthermore, they have been detected in certain fermented products such as soybean paste, yogurt and cheese, and other products such as bread, milk, high-fructose corn syrup, butter and edible oils [4-7]. Their role in food quality is mainly related to sensorial characteristics, particularly in fermented food such as beer and wine [8].

It is well known that α-dicarbonyl compounds do not present adequate photometric or fluorescent properties for their analysis, and then it is necessary a prior derivatization step. The spectrofluorimetric behaviour of two new different possible derivatizing agents with similar structure (2,3-diaminopyridine and 3,4-diaminopyridine) was studied. The stability and the influence of pH were studied. After that, the reactivity of each agent with glyoxal (as representative of α-dicarbonyl compounds) was tested at different pH values, with and without heating and at different temperatures. When 2,3-DAP was used as derivatizing reagent, a quenching of fluorescence could be observed, while an increase was observed for 3,4-DAP at acidic pH. Some experiences were performed to check the reactivity with other α-dicarbonyl compounds (methylglyoxal, diacetyl, 2,3-pentanedione and phenylglyoxal) and 3,4-DAP. Only the corresponding derivative of methylglyoxal was formed, but the fluorescence intensity is minor.

The optimization of physical and chemical variables in the derivatization reaction was done and the conditions selected were: 120 minutes at 90ºC, pH 2 (chloroacetic acid/sodium chloroacetate buffer) and a molar ratio 3:1 (derivatizing agent:analyte). The emission fluorescence was recorded at 371 nm, exciting at 307 nm. The calibration curve was stablished between 0.1 and 1.5 mg L^{-1} and the limit of detection was 9.7 μg L^{-1}.

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RAPID AND ECO-FRIENDLY DETERMINATION OF GLYOXAL IN DIFFERENT MATRICES. DEVELOPMENT OF A NEW SIMULTANEOUS DERIVATIZATION-DLLME METHODOLOGY, FOLLOWED BY HPLC-FD

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Glyoxal is the smallest α-dicarbonyl compound, and it is often detected in fermented foods and beverages due to microbial activity. It appears, for example, in wine, beer and honey. It is an intermediate of Maillard reaction and influences in quality parameters of food, as odor or flavor. Although the human body has effective mechanism to cope with glyoxal, the contribution of α-dicarbonyl compounds in the diet can have negative effects on health [1].

The structure of these compounds makes them difficult to determine by selective methods such as molecular fluorescence. Derivatization, a process that chemically modifies the analyte to produce a derivative with new properties, facilitates or allows their analysis. In this research, we propose a new method of simultaneous derivatization and dispersive liquid-liquid microextraction (DLLME) of glyoxal, which is then determined by liquid chromatography with fluorescence detection.

Previous research has been performed and 3,4-diaminopyridine was found as a good derivatizing agent for glyoxal, and a novel HPLC-FD method for its determination in wine was proposed [2]. However, the aim of this research was to improve this derivatization reaction and apply it to other matrices. Due to the high reactivity of glyoxal, it was found that the derivatization reaction with 3,4-diaminopyridine was produced simultaneously with the DLLME, using 1-buthanol and dichloromethane as extractant mixture, no needing heating, microwave or ultrasound assistance. Total preparation of sample takes less than 20 minutes. Once obtained the derivative, the HPLC-FD method [2] has been applied.

The new developed method has been applied to different matrices with satisfactory results.

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HEADSPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY FOR THE ANALYSIS OF ORGANOTIN COMPOUNDS IN SEDIMENT SAMPLES

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One very important organic pollutant in aquatic sediments is tributyltin (TBT) that is considered together with its two metabolites dibutyltin (DBT) and monobutyltin (MBT) as “the most toxic compound ever deliberately introduced into the marine environment” [1]. During the last century, organotin compounds (OTCs) have been widely used as accelerators, polyvinyl chloride (PVC) stabilizers, biocides, coatings, wood preservatives and antifouling paints etc. As a result, they were introduced into the environment at large quantities [2]. In the environment, TBT, and its two degradation products are preferably associated with fine particles and can therefore usually be found in high concentrations in harbours and estuaries. Therefore, knowledge of sediment concentrations is of great interest. A pre-concentration step typically is required because of the low concentration of the OTCs expected in environmental samples. Solid phase microextraction (SPME) is a simple, fast and solvent-free technique, which combines extraction, concentration and sample introduction into the GC injector using one single device. Gas chromatography–tandem mass spectrometry (GC–MS/MS) operated in selected reaction monitoring (SRM) detection mode can provide high confidence in the identification of target analytes in complex matrices and low detection limits [3].

The aim of this work is to develop a new HS-SPME-GC-MS/MS method for the analysis of organotin compounds in sediment samples. Different extraction and determination conditions, such as solvent volume, amount of sample or extraction time were studied. Finally, an appropriate amount of surrogate standard and of a solvent mixture of acetic acid:methanol:water (1:1:1) were added to 1.5 g of sediment, sonicated for 30 minutes in an ultrasonic bath and centrifuged to obtain a liquid/solid phase separation. The extraction procedure was repeated twice, and the two extraction solutions were combined prior to derivatization.

For HS-SPME, 100 µL of extraction solution and a fixed volume of internal standard were derivatized with 1 mL of 1% (w/v) $NaBPr_4$ solution. Determination was carried out with a Thermo-Finnigan (Waltham, MA, USA) Trace GC chromatograph equipped with a Triplus autosampler, PTV injector and coupled to a triple quadrupole mass spectrometer (TSQ Quantum XLS).

Analytical performance characteristics such as linearity, detection and quantification limits were determined, obtaining satisfactory results. The developed analytical method was validated using NRCPACS-3 (Harbour sediment). Analytical recoveries of DBT and TBT were 107% and 99%, respectively and the method was successfully applied for determination of butyltin compounds in sediment samples.

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DETERMINACIÓN DE POSIBLES BIOMARCADORES DE CÁNCER DE PULMÓN MEDIANTE HS-PTV-GC-MS

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En el trabajo realizado se pone a punto un método analítico basado en *HS-PTV-GC-MS* para la determinación de 2-butanona, tetrahidrofurano, 2-pentanona, pirrol, acetato de butilo, 2-heptanona y 2-etil-1-hexanol en muestras de orina. Este tipo de compuestos se han propuesto como posibles biomarcadores metabólicos de cáncer de pulmón [1-5].

El uso de la generación de espacio de cabeza evita la presencia de interferencias de compuestos no volátiles presentes en la matriz, lo que hace de esta técnica una excelente opción para matrices complejas como son las muestras de orina. Las variables que afectan al espacio de cabeza como volumen de muestra, temperatura y tiempo de equilibrio y adición de sales han sido evaluadas. Además, se ha optimizado el modo de inyección *solvent vent (temperatura, tiempo y flujo de purga y tiempo de inyección)* utilizado en el inyector de temperatura programada, así como la separación cromatográfica (temperatura y tiempo inicial y rampas cromatográficas) y las variables del espectrómetro de masas en el modo de adquisición de datos *SIM/Scan* combinado (tiempo de registro de cada ion y velocidad de barrido).

En las condiciones experimentales optimizadas, se realizaron los calibrados de todos los compuestos, mostrando un comportamiento lineal y sin fallo de ajuste.

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EVALUATION OF THE MAIN ADVANTAGES AND LIMITATIONS OF ACCURATE-MASS SCREENING METHODS FOR TESTING ORGANIC CONTAMINANTS IN FOOD WITH UHPLC-HRMS

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The feasibility of an accurate-mass multi-residue screening method using liquid chromatography high-resolution mass spectrometry has been examined for the determination of over 625 multiclass food contaminants (426 pesticides, 117 veterinary drugs, 42 food-packaging contaminants, 21 mycotoxins, 10 perfluorinated compounds, 9 nitrosamines and 5 sweeteners). The proposed approach was based on the use of ultra-high performance liquid chromatography electrospray (quadrupole) time-of-flight mass spectrometry, operating in positive and/or negative ionization mode, and with data acquired in full-scan mode. Compelling aspects such as chromatographic separation and the selectivity and confirmation capability provided by HRMS with different acquisition modes (full-scan or full-scan combined with collision induced dissociation (CID) with no precursor ion isolation), along with caveats such as sensitivity or automated data processing are examined and discussed. The identification of compounds was carried out using retention time matching and accurate mass measurements of the targeted ions for each analyte (mainly (de)protonated molecules). Compounds with the same nominal mass (isobaric species) were very frequent due to the wide number of compounds used. 76% of database compounds were involved in isobaric groups and 99% of isobaric species were distinguished by retention time, resolving power, isotopic profile or fragment ions. Only three pairs could not be resolved using the proposed methodology. In-source CID fragmentation was evaluated in depth, varying fragmentor voltages from 160 to 250 V. The results obtained in terms of fragmentation information were not as thorough as those obtained using CID MS/MS experiments without precursor ion isolation (*all ion mode*). This acquisition mode is definitely the best suited for this type of large-scale screening method instead of classic product ion scan, as provides excellent fragmentation information for confirmatory purposes for an unlimited number of compounds. The main weaknesses of the approach are basically the relatively low sensitivity for selected compounds which does not map well against electrospray ionization and also quantitation issues such as those produced by signal suppression effects due to either matrix effects from coeluting matrix components or from coeluting analytes present in the standards solutions which often occur as they contain hundreds of the analytes included in the database.

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APLICACIÓN DE LA MICROEXTRACCIÓN USAEME-SFOD ACOPLADA CON HPLC PARA LA DETERMINACIÓN DE FRAGANCIAS POTENCIALMENTE ALÉRGENAS EN AGUA Y COSMÉTICOS

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Las fragancias son componentes en muchas formulaciones presentes en nuestra vida diaria (perfumes, cosméticos, productos de cuidado personal, limpieza, etc…); y aunque son generalmente inocuas, en ocasiones pueden causar irritación de la piel o reacciones alérgicas. En la actualidad, son 24 compuestos químicamente bien definidos y dos extractos de musgo naturales los que forman la lista de sustancias reconocidas como potencialmente alérgenas relacionadas con las fragancias (PAS, *potentially allergenic substances*). La Unión Europea requiere la declaración de la presencia de estas sustancias en las etiquetas cuando su concentración es mayor del 0.001% en productos que no se aclaran y mayor del 0.01% en productos que se aclaran [1]. Por otro lado, el contacto con aguas contaminadas debe ser considerado también una ruta importante de exposición a estos compuestos.

Hasta la fecha los métodos para la determinación de PAS utilizan principalmente cromatografía de gases con detectores de masas, habiéndose acoplado a diferentes procesos de pre-tratamiento de muestras con el objetivo de concentrar y separar los analitos de otros componentes. Otras técnicas, entre ellas la cromatografía de líquidos de alta resolución (HPLC), han sido utilizadas, pero sin pre-tratamiento [2]. El objetivo de este trabajo es el desarrollo de un método simple, fiable y sencillo para la determinación de 18 PAS aplicando la microextracción en fase líquida (LPME) como técnica de pre-tratamiento antes de la determinación por HPLC. La LPME es una versión miniaturizada de la clásica extracción líquido/líquido. En ella únicamente unos microlitros de disolvente inmiscible en agua (extractante) se ponen en contacto con la fase acuosa (muestra) para extraer los compuestos de interés. Desde su introducción, se han desarrollado diferentes versiones de LPME. En el método puesto a punto se utiliza la microextracción mediante emulsificación asistida por ultrasonidos seguida por la solidificación de la gota orgánica (USAEME- SFOD). En esta variante se aplican ultrasonidos para formar una emulsión del extractante en la fase acuosa, acelerando así la transferencia de los analitos. Tras ello, se centrifuga la muestra obteniéndose una única gota de extractante, que gracias a su temperatura de fusión, puede ser congelado y separado mediante una espátula. Tras su descongelación, el extractante es disuelto en metanol para su compatibilización con la fase móvil utilizada en el HPLC y directamente inyectado en el equipo

Bajo las condiciones óptimas, el método mostró una buena linealidad en los rangos seleccionados (R^2 de 0.948 a 0.999). Los límites de detección de los analitos variaron de 0.001 a 0,154 µg mL^{-1} y los factores de enriquecimiento de 9 a 237. La precisión del método fue evaluada a dos niveles obteniendo buenos resultados (DER, 3,3-14,4 %). Los ensayos de recuperación realizados en aguas de baño de bebé y de colonia mostraron una exactitud aceptable. Finalmente, el método desarrollado fue aplicado a diferentes muestras acuosas y cosméticas comerciales, pudiéndose llegar a determinar los analitos en los niveles regulados por la ley.

Agradecimientos
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CARACTERIZACIÓN ESPACIO-TEMPORAL DE LA EMISIÓN DE BANDAS MOLECULARES EN PLASMAS DE COMPUESTOS ORGÁNICOS INDUCIDOS POR LÁSER DE FEMTO Y NANOSEGUNDOS

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La espectroscopía de plasmas producidos por láser (LIBS) es una de las técnicas analíticas más poderosas que pueden usarse para obtener información elemental directa de muestras sólidas debido a su versatilidad, que le ha permitido ser aplicada al análisis directo de fondos marinos o a la superficie de Marte. A pesar de su uso exclusivo como técnica de análisis elemental, es posible extraer información molecular de especies di- o triatómicas que resultan de elevado interés para conocer la cinética de descomposición de ciertos compuestos (explosivos, por ejemplo) o incluso para obtener una información adicional que permite diferenciar isómeros estructurales en base a las relaciones de intensidad de sus emisiones moleculares (C2, CN, OH, NH, …).

Sin embargo, la mayoría de dichos análisis se llevan a cabo a presión atmosférica, a la cual la emisión de estas especies puede provenir también de otras fuentes como el propio aire, que también se ioniza y pasa a formar parte de la composición del plasma estudiado, lo que genera dudas respecto a las fuentes de origen de las bandas estudiadas. Es por esto que estudios fundamentales de plasmas formados bajo distintas composiciones del ambiente en el que se expanden pueden proporcionar respuestas a estas preguntas, es decir, ¿hasta qué punto el medio condiciona el espectro registrado?

En este trabajo, se presenta un estudio mediante espectrometría óptica de plasmas de compuestos orgánicos que se expanden bajo distintas condiciones de vacío, desde presión atmosférica hasta presiones de 10^{-3} milibares con el objetivo de demostrar la variación en la composición según cada situación. Además, el estudio se completa añadiendo resolución espacial, que permite seccionar finamente el plasma para atender a la distribución puntual de las especies dentro del mismo a fin de conocer la posible existencia errores derivados de la habitual colección completa del plasma y, por último, una comparativa entre los resultados obtenidos empleando para la excitación láseres pulsados de nanosegundos y de femtosegundos, ya que los distintos mecanismos seguidos en ambos casos provocan diferencias notables en las características de los plasma tales como su morfología y la pluma formada.

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ACCURACY EVALUATION OF ULTRASOUND PROBE SONICATION AND MICROWAVE-ASSISTED EXTRACTION SYSTEMS FOR RAPID SINGLE EXTRACTION OF METALS IN SOILS

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The accumulation of metals in soils and sediments causes a potential risk to human health due to the transfer of these elements to other environmental compartments. However, metals are present in soils under different chemical forms or types of binding, so the total metal content is a poor indicator of their bioavailability, mobility or toxicity, since these properties basically depend on the chemical association of the different soil components. Therefore, the environmental impact of metalliferous soils is better assessed on the basis of the environmental accessibility of metals, i.e. the bioavailable forms for plants.

Harmonization of single extraction tests for the determination of extractable metals in soils has become of paramount importance, since they are used for discrimination of different solid-phase associations of metals in soils and sediments. Nevertheless, in the conventional extraction schemes, long extraction times by mechanical shaking and large quantities of samples and reagents are typically employed for the determination of the extractable metal content [1, 2].

In the present work, ultrasonic probe sonication (UPS) and microwave-assisted extraction (MAE) have been evaluated as alternatives to the conventional Standards, Measurements and Testing program (SM&T) procedures for single extraction of metals in soils, in order to reduce the extraction time and the consumption of samples and extracting agents. Optimization studies were carried out on the certified reference materials (CRMs) BCR 483 (Sewage sludge amended soil) and BCR 700 (Organic rich soil) for accuracy evaluation of the proposed methods. Extractable concentrations of Cd, Cr, Cu, Ni and Zn using 0.01 mol L^{-1} calcium chloride ($CaCl_2$) (in BCR 483), and also of Pb with 0.43 mol L^{-1} acetic acid (CH_3COOH) and 0.05 mol L^{-1} ethylenediaminetetraacetic acid (EDTA) at pH 7.0 (in both CRMs) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

The extraction time for the $CaCl_2$ extraction method was reduced from 3 h conventional procedure) to 2 min by UPS or 5 min at 50 ºC by MAE. Similarly, the time required for acetic acid extraction was also reduced from 16 h to 15 min by UPS or 15 min at 120 ºC by MAE. Finally, the extraction time with EDTA was reduced from 1 h (conventional procedure) to 2 min by UPS or 5 min at 50 ºC by MAE [3]. For these extraction conditions proposed, quantitative extraction recoveries were obtained for most elements studied. In addition, the amount of sample and extracting solutions was drastically reduced, maintaining the sample weight/extractant volume ratio.

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DEVELOPMENT AND CHARACTERIZATION OF CARBON BASED ELECTRODES FROM PYROLYZED PAPER FOR BIOSENSING APPLICATIONS

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The current communication details the study of electrochemical behavior of new carbon electrodes based on pyrolysis of different paper sources to be used in biosensor applications. The resistivity of the pyrolyzed papers was initially used as screening parameters to select three paper samples (imaging card paper, multipurpose printing paper, and 3MM chromatography paper) and assemble working electrodes that were further characterized by a combination of microscopy, electrochemistry, and spectroscopy. Although slight differences in performance were observed, all carbon substrates fabricated from pyrolysis of paper allowed the development of competitive biosensors for uric acid. The resulting electrodes feature low resistivity, large surface area, and uniform thickness. More importantly, the electrodes can be used for the development of biosensors by simply immersing them in a solution containing a selected enzyme. Several studies related to the selection of the substrate, the characterization (microscopy and electrochemical) as well as their potential for the development of an electrochemical biosensor for uric acid were performed. The obtained results demonstrate that standard varieties of commercially available paper (and more so 3MM chromatography paper) can be fashioned into a simple, inexpensive carbon electrode for sensing applications.

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SÍNTESIS DE NUEVOS MATERIALES PARA LA DETERMINACIÓN DE MERCURIO Y METILMERCURIO EN MUESTRAS MEDIOAMBIENTALES

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Los niveles de mercurio en el medio ambiente debido a la actividad humana han aumentado considerablemente en los últimos años debido a procesos como la utilización de combustibles fósiles, la minería o la incineración de residuos. El mercurio tiene gran facilidad para formar especies orgánicas en los medios naturales, siendo éstas un mayor peligro para la salud debido a su elevada toxicidad. El metilmercurio es generado en los medios naturales por acción microbiana o por el metabolismo de los organismos marinos, con una mayor acumulación en los animales de gran tamaño, como el atún o el pez espada. La Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) ha desaconsejado el consumo de atún y pez espada por parte de embarazadas y niños menores de tres años [1]. De entre todos los métodos de extracción de metilmercurio, la extracción en fase sólida empleando polímeros de impronta molecular (MIP-SPE) como sorbentes altamente selectivos permite analizar bajas concentraciones de la especie de interés y la eliminación de interferentes [2]. Son recientes los trabajos en los que los quantum dots (QDs) se emplean como sensores ópticos. El uso más común de los QDs como quimiosensor implica el hecho de que la emisión de los QDs es muy sensible a cambios en su superficie. La funcionalización de los QDs mediante polímeros de impresión molecular (QDs-MIP) ha resultado también como un método adecuado para la determinación de numerosos compuestos [3-4]

En este trabajo se han desarrollado un MIP para la determinación de MeHg+ en productos pesqueros y de Hg inorgánico en muestras de aguas residuales, empleando fenobarbital como agente complejante, ácido metacrílico como monómero y etilenglicoldimetacrilato como entrecruzante. También se han funcionalizado quantum dots de ZnS dopados con Mn para la determinación de Hg por fluorimetría.

El fenobarbital empleado como ligando para la síntesis de los polímeros de impronta molecular y los quantum dots funcionalizados ha sido estudiado con anterioridad como método de detoxificación en casos de envenenamiento con mercurio [5] y para la síntesis de MIPs utilizados para la propia determinación del fenobarbital [6].

El MIP sintetizado ha sido caracterizado mediante análisis elemental, espectroscopía de infrarrojo, fluorescencia de rayos X y microscopía electrónica de barrido. La estructura y tamaño de los quantum dots ha sido determinada mediante difracción de rayos X. La determinación de Hg y MeHg tras la MIP-SPE se llevó a cabo mediate técnicas espectroscópicas acopladas a sistemas de generación de vapor frío (Absorción Atómica de Fuente Contínua de Alta Resolución, HRCSAAS, e ICP-OES), o mediante un espectrofotómetro de fluorescencia en el caso de la utilización de los quantum dots. Se han obtenido extracciones cuantitativas de Hg y MeHg a un pH óptimizado de 8.0 y mediante una extracción con tolueno y HCl 6M se ha logrado determinar la concentración de metilmercurio en materiales de referencia para el análisis de productos pesqueros (BCR-463 y TORT-2) con buena precisión y exactitud. Por otra parte, se ha comenzado con la optimización del método fluorimétrico mediante QDs recubiertos de polímero para la determinación de mercurio.

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DEVELOPMENT OF AN IMPROVED METHODOLOGY FOR THE DETERMINATION OF BISPHENOL A BASED ON LIQUID CHROMATOGRAPHY LINEAR ION TRAP ORBITRAP MASS SPECTROMETRY

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Bisphenol A (BPA) is a well-known endocrine disrupting compound (EDC) used as a monomer for the production of epoxy resins, phenol resins, polycarbonates, polyesters and lacquer coatings, and even food cans. Because of its toxicity at low concentrations, the analysis of BPA in environmental, food, industrial and biological studies is required.

In order to achieve these low levels, the choice of an adequate instrumental technique is critical. BPA has been usually determined by LC-MS/MS using a triple quadrupole analyzer (QqQ) because of its selectivity and sensitivity in MRM mode. However, QqQ shows low sensitivity in full-scan mode which limits its capabilities for screening applications [1]. Liquid chromatography high resolution mass spectrometry (LC-HR-MS) provides high mass accuracy and higher sensitivity both in full scan mode and MRM mode. Consequently, better results can be obtained in the analysis of target and untarget compounds in complex matrices. The linear ion trap Orbitrap (LTQ Orbitrap) carries out single-stage mass analysis, two-stage mass analysis (MS/MS) and multi-stage mass analysis (MS^n) [2]. As far as we know, few previous works based on the BPA determination by this instrumental methodology can be found in the literature; therefore, its application in the analysis of this pollutant could be interesting.

In this case, three acquisition modes of LC-HR-MS (*full scan, SIM and MS/MS*) using a LTQ-Orbitrap (Thermo Fisher Sci.) were tested. For MS/MS determination collision energy was optimized. This acquisition mode seemed to be the most adequate for the analysis of BPA because of its sensitivity. Consequently, a new and improved instrumental methodology for the determination of bisphenol A by LC-HR-MS/MS was optimized and validated.

Some variables of the ESI ionization source such as nebulizer gas and capillary-source distance) were assayed. Furthermore, LC parameters like mobile phase modifiers (0.05% ammonium) and flow (0.25 ml min^{-1}) were also studied.

The developed LC-HR-MS/MS methodology has demonstrated a good suitability for BPA analysis. Linearity was showed from IDL to 1000 μgL^{-1} with good fits (r^2=0.9998). Repeatability and intermediate precision were <8%. The IDL and IQL obtained for BPA were 0.6 and 1.5 ng respectively.

These analytical parameters were compared with the obtained using a LC-QqQ-MS/MS methodology [3]. Although similar precision were obtained with LTQ-Orbitrap, lowest IDL and IQL were achieved, which allows the identification and quantitation of this pollutant at ultratace levels.

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MICROFLUIDIC PAPER BASED ANALYTICAL DEVICE FOR NITRITE DETERMINATION WITH TETRAZINE

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We present a new chemistry to determine nitrites implemented in a paper-based microfluidic analytical device (µPAD). The design of the µPAD shown in Figure 1. The device is fabricated in cellulose paper with a sample reception area and three replicates detection areas with recognition chemistry immobilized by adsorption. The method involves the use of nitrites in acid medium reaction to generated nitrous acid, which produces the tetrazine oxidation that change from colourless to pink the detection zone. The µPAD is imaged with a digital camera and the S coordinate (saturation) of HSV colour space is used as analytical parameter for quantitative analysis of nitrites. A cellulosic paper membrane (1240 from Filter-Lab) was the optimum substrate for reagent immobilization, colour change in the detection area, capillary action and evaporation of the sample. Other parameters such as concentration, layers and volume of tetrazine, pH, sample/standard volume, and colour development time were studied. The detection limit for this method is 2.05 µM nitrite. To estimate the selectivity of the method an interference study of common ions in water samples was performed. The procedure was applied to natural water and compared with reference procedures.

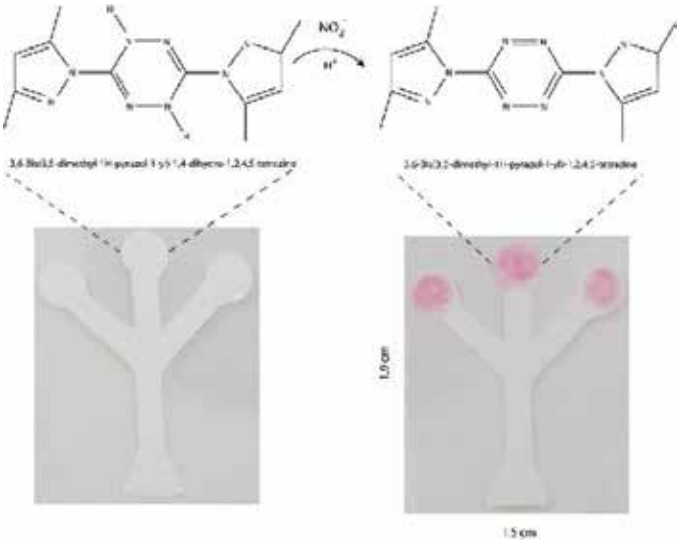

Figure 1

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DETERMINACIÓN ENZIMÁTICA DE FOSFOLíPIDOS QUE CONTIENEN COLINA MEDIANTE LA MEDIDA DE VARIACIÓN DE FLUORESCENCIA DE LA COLINA OXIDASA

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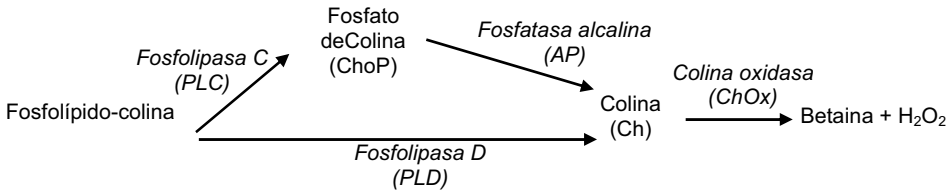
Nuestro grupo de investigación tiene una amplia experiencia en el uso de los cambios de la fluorescencia de las flavoenzimas (tanto intrínseca como extrínseca) durante la reacción enzimática para la determinación de numerosos sustratos (glucosa, colina, colesterol,…)[1]. La metodología tiene muchas ventajas, entre las que destacan que no hay que añadir reactivos para la detección de la reacción y que la enzima se regenera lo que ha permitido desarrollar sensores para los sustratos mencionados.
Los fosfolípidos son un grupo de compuestos con mucho interés biológico, algunos de los cuales tienen colina en su estructura. Dicha colina puede liberarse mediante la acción de una enzima lipasa a través de dos posibles rutas dependiendo de la lipasa:



Los fosfolípidos que contienen colina pueden ser determinados haciendo reaccionar con ChOx la colina liberada en la etapa de hidrólisis y midiendo la variación de fluorescencia de la enzima durante la reacción.Esto puede hacerse de dos maneras:
a.- **en dos pasos,** de forma que primero se realiza la hidrólisis durante un determinado tiempo y posteriormente la reacción de oxidación con ChOx. Esta metodología ha sido desarrollada con éxito por el grupo de investigación. Se ha estudiado tanto el tiempo de hidrólisis, como disolvente adecuado, las condiciones de pH y concentración de las diferentes enzimas implicadas, permitiendo la determinación de fosfatidilcolina (PC) y fosfato de colina (ChoP) [2],evaluando tanto las posibilidades de la fluorescencia intrínseca como extrínseca.
b.- **en un paso**, en el que ambos procesos enzimáticos ocurren simultáneamente. Mediante esta opción se ha podido abordar la determinación de PC así como la determinación conjunta de ChoP y Ch en leche infantil en un mismo ensayo. Asímismo se ha conseguido la inmovilización en un soporte de bisacrilamida de las enzimas AP y ChOx permitiendo el desarrollo de una lámina sensora.

Recientemente se ha desarrollado esta metodología para la determinación de un grupo de fosfolípidos de características semejantes al Factor Activador de Plaquetas (FAP). Se han estudiado las mejores condiciones de hidrólisis, pH, concentración de enzimas,… obteniendo resultados satisfactorios.

Agradecimientos
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UTILIZACIÓN DE LA ESPECTROSCOPÍA DE ALTA RESOLUCIÓN CON FUENTE CONTINUA (HRCSAAS) PARA EL ANÁLISIS DE AZUFRE Y FÓSFORO EN FERTILIZANTES

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El azufre y el fósforo son dos de los elementos más importantes para los seres vivos y más abundantes en la corteza terrestre, pero su determinación por técnicas atómicas o de masas presenta ciertas dificultades, por lo que se suelen utilizar los métodos tradicionales como turbidimetrías, colorimetrías o valoraciones.

La espectroscopía de alta resolución con fuente continua (HRCSAAS) posibilita la determinación multielemental utilizando una única lámpara de xenón que actúa como una fuente continua. Además permite el uso, no sólo de las líneas atómicas para cada elemento, sino también de las bandas moleculares, así como hacer correcciones espectrales automáticas durante la medida.

En el presente trabajo tanto para el fósforo como para el azufre se optimizaron las condiciones de la llama (altura sobre el mechero, flujo de aire, flujo de acetileno), se seleccionaron las líneas de medida y los píxeles analíticos y de corrección. Para el análisis del fósforo se evaluaron algunas de las bandas rotacionales de PO, de las que finalmente se escogió la situada a 246,40 nm. En el caso del azufre se emplearon las bandas de absorción del CS de las que se seleccionó la situada a 258,056 nm. En cuanto a los píxeles analíticos tanto para el fósforo como para el azufre se utilizaron los 5 píxeles centrales mientras que para los píxeles de corrección en el caso del azufre se escogieron los píxeles 95-97 y 109-111, y para el fósforo se utilizó la corrección automática. Al optimizar las condiciones de la llama se obtuvo para el fósforo una altura de medida óptima sobre el mechero de 11 mm y un flujo de acetileno de 70 L/h mientras que para azufre la altura óptima de medida sobre el mechero fue de 16 mm y el flujo de acetileno de 120 L h^{-1}. El flujo de aire utilizado fue igual para ambos elementos 417 L h^{-1}, así como los tiempos de medida y de retardo.

En estas condiciones se calcularon los límites de detección y cuantificación para fósforo (40,5 y 135,1 mg L^{-1}) y azufre (17,7 y 59,0 mg L^{-1}), los coeficientes de variación (8,1% para fósforo y 6,3% para el azufre, n=11) y la recuperación con valores próximos al 100%. Finalmente se realizó la determinación de ambos elementos en muestras de fertilizantes diluidas con agua Milli-Q o tratadas con H_2O_2 y HNO_3. Se concluyó que no existían diferencias significativas entre las concentraciones medidas en las muestras tras la oxidación, y por tanto que los elementos se encontraban en los fertilizantes estudiados en formas oxidadas como fosfatos y sulfatos.

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DETERMINATION OF PLASTIC ADDITIVES IN PACKAGING BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TIME OF FLIGHT MASS SPECTROMETRY

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Additives are incorporated into polymeric packaging in order to modify or enhance their properties as well as to increase their shelf life. Depending on the additive function they can be stabilizer, modifier or filler. Modifier additives, such as slip or anti-blocking agents, improve and alter the polymeric properties; while stabilizer additives such as light stabilizers or antioxidants preserve the original features of the polymer manufactured. For quality and regulatory reasons, it is very important to determine the level of these additives in polymers by both manufacturers and regulators in order to ensure that plastic packaging is adjusted to its purpose safely.

A simple and sensitive analytical method for the determination of several plastic additives in multilayer packaging, based on solid liquid extraction (SLE) and ultra-high performance liquid chromatography coupled to variable wavelength detector and time of flight mass spectrometry detectors, is presented in this communication.

The proposed method allows the simultaneous determination of slip agents, which are usually determined by GC-FID, and the rest of additives, which are determined only by liquid chromatography, in less than 10 min. Furthermore, this method is between 21 and 5000 times more sensitive than GC and LC methods previously reported [1-3] showing LOQ between 0.6 and 8 ng/ml for most additives. In addition, good repeatability and intermediate precision values were achieved for all of them even at concentrations close to LOQ, with RSDs below 7% and 20%, respectively.

For sample preparation, focused ultrasonic solid liquid extraction (FUSLE) and SLE were optimized and evaluated to extract plastic additives from packaging. Extraction efficiency values were compared to those obtained by using pressurized liquid extraction (PLE). Both extraction methods showed excellent extraction efficiency for slip agents because those additives bloom to the surface once the film is produced and are less branched. However, Ix1010 was the most difficult to extract because of its large size.

FUSLE was rejected because of its ineffectiveness since it did not produce any significant acceleration of the extraction process. Regarding PLE, this method makes impossible the determination of oxidized/reduced analyte ratio of antioxidants such as Irgafos126 and Irgafos168 in the packaging because the phosphite group of these antioxidant additives are partially or completely oxidized at the high temperatures used during extraction.

Finally, the selected method was applied to the analysis of nine multilayer packaging samples made of polyethylene film, aluminum foil, polyester and/or paper. All samples showed the same additives: one slip agent (erucamide), three antioxidants (Irgafos 168, Irgafox 1076 and Irgafox 1010) and one oxidation by-product of Irgafos 168, in a concentration of total additives ranging from 142 to 910 µg/g, being Irgafox 1076 the predominant and Irgafox 1010 the less abundant.

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POLYPHENOLIC CONTENT AND IN VITRO ANTIOXIDANT ACTIVITIES OF GRAPE SEEDS EXTRACTS FROM DIFFERENT WHITEGRAPE VARIETIES

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Grape seeds are waste products of the winery and grape juice industry; particularly, grape seeds represent a high percentage (20 to 26 %) of the grape marc obtained as a residue of winemaking. The management of these residues produces problems due to their seasonal character and they have been just discarded for several years. However, these seeds contain fatty acids, tocopherols, and a variable proportion of polyphenols depending on the variety. In this work, the seeds obtained from monovarietal grape marcs of twelve different varieties of *Vitis vinifera* L. from Galicia (NW Spain) have been analyzed in order to get the total polyphenolic content and the concentration of the major polyphenols presented in each grape seeds type. The studied grape seed samples were isolated from industrial solid wastes from native varieties obtained in wineries during the normal process of commercial white winemaking: Albariño, Treixadura, Godello, Loureiro and Caíño Blanco; and from experimental grape marcs, obtained for research purposes from the *Ribadumia Enological Station* (Pontevedra): Torrontés, Gewürztraminer, Chardonnay, Riesling, Pinot gris and Pinot Blanc. The samples were obtained in 2012 and 2013 vintages.

The aim of this work is therefore to extract, determine and quantify polyphenols in grape seeds, as well as the total polyphenol content, and the antioxidant activity, to relate them with the variety, origin and year of grape harvest. Phenolics have been extracted from seeds by means of an optimized Pressurized Liquid Extraction (PLE) methodology [1] and analyzed by HPLC-DAD. In addition, in vitro antioxidant capacity has been determined in all seed extracts by the antiradical activity measure procedure based on DPPH. The correlation between the phenolic content (total and individual key polyphenols) and the antioxidant activity is deeply investigated and discussed because it is not always as direct as it can be supposed.

Foreseeable, grape seeds contain most of the polyphenols of the whole grape marc total content. Thus, in this contribution, solid wastes from the white wine industry (grape marc and grape seeds) were also compared as potential sources of bioactive phytochemicals on the basis of their content in phenolics and in vitro antioxidant activity. The results showed that extracts from white grape seeds, whatever the native variety industrially exploited, contain large amounts of polyphenols, in spite of slight differences in the individual polyphenolic profile. The average antioxidant activity is also high for all of them.

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QUANTITATIVE DETERMINATION OF LACTIC AND ACETIC ACID IN CIDER BY ^{1}H NMR SPECTROMETRY

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Lactic acid gives a doublet signal at 1.42 ppm in the spectrum and acetic acid a singlet signal at 2.09 ppm, whose areas are used to determine the concentration of these compounds. 3-(Trimethylsilyl)-2,2,3,3-d^4-propionic acid sodium salt is added to the cider with a double purpose: as a reference for 0.00 ppm and as an internal standard for ^{1}H NMR measurement.

The method[1] consists in adding 600 mL of the calibration standard or the cider in a 5 mm outer diameter NMR tube and adding 100 mL of the TSP-D_2O solution. The final concentrations are TSP 1.426 ± 0.014 g L^{-1} and D_2O 10% (v/v). The 500 MHz ^{1}H NMR spectra are recorded under the following conditions: temperature of 30 ºC, 64 scans of 32K data points, with a spectral width of 8012 Hz (16 ppm), acquisition time of 4.0 s, recycle delay of 2.0 s, flip angle of 90º and constant gain of 28.5. Required time per sample was about 8 min.

A total of 10 standards are prepared with lactic acid concentrations in the 0.5-5.0 g L^{-1} and another 10 standards are prepared with acetic acid (previously standardized against NaOH) in the range of 0.3-3.0 g L^{-1} of concentration. The pH of all the standards is adjusted at 1.0 by adding concentrate H_2SO_4.

The method is applied to 10 samples of commercial ciders and enzymatic analysis methods are used to validate the method. The results obtained are the following:

	Lactic acid L	**Lactic acid D**	**Lactic acid TOTAL**	**Lactic acid TOTAL**	**Acetic acid**	**Acetic acid**
Sample	Enzymatic	Enzymatic	Enzymatic	^{1}H NMR	Enzymatic	^{1}H NMR
1	3.02	3.19	6.21	6.86	2.52	2.56
2	2.87	2.84	5.71	6.18	1.45	1.55
3	4.86	0.29	5.15	5.88	1.68	1.78
4	0.91	0.96	1.87	1.98	1.98	2.06
5	8.42	0.31	8.73	9.45	0.3	0.33
6	6.65	0.32	6.97	7.29	0.92	0.97
7	1.12	0.83	1.95	2.39	1.3	1.24
8	2.12	2.3	4.42	5.09	1.71	1.75
9	2.18	1.97	4.15	4.85	0.48	0.55
10	4.38	0.33	4.71	4.93	0.84	0.87

Good correlations are found between the lactic and acetic acids concentrations obtained by ^{1}H NMR and those obtained by enzymatic methods in different commercial ciders. For lactic acid, the 95% confidence interval for the slope is 1.05 ± 0.08 (includes 1) and for the intercept 0.27 ± 0.42 (includes 0). For acetic acid, these intervals are 1.01 ± 0.06 for the slope and 0.03 ± 0.08 for the intercept. The described method is fast and direct.

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USO DE RELACIONES DIAGNÓSTICO, BIOMARCADORES Y REDES NEURONALES DE KOHONEN DE 3 VÍAS PARA LA MONITORIZACIÓN DE LA EVOLUCIÓN TEMPORAL DE LOS VERTIDOS DE PETRÓLEO

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La identificación de vertidos de petróleo se basa habitualmente en el tratamiento avanzado de datos cromatográficos. En esta comunicación se presenta un método quimiométrico novedoso y simple basado en redes neuronales de Kohonen para manejar matrices de datos tridimensionales, dicho método se denomina MOLMAP ('Matrix reOrganization Layout to Map Analytical Parameters') [1].

Se consideraron un conjunto de 28 relaciones diagnóstico calculadas entre diversos PAHs, y sustancias biomarcadores típicamente asociadas a hidrocarburos para caracterizar seis productos vertidos en condiciones controladas a lo largo de 4 meses.

De los estudios se dedujo que algunas relaciones diagnóstico consideradas constantes tradicionalmente no eran lo suficientemente estables. En particular, algunos hidrocarburos aromáticos policíclicos (PAHs) alquilados (por ejemplo, 1-metildibenzotiofeno, 4-metilpireno, 27bbSTER y los esteroides triaromáticos TA21 y TA26) parecían menos resistentes al envejecimiento a medio plazo que los biomarcadores. Se ha encontrado que los productos vertidos se pueden diferenciar usando una (o dos) relaciones diagnóstico. Por ejemplo, 30O, 28ab (y 25nor30ab), C3-DBT/C3-phe, 27Ts, TA26 y 29Ts caracterizan Ashtart, Brent, Maya, Sahara, IFO y Prestige, respectivamente.

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ANALYSIS OF EMERGING POLLUTANTS IN ENVIRONMENTAL WATERS BY USING PHOTOINDUCED FLUORESCENCE COMBINED WITH U-PLS/RBL SECOND ORDER MULTIVARIATE ALGORITHM

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Over the past few years, drugs have been considered emerging pollutants due to their continuous input and persistence in the aquatic ecosystem even at low concentrations. They have been detected worldwide in environmental matrices, indicating their ineffective removal from water and wastewater using conventional treatment methods. For that reason, the number of analytical methods for their analysis has been increasing in the last decades. Usually, most of the analytical methodology employed for the determination of emerging pollutant in environmental waters is based on chromatographic separations connected to detection with mass spectrometry (LC-MS) [1], which is a very powerful tool due to their selectivity and sensitivity. However, LC-MS requires a sophisticated and expensive instruments that, unfortunately, are not available in many analytical laboratories. For this reason, in the present research work we propose a novel, quick and easy method based on the acquisition of fluorescent signals and their treatment with second-order algorithm. Following this methodology, three emerging pollutants, representative of different groups of therapeutic drugs, were investigated: the anticonvulsant carbamazepine (CBZ), the antibacterial fluoroquinolone ofloxacin (OFL), and the non-steroidal anti-inflammatory piroxicam (PX).

CBZ is one of the most frequently detected drugs in environmental waters all over the world, and not present native fluorescence [2]. For that reason, the determination has been carried out by measuring excitation-emission photoinduced fluorescence matrices of the products formed upon ultraviolet light irradiation, in acidic media. In this conditions all target compounds have been transformed into fluorescent photoproducts. The selectivity of the method is achieved through the use of unfolded partial least squares algorithm (U-PLS). The proposed method was validated in absence and presence of potential interferences. In the last case, the algorithm U-PLS was coupled to the routine RBL to achieve the second order advantage, which permits quantitating analytes in test mixtures in the presence of interferences which have not been included in the calibration phase.

Finally, CBZ, OFL and PX were analysed in real waters from Argentina, including river (Paraná River), underground water (Funes City and Santa Rosa City) and tap water (Venado Tuerto City). They were prepared by spiking them with the analytes at two different concentrations level between 0.08 and 14 ng mL^{-1}. The samples were initially filtered to remove the impurities in suspension and, to improve the sensitivity of the proposed method, most samples were subjected to solid-phase extraction (SPE) with C18 disks. The recoveries obtained were comprised between 83-119 %, and the detection limits in preconcentrated real waters samples (1:125) ranged from0.04 to 0.3 ng mL^{-1}.

Acknowledgments
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CONTROL DE CALIDAD INTERNO MEDIANTE CARTAS DE CONTROL DE INDIVIDUALES, EN BIOENSAYOS DE LÍNEAS PARALELAS

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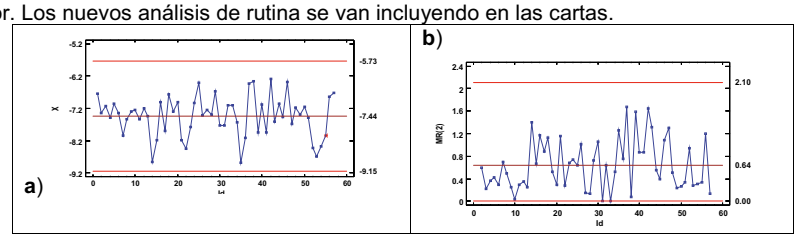
El control estadístico de la calidad es relativamente reciente; por primera vez, Walter Shewhart en 1931 aplicó metodología estadística a procesos industriales. Las Cartas o Gráficos de Shewhart permiten monitorizar el comportamiento, en cuanto a centralización y variabilidad, de las variables críticas del proceso desde el inicio hasta el final del mismo. El objetivo de este trabajo es implementar Cartas de control de medidas individuales, con el fin de establecer un procedimiento de control interno para ensayos rutinarios. Se aplica a bioensayos realizados para determinar la potencia de anticuerpos frente a antígeno de superficie del virus de la hepatitis B en inmunoglobulinas humanas de administración parenteral. Se utiliza el modelo de líneas paralelas, siguiendo el protocolo establecido por las directrices europeas, *European Pharmacopoeia* [Ph. Eur., 2014].

La validez de los ensayos se prueba mediante Análisis de Varianza, considerando las siguientes fuentes de variación: *i*) Preparaciones (muestra y estándar), *ii*) Regresión (lineal), *iii*) No paralelismo, *iv*) No linealidad, *v*) Tratamientos y *vi*) Error Cuadrático Medio (MRS).En el análisis de los datos se realizan los correspondientes contrastes de hipótesis utilizando MRS como denominador de la *F* de Snedecor. El ensayo se considera válido cuando los valores de la *F* de Snedecor del ANOVA llevan a la conclusión de aceptar como significativos los contrastes de hipótesis para la regresión, el No paralelismo y la No linealidad. La muestra está formada por 57 bioensayos independientes. La variable objeto de análisis es el *Error Cuadrático Medio* (MRS).La media de MRS de los 57 bioensayos está comprendida en el intervalo [0.00014; 0.00184] siendo el valor medio de la muestra 0.00070. La desviación estándar de la asimetría es muy alta (3.416) lo cual indica que la distribución no es simétrica. Dado que las gráficas de control para medidas individuales son muy sensibles a la falta de normalidad de los datos, los valores de MRS deben tratarse mediante una transformación que genere un conjunto de datos distribuido normalmente. En este trabajo se ha realizado la transformación de Krishnamoorthy [Krishnamoorthy K, 2006], derivada de Peizer et al [Peizer et al., 1968], la transformación logaritmo natural de MRS y la transformación logaritmo decimal de MRS. De acuerdo con los resultados cualquiera de las transformaciones anteriores podría haber sido utilizada para obtener las cartas de Control de Control, por sencillez se ha optado por LOG(MSR). Como base para la estimación de la variabilidad del proceso se utiliza la Carta de rango móvil MR(2), con dos observaciones sucesivas para la estimación. El análisis de datos se ha realizado con Statgraphics *Centurion*®. Para la construcción de las cartas se acepta que los datos proceden de una distribución N(-7.437, 0.5703) en la que los parámetros fueron estimados a partir de los datos transformados. En la figuras se muestran las cartas:**a**) Carta individuales X, y **b**) carta MR(2), indicando la línea central y los límites de control superior e inferior. Los nuevos análisis de rutina se van incluyendo en las cartas.



Ninguno de los 57 valores fue excluido de ninguna de las cartas, como se muestra en las gráficas, por lo que no se puede rechazar con un nivel de confianza del 95% la hipótesis nula H_0: el proceso está en estado de control estadístico. Los nuevos análisis se van incluyendo en la carta, frente a la alternativa H_1:el proceso no está en estado de control estadístico.

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GREEN DETERMINATION OF COPPER-MANCOZEB PESTICIDE FORMULATIONS BY NEAR INFRARED SPECTROSCOPY

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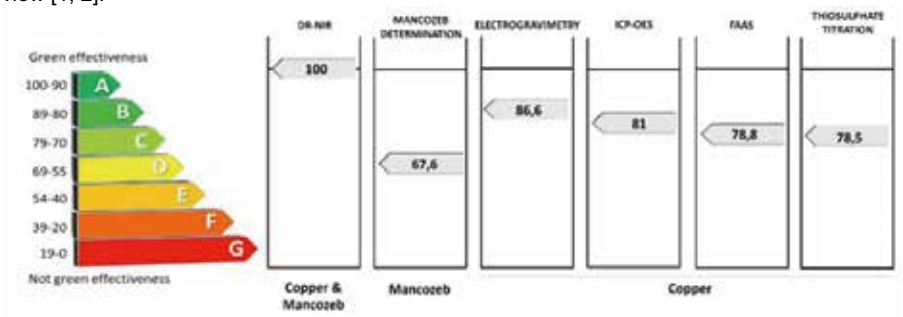
Quality control of copper oxychloride – mancozeb formulations remains a challenge for the phytosanitary industry. The reference methodologies proposed by CIPAC for the analysis of both active ingredients are based on classical titration methods which involve the consumption of solvents and reagents, energy and time and the generation of considerable amounts of wastes. In this work, the direct determination of copper and mancozeb in phytosanitary products is proposed by using diffuse reflectance near infrared spectroscopy (DR-NIR) and partial least square (PLS) calibration. The accuracy of the DR-NIR methodology was evaluated by comparison of the copper and mancozeb concentrations obtained for an independent external validation set with those obtained by the CIPAC reference methods based on thiosulphate (copper) and iodometric of the evolved carbon disulphide (mancozeb) titrations. The relative error ranged between -1.3 and +2.2 % and -1.4 and 5.2 % for copper and mancozeb respectively.

The three methodologies, together with additional ones based on electrogravimetry, flame absorption atomic spectrometry (FAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES), were evaluated from a Green Analytical Chemistry (GAC) point of view [1, 2].



The obtained results are shown in the figure. As it can be seen, the DR-NIR methodology provide a score of 100 points, the top of the Green Certificate ranking [3], because the use of reagents and the generation of residues is avoided, being the energy consumption lower than 0.1 kWh. On the other hand, the electrogravimetry procedure, FAAS, ICP-OES and the CIPAC mancozeb reference methodology provided low score values, of 86.6, 78.8, 81 and 67.6, respectively.

Moreover, the developed DR-NIR procedure provided a direct, non-destructive accurate, simultaneous, fast and green determination of copper oxychloride and mancozeb, being a powerful tool in the quality control in the pesticide industry.

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DISTRIBUCIÓN Y CUANTIFICACIÓN DE HIDROCLOROTIAZIDA EN COMPRIMIDOS COMERCIALES DE HIDROSALURETIL MEDIANTE ESPECTROSCOPIA RAMAN Y HPLC

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Se propone una metodología basada en el registro del espectro Raman para verificar la uniformidad de distribución de la hidroclorotiazida, principio activo del preparado comercial sólido Hidrosaluretil. Este medicamento se utiliza para tratar enfermedades tales como la hipertensión arterial, los edemas, la diabetes y la hipercalciuria ideopática. El contenido nominal del principio activo en estos comprimidos es de 50 mg, que representa un 25 % de la composición total del producto [1].

Como primera etapa se ha estudiado el efecto del fraccionamiento sobre la masa de cada mitad siguiendo las normativas de la Farmacopea Europea [2] y la FDA (Food and Drug Administration) [3]. Se comparan los resultados de romper el comprimido con la mano o con la ayuda de un cuchillo.

Se comprobó que un método HPLC descrito anteriormente en la bibliografía no cumplía con los criterios establecidos por la farmacopea para la resolución mínima exigible entre hidro- y clorotiazida, por lo que ha sido necesario poner a punto un nuevo método HPLC para su uso como método de referencia. Así mismo, se han estudiado diversas alternativas para la extracción cuantitativa del principio activo en el preparado comercial.

Los resultados del análisis por HPLC se han utilizado para verificar el cumplimiento de los criterios de uniformidad de contenido, y como valores de referencia en el desarrollo posterior de un método cuantitativo a partir de los espectros Raman utilizando regresión parcial por mínimos cuadrados (PLS).

Para la validación del método por espectroscopia Raman se han analizado diez comprimidos de cinco lotes diferentes del producto comercial. A cada comprimido se le ha realizado un mapeo de 16 puntos distribuidos sobre la superficie mediante una gradilla de 8 puntos por mitad. Estos 16 espectros se han procesado con dos metodologías distintas: a) midiendo la intensidad o área de un pico del API no interferido por ningún otro componente del preparado, y b) a partir de un modelo cuantitativo obtenido mediante calibración multivariante por regresión de mínimos cuadrados parciales (PLS). En el primer caso tenemos una medida semicuantitativa de la distribución del API en la superficie, mientras que en el segundo tenemos un valor objetivo de concentración.

En esta comunicación se presentan los resultados y se discuten las ventajas e inconvenientes entre la aproximación basada sólo en la intensidad del pico del API, o la calibración multivariante por PLS. Así mismo se discute la posibilidad del uso del Raman como técnica analítica para la estimación de la uniformidad de contenido.

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QUANTIFICATION OF FLAVONOID COMPOUNDS USING SPECTROFLUORIMETRY COUPLED TO CHEMOMETRIC TOOLS

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Flavonoids are a group of polyphenolic compounds present in medicinal plants, vegetables, fruits and a variety of beverages (tea, coffee, wines and fruit drinks) [1, 2]. Some of them have been determined using fluorescence methods based on the formation of complexes and they were quantified as total content of flavonoid [3, 4].

The objective of this study was to determinate flavonoids mixtures using spectrofluorimetry coupled to chemometric tools. Firstly, a method to improve the fluorescence signal of flavonoid was developed. The pH of the medium, instrumental parameters, percentage of methanol and volume of buffer solution were optimized. The optimal conditions were obtained using a moderate basic medium, because these compounds are oxidized in these conditions as proven by kinetic measurements. The optimal working conditions were: pH between 9 and10, a photomultiplier voltage of 650 V, excitation and emission slits of 10 nm, a percentage of methanol lower than 10 % and a percentage of buffer solution of 87%. In these conditions, the analytes presented a considerable stability. However, only three of the five analytes studied presented fluorescence emission (quercetin, kaempferol and myricetin), whereas other two (apigenin and luteolin) do not fluoresce in these conditions. The fluorescence of myricetin being considerably lower than the fluorescence of quercetin and kaempferol.

A sample set of mixtures of quercetin and kaempferol was built for calibration with the PARAFAC, U-PLS/RBL and N-PLS/RBL models. Excitation – emission fluorescence matrices were obtained for each one of the standards and processed with second-order algorithms. The selection of the optimal factors were performed for each model.

The results obtained showed that PARAFAC could quantify flavonoids as total content of quercetin and kaempferol, but it couldn't differentiate among them. However, U-PLS/RBL and N-PLS/RBL allowed quantify quercetin and kaempferol separately. The method offered satisfactory results in the analysis of a set of test samples and recovery values found were between 89 – 118 %.

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DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN ENVIRONMENTAL SOLID SAMPLES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOLLOWING MICROWAVE ASSISTED AND CONTINUOUS SOLID-PHASE EXTRACTION

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Endocrine disrupting compounds (EDCs) are exogenous chemical substances or mixtures thereof that alter some functions of the endocrine system and have been postulated as the cause of a large number of adverse health effects most of which involve reproductive abnormalities potentially leading to population declines. The effects ascribed to EDCs in humans include temporary reductions in sperm counts and its quality, increased incidence of testicular, prostate and breast cancer, altered sex ratios, neurological disorders, polycystic ovaries, endometriosis, cardiovascular disease, thyroid disorders and immune deficiencies. A high variety of products commonly used in daily life (e.g., detergents, personal care products such as cosmetics, pharmaceuticals, industrial formulations) contain EDCs of diverse structure. As a result, EDCs are frequently encountered in the aquatic environment, which they reach largely through effluents from wastewater treatment plants and run-offs from farmlands. Because EDCs can potentially affect the environment at very low concentrations, their analysis requires using methods with very low detection limits, which is especially difficult with complex matrices such as environmental solid samples (e.g., soils, sediments, sewage sludge). The EDCs 4-tert-octylphenol and nonyphenol are ubiquitous contaminants; so much so that the European Directive 2003/53/ European Commission (EC) has established restrictions on the marketing, uses and preparation of certain hazardous substances including both (EC, 2003). The Working Document on Sludge 3rd Draft of the EC suggests a concentration limit of 50 mg kg^{-1} for nonylphenol in sludge (EC, 2000). In literature, there are several analytical methodologies already available for the determination of EDCs in soil, sediments and sewage sludge samples with acceptable limits of detection. Most commonly used procedures applied for the extraction of the target compounds in solid samples are based on Soxhlet extraction, sonication, microwave assisted extraction, pressurized liquid extraction and supercritical fluid extraction. Clean up of the samples is generally performed using classical approaches such as solid phase extraction or using semi-automatic techniques such as a gel permeation chromatography.

A sensitive method has been developed and validated for the determination of diverse groups of EDCs, bisphenol A, alkylphenols, phenylphenols, p-hydroxybenzoic acid esters (parabens, preservatives), and antimicrobials such as triclosan in environmental solid samples (soil, sediment and sewage sludge). Samples were extracted by microwave-assisted extraction followed by continuous solid-phase extraction cleanup. For determination of compounds, sample extracts were further derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide+1% trimethylchlorosilaneto improve the analytical sensitivity. The chemicals were determined by gas chromatography–mass spectrometryin SIM mode. The proposed method provides a linear response over the range 2.0-5000 ng kg^{-1} and features limits of detection from 0.5 to 4.5 ng kg^{-1} depending of the particular EDC. The method was successfully applied to the determination of target compounds in agricultural soils, pond and river sediments, and sewage sludge. The sewage sludge samples were found to contain all target compounds except benzylparaben at concentration levels from 36 to 164 ng kg^{-1}. By contrast, the other types of samples contained fewer EDCs and at lower concentrations (5.6-84 ng kg^{-1}).

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MICROENCAPSULACION DE BIOATRAYENTES ESPECÍFICOS: VENTAJAS EN TOXICOLOGÍA MEDIOAMBIENTAL

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Los artrópodos como la *Musca doméstica* constituyen una problemática importante en salud ambiental. Los criaderos de moscas son portadores de microorganismos que causan enfermedades como la salmonella, la fiebre tifoidea y otras enfermedades diarreicas [1]. En la naturaleza existen plantas y hongos que producen compuestos químicos volátiles que atraen a las moscas. En nuestro laboratorio, se han identificado un gran número de estos compuestos, haciendo uso de la técnica de espacio en cabeza acoplada a un equipo de cromatografía gaseosa-espectrometría de masas (HS-GC/MS) [2]. Asímismo, se han realizado ensayos de atracción de dichos compuestos sobre la mosca doméstica [3].

El objetivo de este trabajo es la microencapsulación de bioatrayentes específicos de la mosca doméstica y su liberación. Los bioatrayentes se han seleccionado en función de los resultados obtenidos en los ensayos llevados a cabo con mosca doméstica.

Se ha optimizado el procedimiento de encapsulación de los compuestos volátiles, utilizando como agentes encapsulantes las ciclodextrinas, compuestos solubles en agua, biodegradables y con un bajo coste económico. Una vez obtenidos los productos encapsulados se ha comprobado la encapsulación mediante calorimetría diferencial de barrido y se ha procedido a llevar a cabo estudios de liberación en agua a temperatura ambiente. Para ello se ha utilizado la técnica de HS-GC/MS, y se ha llevado a cabo la optimización de las variables que afectan al sistema (tiempos de: equilibrado, llenado del loop y de inyección y, temperaturas de: horno, loop y línea de transferencia) mediante un diseño experimental.

Este trabajo servirá de base para en un futuro próximo crear un producto biocida microencapsulado más eficaz y específico, constituido por bioatrayentes y pesticidas. Se seleccionarán estos últimos entre los más utilizados en sanidad ambiental: piretroides carbamatos y nicotinoides. Indicar que nuestro grupo de investigación ha sintetizado productos microencapsulados con estas familias de pesticidas [4].

El producto microencapsulado ofrecerá una liberación controlada del pesticida y de los bioatrayentes, lo que minimizará el riesgo que estos insecticidas suponen para la salud humana y el medioambiente [5, 6].

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APLICATION OF TOTAL REFLECTION X-RAY FLUORESCENCE TO THE ASSESSEMENT OF METAL MOBILITY AND BIOAVAILABILITY FROM SOILS FOLLOWING RAPID SINGLE EXTRACTION TESTS

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Total reflection X-ray fluorescence is an emerging technique that allows performing mutielement determinations at trace level using microsamples deposited onto a smooth surface. Evaluation of metal mobility and bioavailability from soils has been extensively addressed using a variety of operational approaches. In this work, a portable TXRF spectrometer was employed along with a rapid and miniaturized extraction method assisted by ultrasound for *in situ* assessment of extractable metals (Cr, Mn, Fe, Ni, Cu, Zn and Pb) in EDTA and acetic acid from soils. Ultrasonic extractions were compared with the single extraction scheme proposed by the Standards, Measurements and Testing program (SM&T) from the European Commission. A dramatic decrease in the extraction time was achieved using ultrasonic extraction while achieving similar extractability as compared to the conventional approach. In addition, the use of TXRF for detection eliminates the need for a filtration step, which reduces further the treatment time. Method performance was evaluated with CRM BCR 483 (sewage sludge amended soil). Application to two field sampled soils (*i.e*, roadside and cultivated soils) was carried out. Under optimal conditions, no significant differences between the extractable contents obtained with the conventional and ultrasonic extraction approaches was found in most cases. Metal mobility in roadside soil was Pb>Cu>Mn≈Zn>Fe using EDTA as extraction agent.

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EVALUACIÓN EXTERNA DE LA CALIDAD DE LOS DATOS DE CONTAMINANTES EN LOS PROGRAMAS DE VIGILANCIA DE LA CONTAMINACIÓN MARINA DEL IEO

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Desde principios de los años 90, el Grupo de Contaminación Marina del Instituto Español de Oceanografía (IEO) del Centro Oceanográfico de Vigo, es el responsable de diseñar los muestreos y de cuantificar e interpretar los datos de contaminantes como contribución de España al Convenio Regional para la Protección del Medio Marino del Atlántico Nordeste (Convenio OSPAR).

Los datos de concentraciones de contaminantes obtenidos cada año deben ir acompañados de la información de control de calidad interno y externo del laboratorio que lleva a cabo los análisis químicos correspondientes. Esta información es necesaria para certificar la calidad analítica de los datos de contaminantes desde el punto de vista de exactitud, precisión y fiabilidad. Un buen nivel de calidad analítica demostrable es condición indispensable para que los datos de concentraciones de contaminantes de cada país sean incluidos en las evaluaciones internacionales conjuntas que el Convenio OSPAR lleva a cabo periódicamente.
Es importante señalar que estas series históricas de contaminantes en organismos y sedimentos marinos del IEO han sido de gran utilidad en el proceso de implementación y desarrollo de la Directiva Marco sobre la Estrategia Marina, que es actualmente el principal instrumento ambiental de la política marítima de la Unión Europea. En el Grupo de Contaminación Marina, todo el trabajo se desarrolla de acuerdo con unos protocolos preestablecidos, dentro del sistema de Control de Calidad, que incluyen todas las etapas del proceso desde el momento del muestreo hasta la remisión de los resultados finales. Como parte de su sistema de calidad, y para garantizar la necesaria fiabilidad de todos los datos producidos, las etapas analíticas son sometidas a un riguroso control de calidad efectuado a dos niveles: Control de Calidad interno o intralaboratorio y Control de Calidad externo o interlaboratorio.

El Control de Calidad interno es el control rutinario de la exactitud y precisión de las técnicas y metodologías más habituales, incluyendo el uso de materiales de referencia certificados, análisis de muestras por duplicado, determinación de blancos, análisis de muestras ciegas o gráficos de control. Además de seguir las recomendaciones aconsejadas en las Buenas Prácticas de Laboratorio tanto en la limpieza y conservación del material como en el control del ambiente atmosférico. El control de calidad externo permite garantizar la comparabilidad de los datos producidos por diferentes laboratorios constituyendo el medio por el cual el laboratorio conoce objetivamente y demuestra externamente la fiabilidad de sus resultados y la exactitud analítica de sus métodos. Se lleva a cabo participando en los ejercicios de intercalibración que determinan la capacidad del laboratorio participante para conseguir resultados comparables y permiten detectar si sus métodos son válidos o necesitan ser perfeccionados.

En esta comunicación se presenta un resumen de los resultados obtenidos en los últimos 20 años en los ejercicios de intercalibración organizados por QUASIMEME (Quality Assurance of Information in Marine Environmental in Europe) de los principales grupos de contaminantes inorgánicos y orgánicos en organismos y sedimentos marinos analizados sistemáticamente en los Programas de Vigilancia de la Contaminación Marina del IEO.

En estos ejercicios de intercalibración se emplea el parámetro Z para calificar la exactitud de los resultados comparando el valor obtenido con el asignado. Se considera que los valores remitidos por los participantes son satisfactorios cuando $|Z| \leq 2$. A lo largo de estos años, aunque se han modificado los métodos y se han actualizado los equipos instrumentales, se han obtenido un alto porcentaje de Z inferiores a 2 lo que indica que los resultados enviados por el laboratorio se consideran altamente satisfactorios.

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VARIACIÓN ESTACIONAL DE LOS NIVELES DE METALES PESADOS EN MEJILLÓN DE ROCA DE LA COSTA DE GALICIA Y CANTÁBRICO

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Los programas de vigilancia de la contaminación marina utilizan una gran variedad de organismos centinelas, entre ellos el mejillón de roca (*Mytilus galloprovincialis*), como bioindicadores de contaminantes químicos. Estos moluscos son organismos sedentarios, filtradores, tienen una amplia distribución geográfica y sobre todo poseen una gran capacidad para concentrar en sus tejidos la mayor parte de los contaminantes presentes en el mar siendo los niveles de contaminantes proporcionales a los del medio en el que desarrollan su ciclo vital. Durante el año 2013 se ha muestreado bimestralmente mejillón de roca en cinco estaciones pertenecientes a la red de seguimiento del IEO de la zona costera de Galicia y Cantábrico. Los puntos seleccionados han sido: Oia, Raxó, Mera, Avilés y Pedreña, correspondiendo a zonas urbanas e industriales, pequeñas poblaciones e incluso zonas limpias para permitir hacer estudios comparativos entre ellas.

En el laboratorio se han preparado tres homogeneizados constituidos por un mínimo de 50 individuos del intervalo de tallas disponibles (35-60 mm) en el punto de muestreo. Posteriormente, se realiza la trituración, liofilización y homogenización como paso previo al análisis. El método empleado para el análisis de metales pesados se basa en una digestión con ácido nítrico en reactores de teflón a presión en horno microondas. La cuantificación se lleva a cabo mediante espectrofotometría de absorción atómica con cámara de grafito con efecto Zeeman (As, Cd, y Pb) o con llama (Cu y Zn). El Hg se determina mediante espectrometría de absorción atómica por inyección de flujo por el método de vapor frío. La validación de los resultados se realiza mediante el análisis simultáneo de muestras y material de referencia certificado así como por la participación sistemática del laboratorio en ejercicios de intercalibración internacionales tales como QUASIMEME. Además de la cuantificación de los niveles de metales pesados, se valoraron una serie de parámetros biológicos con el fin de establecer la relación entre la variabilidad estacional en los niveles de metales y la secuencia de los procesos biológicos que componen el ciclo anual en estos animales. Los parámetros biológicos estudiados guardan relación con el estado nutritivo y gametogénico, los cuales están altamente relacionados. Se determinaron los índices biométricos tanto de carne como de valva; los niveles de componentes bioquímicos principales como proteínas, carbohidratos y lípidos; y el grado de maduración sexual junto con el porcentaje de ocupación volumétrica de los gametos.

La evolución temporal en las estaciones estudiadas, en líneas generales, presenta el mismo comportamiento siendo más señaladas para unos metales que para otros. Este patrón anual parece estar relacionado con la gametogénesis que, para esta especie, tiene lugar desde finales de otoño hasta la primavera. La caracterización biológica de las poblaciones de mejillones estudiadas muestra que los índices biológicos presentan una gran variabilidad entre los diferentes sitios de muestreo. Además, la variación de estos parámetros biológicos a lo largo del año es diferente dependiendo de la estación, lo cual parece estar relacionado con las diferentes condiciones tróficas de los lugares de procedencia.

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DETERMINATION OF ORGANOLEAD AND ORGANOMANGANESE COMPOUNDS IN SEAWATERS BY ULTRASOUND-ASSISTED EMULSIFICATION MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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The toxicity of organometallic species, even at low concentrations, demands new analytical methods for their monitoring. Organolead and organomanganese compounds are one of the most widespread organometallic compounds in environmental samples, due to their extensive use in gasoline and other fuels along the 20th century [1, 2].

A simple method based on ultrasound-assisted emulsification microextraction (USAEME) has been developed for the simultaneous determination of organolead and organomanganese compounds in seawater samples. Trimethyllead (TMeL), triethyllead (TEtL), tetraethyllead (TeEtL), cyclopentadienylmanganese tricarbonyl (CMT) and its methyl derivative, (methylcyclopentadienyl)manganese tricarbonyl (MMT) were separated and determined using gas chromatography and mass spectrometry (GC-MS).

Before being submitted to preconcentration, a derivatization step should be applied to TMeL and TEtL. Phenylation reaction with sodium tetraphenylbotate improved their chromatographic response and extraction efficiency, while being easily performed in aqueous medium. USAEME has proved to be an efficient, economic and fast preconcentration procedure requiring simple equipment, which is available in most analytical laboratories. Sample preparation, preconcentration, separation and detection could be performed in less than 20 min.

The absence of matrix effect among different samples allowed quantification against external standards prepared in seawater simulant solutions, reaching detection limits that ranged from 7.0 to 41 ng L^{-1}. The method was validated by means of recovery studies, and recoveries in the 84-118% range were obtained. Twelve seawater samples, collected in different points of the Cartagena Bay, were analyzed using the proposed procedure, being none of the target analytes found, at least above their corresponding detection limits.

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USE OF A NEW ENRICHMENT NANOSORBENT FOR SPECIATION OF MERCURY BY FI-CV-ICP-MS

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Mercury is one of the most toxic environmental pollutants and its effects on human and ecosystem health are well known. All mercury species are toxic, with organic mercury compounds generally being more toxic than inorganic species. Chromatography techniques (GC, HPLC) coupled to element specific detectors, are able to separate mercury species in order to elucidate mercury transformation and transport processes where the determination of all mercury species is desirable. However, in practice, especially in sampling campaigns for sea water analysis where a large number of samples are collected over a longer period of time, a combination of methods is usually applied to accurately determine the most toxic mercury species. These include non-chromatographic methods based on the different chemical and/or physical behavior of the mercury species. These non-chromatographic methods can be less time consuming, more cost effective and available, and present competitive limits of detection. Especially when mercury could vapor (CV) generation technique is employed, which reduces salt effect on the analytical signal and improve the sensibility. Among non-chromatographic methods, solid phase extraction and microextraction (SPE and SPME) which is becoming increasingly popular for sample preparation in organic analysis, found its way to speciation analysis of organometals. SPE/SPME is the most popular sample preconcentration method for its simplicity, high enrichment factor, low or no consumption of organic solvents and feasibly to be automated. On the other hand, the exploration of new materials, especially nanometer sized materials, as the support phase is another active research area in SPE/SPME for mercury determination. The use of nanoparticles leads to higher extraction capacity/efficiency and rapid dynamics of extraction originated from the higher surface area to volume ratio and short diffusion route.

In this work, a new enrichment nanosorbent functionalized with 1,5 bis (2-pyridyl) methylene thiocarbohidrazide was synthesized and characterized. From the study of its adsorption capacity toward metal ions, Hg^{2+} was observed to be one of the most retained 173.1 μmol g^{-1} at pH 5. Thus, a flow injection solid phase extraction and cold vapor generation method for its determination and speciation based on the use of this new chelating nanosorbent was optimized. The method developed has showed to be useful for the automatic pre-concentration and sequential speciation of mercury and methylmercury in environmental and biological samples. The system was based on chelating retention of the analytes onto a mini-column filled with the new nanosorbent and their sequential elution by using two different eluents, 0.2 % HCl for CH_3Hg^+ and 0.1 % thiourea in 0.5 % HCl for Hg^{2+}. The determination was performed using inductively coupled plasma mass spectrometry. Under the optimum conditions and 120 s preconcentration time, the enrichment factors were 4.7 and 11.0; the detection limits (3σ) were 0.002 and 0.004 μg L^{-1}; the determination limits (10σ) were 0.011 and 0.024 μg L^{-1}; and the precisions (calculated for 10 replicate determinations at a 2 μg L^{-1} standard of both species) were 2.8 and 2.6 % (RSD); for CH_3Hg^+ and Hg^{2+}, respectively. Linear calibration graphs were obtained for both species from the determination limits to at least 70 μg L^{-1}. For the quality control of the analytical performance and the validation of the newly developed method, the analysis of two certified samples, LGC 6016 estuarine water and SRM 2976 mussel tissue were addressed. The results showed good agreement with the certified values. The method was successfully applied to the speciation of mercury in sea-water samples collected in the Málaga Bay.

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BIOENSAYOS DE ACUMULACIÓN DE ARSÉNICO E IMPACTO POR CONTAMINACIÓN EN LARVAS DE LUBINAS

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La calidad del agua y los sedimentos es vital para la supervivencia y el bienestar de los recursos vivos, especialmente en las zonas costeras y estuarios, y se encuentra afectada por muchos factores, entre ellos el grupo de contaminantes formados por los metales pesados (y algunos metaloides) [1]. La contaminación metálica influye a largo plazo en la estructura de los ecosistemas marinos, por lo que está considerada como uno de los problemas medioambientales más serios en el medio acuático [2]. Algunos de estos elementos se acumulan rápidamente en los tejidos de los animales acuáticos pudiendo llegar a ser tóxicos para los peces y, por consiguiente, para los humanos a través de la cadena alimentaria. Entre estos elementos se encuentra el arsénico, considerado una de las sustancias más tóxicas que se liberan directa o indirectamente a los ecosistemas acuáticos [3], y cuyo efecto sobre la biota puede ser muy elevado. Por ello, en el presente trabajo de investigación se propuso estudiar a escala de laboratorio la bioacumulación de arsénico en larvas de lubina (*Dicentrarchus labrax*, Linneaus 1758), un estadio muy sensible a los efectos externos. Para ello, se diseñó un ensayo de bioacumulación en el que los organismos seleccionados fueron expuestos a diferentes concentraciones de este contaminante, mediante la adición de As_2O_3 (trióxido de arsénico) en los tanques de ensayo, durante períodos de 24 y 96 h. Durante la realización de los bioensayos se controlaron los parámetros físico-químicos de los tanques, los cuales fueron óptimos para la supervivencia de los individuos durante todo el ensayo, así como el carbono orgánico disuelto (COD) de las muestras de agua. Para controlar la concentración de arsénico en disolución se tomaron muestras de agua que fueron analizadas por Voltamperometría de Redisolución Anódica de Impulso Diferencial (DPASV) y Espectroscopía de Emisión Atómica con Plasma Acoplado Inductivamente (ICP-AES), según la concentración de los tanques. Los estudios de bioacumulación de As (mg/kg) en las muestras biológicas se realizaron mediante un pretratamiento de las muestras de larvas y posterior análisis por Espectrometría de Masas con Fuente de Plasma Acoplado Inductivamente (ICP-MS). A partir de los resultados obtenidos se pudieron establecer las correlaciones existentes entre los niveles de metal adicionados en los ensayos de bioacumulación presentes en las aguas y las concentraciones de metal bioacumuladas en las muestras biológicas, llegando a la conclusión de que el As se bioacumula significativamente a valores altos de concentración, así como al aumentar el tiempo de exposición. Además, la concentración de As en agua, no mostró un incremento significativo según el tiempo de exposición, por lo que ni precipita ni es absorbido en la superficie de la materia en suspensión. Al estar realizado este trabajo dentro de un proyecto más amplio del grupo de investigación, se pudo disponer de los resultados de un estudio similar realizado con larvas de dorada (*Sparus aurata*, Linnaeus 1758), realizando así una comparativa sobre la bioacumulación de arsénico en las larvas de ambas especies y concluyendo que no existen diferencias significativas entre ambas especies.

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SPECIATION ANALYSIS OF INORGANIC ANTIMONY IN SEDIMENT SAMPLES FROM SÃO PAULO ESTUARY, BAHIA STATE, BRAZIL

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This paper proposes an extraction procedure for the speciation analysis of inorganic antimony in sediment samples using slurry sampling and hydride generation atomic absorption spectrometry. The optimization step of extraction of the species was performed employing a full two-level factorial design (2^3) and a Box-Behnken matrix, where the studied factors in both experiments were: extraction temperature, ultrasonic radiation time and hydrochloric acid concentration. Using the optimized conditions, antimony species can be extracted in closed system using a 6.0 M hydrochloric acid solution, at temperature of 70^{o}C and an ultrasonic radiation time of 20 min. The determination of antimony is performed in presence of 2.0 M hydrochloric acid solution using HG AAS by external calibration technique with limits of detection and quantification of 5.6 and 19.0 ng L^{-1} and a precision expressed as relative standard deviation of 5.6% for an antimony solution with concentration of 6.0 µg L^{-1}. The accuracy of the method was confirmed by analysis of two certified reference materials of sediments. For a sample mass of sediment of 0.20 g, the limits of detection and quantification obtained were 0.70 and 2.34 ng g^{-1}, respectively. During speciation analysis, antimony(III) is determined in presence of citrate, while total antimony is quantified after reduction of antimony(V) to antimony(III) using potassium iodide and ascorbic acid. The method was applied for analysis of six sediment samples collected in São Paulo Estuary (Bahia State, Brazil). The antimony contents obtained varied from 45.3 to 89.1 ng g^{-1} for total antimony and of 17.7 to 31.4 ng g^{-1} for antimony(III). These values are agreeing with other data reported by literature for this element in uncontaminated sediment samples.

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ESTUDIOS DE BIOACUMULACIÓN DE PLOMO EN LARVAS DE LUBINA DE GRAN VALOR COMERCIAL

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La contaminación por metales pesados es uno de los mayores problemas ambientales en la actualidad debido a su alto grado de persistencia en el medio y su tendencia a la bioacumulación en la cadena trófica, por lo que son considerados un riesgo importante para los seres vivos. Estos metales, entre los que destaca el plomo, son considerados extremadamente peligrosos, pues a pesar de sus bajos niveles de concentración en los sistemas acuáticos naturales, poseen importantes implicaciones a nivel químico y biológico debido a su elevada toxicidad [1].

Por ello, se propuso estudiar a escala de laboratorio la bioacumulación de plomo en larvas de lubina (*Dicentrarchus labrax*, Linneaus 1758), el cual es un estadio muy sensible a los efectos externos. Para ello, se diseñó un ensayo de bioacumulación cuyos organismos seleccionados fueron expuestos a diferentes concentraciones de este metal durante periodos de 24 y 96 h. Este metal se adicionó como acetato de plomo (Pb $(CH_3COO)_2$) en los tanques de ensayo con objeto de estudiar la bioacumulación producida por diferentes concentraciones de plomo en las larvas con respecto al tiempo de exposición. Durante la realización de los bioensayos se controlaron diariamente los parámetros físico-químicos y se evaluó el carbono orgánico disuelto (COD) con el fin de hacer un seguimiento del contenido en materia orgánica de los tanques. Por otro lado, se realizó un seguimiento de las concentraciones de plomo en el agua de los tanques mediante Voltamperometría de Redisolución Anódica de Impulso Diferencial (DPASV) y Espectroscopía de Emisión Atómica con Plasma Acoplado Inductivamente (ICP-AES), debido a los diferentes límites de detección de ambas técnicas. Por último, se realizó un pretratamiento de las muestras de las larvas obtenidas y se analizaron mediante Espectrometría de Masas con Fuente de Plasma Acoplado Inductivamente (ICP-MS) con el fin de realizar los estudios de bioacumulación de Pb (mg Kg^{-1}) en las muestras biológicas.

A partir de los resultados obtenidos se pudieron establecer las relaciones existentes entre los niveles de metal adicionados en los test de toxicidad presentes en las aguas y las concentraciones de metal bioacumuladas en las muestras biológicas, determinándose que se produce una bioacumulación significativa del plomo a valores altos de concentración, así como al aumentar el tiempo de exposición.

Como conclusión, se puede decir que con el presente trabajo de investigación se ha ampliado el conocimiento sobre la bioacumulación del plomo en larvas de lubina en procesos de contaminación.

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DETERMINATION OF IODIDE, IODATE AND TOTAL IODINE IN ESTUARINE WATERS BY VOLTAMMETRIC STRIPPING USING A VIBRATING SILVER AMALGAM MICROWIRE ELECTRODE

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Iodine is one of the most abundant and biologically essential minor elements in seawater. Deficiency of iodine can be related to mental retardation, goiter, primary hypothyroidism and also gastric cancer mortality [1, 2]. It exists mainly as iodide and iodate along with a small fraction of organic iodine compounds in seawater. Iodide has marine importance as it is a nutrient for fish and is used for instance in marine fish farms. The original methods using hanging mercury drop electrode (HMDE) have been successfully applied to determine iodide in seawater directly with high sensitivity and accuracy [3, 4]. However, in an attempt to minimise the use of mercury in analytical chemistry, a vibrating mercury-coated silver microwire electrode is used here for the determination of iodide speciation by cathodic stripping voltammetry in natural waters. Microwire electrodes were made from silver wires (diameter: 12.5 μm) and sealed in a polyethylene holder. The silver electrodes were electrochemically coated with mercury before use. The electrode surface was stable for extended periods of analyses (at least one week) and was then replaced by a new one. The optimised conditions include a pH 8, a frequency of 500 Hz and a deposition time of 60 s, among others. The influence of conditioning potential and duration of this potential were optimised showing that the microwire can be reactivated prior to each scan by applying a negative potential (-3 V) for 1 s. The detection limit for iodide in seawater was found to be 0.7 nM I^- at a deposition time of 60 s. The response increased linearly with the concentration of iodide in seawater up to 100 nM I^-. The method was successfully applied on various water samples collected from the estuary of the river Mersey (Liverpool Bay) giving iodide concentrations between 71 nM and 252 nM. Speciation studies were also developed for the determination of iodate and total iodine in estuarine waters. The work demonstrates that it is possible to much reduce the amount of mercury of the mercury drop electrode with the re-usable mercury-coated microwire for determination of iodide.

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BIOENSAYOS DE ACUMULACIÓN DE COBRE E IMPACTO EN LARVAS DE LUBINA (DICENTRARCHUS LABRAX) DE ALTO INTERÉS COMERCIAL

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La importancia de monitorizar y evaluar la contaminación metálica en muestras acuáticas es incuestionable dado el efecto nocivo que supone su presencia a determinadas concentraciones sobre la calidad medioambiental y sobre los organismos vivos. De igual forma, la capacidad de algunos metales pesados de presentar el fenómeno de la biomagnificación o bioacumulación, los convierten en sustancias prioritarias [1] en el ámbito de la política de aguas en la que se incluyen de forma explícita estos elementos así como algunos de sus compuestos [2].
Por ello, en este proyecto de investigación se han llevado a cabo estudios de bioacumulación de cobre tanto en su forma iónica y como nanopartículas. Dicho contaminante pueden ser absorbido por los peces suponiendo un riesgo tanto para la salud de los organismos expuestos, como para la de los humanos consumidores de los mismos.

La especie seleccionada en este estudio ha sido la lubina (*Dicentrarchus labrax*), por estar muy extendido su consumo, ser muy útiles como modelos para investigación básica y por tener un notable interés filogenético, lo que representa un valor añadido para profundizar en el estudio de sus características biológicas y en el efecto de la contaminación metálica. Se ha seleccionado el estado larvario por ser más sensibles a los procesos de contaminación.
Así, se diseñaron ensayos de bioacumulación a escala de laboratorio donde los organismos seleccionados fueron expuestos a diferentes concentraciones de cobre durante periodos de 24 y 96 h, con objeto de estudiar la bioacumulación producida con respecto al tiempo de exposición.

A partir de los resultados obtenidos se pudieron establecer las correlaciones existentes entre los niveles de metal adicionados en los ensayos de bioacumulación presentes en las aguas y las concentraciones de metal bioacumuladas en las muestras biológicas.
En relación con la especie contaminante estudiada, se observó que para igual cantidad de Cu, la bioacumulación fue algo menor si el metal se encuentra en la fracción coloidal como nanopartícula, que si está en su forma iónica como metal iónico, siendo ésta una conclusión de gran relevancia a nivel medioambiental y desde el punto de vista de la especiación, pues en la actualidad el uso de esas nanopartículas en la industria es cada vez más elevado, teniendo así mismo consecuencias medioambientales.

Por último, cabe destacar que se realizó la comparación de estos resultados con aquellos obtenidos en otros estudios de biacumulación con larvas de lubina y otros metales, realizados en el grupo de investigación.

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El presente trabajo de investigación ha sido realizado gracias a la financiación recibida por el proyecto "Bioindicadores de contaminación metálica en sistemas acuáticos. Criterios de calidad ambiental asociados a alteraciones histopatológicas y bioquímicas en peces de interés comercial", cofinanciado por la Consejería de Economía, Innovación y Ciencia de la Junta de Andalucía (Proyecto de Excelencia RNM-6641) y por el Ministerio de Ciencia e Innovación (Proyecto CTM 2010-17474).

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ADSORCIÓN Y DESORCIÓN DE CROMATO POR DOS SUELOS VOLCÁNICOS DEL ÁFRICA ECUATORIAL

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Los suelos volcánicos cubren el 1% de la superficie de la Tierra pero mantienen al 10% de la población mundial [1], siendo intensamente cultivados debido a su fertilidad. Diversos componentes de los suelos volcánicos pueden adsorber aniones de forma específica o no específica. Según Nanzyo [2], la capacidad de retención de fosfato de los Andosoles es atribuible a la presencia de componentes como alofano, imogolita, complejos Al-humus, ferrihidrita, aluminio de cambio y polímeros hidroxialumínicos en las intercapas de silicatos 2:1. Otros aniones pueden ser adsorbidos de forma similar al fosfato.

El cromo es un elemento relativamente abundante en la corteza terrestre, donde su concentración media es de 100 mg kg^{-1} [3]. Su concentración es elevada en rocas básicas y ultrabásicas y en suelos derivados de las mismas. Su presencia en el suelo, además de un origen litológico, puede relacionarse con diversas actividades antrópicas. Puede presentarse en el suelo en estados de oxidación +3 y +6, siendo la segunda forma más móvil y más tóxica. La alta toxicidad y movilidad del cromo hacen que su adsorción por el suelo sea de vital importancia.

Se estudia la adsorción/desorción de cromato por dos horizontes A de suelos representativos de áreas volcánicas del África ecuatorial: un Andosol vítrico [4] desarrollado sobre materiales basálticos en Santo Tomé y Príncipe (0º20'00"N, 6º39'00"E) y un Andosol silándico desarrollado sobre cenizas volcánicas en Ruanda (1º36'32"S, 29º32'57"E). Los dos suelos son ácidos, con una textura franco limosa o franco arenosa. El Andosol vítrico de Santo Tomé presenta concentraciones moderadas de Fe y Al no cristalinos, pero una gran cantidad de oxihidróxidos de hierro cristalinos. Por el contrario, en el Andosol silándico de Ruanda predominan las formas no cristalinas de hierro sobre las formas cristalinas.

Se determinaron las isotermas de adsorción de Cr por los dos suelos, llevándolos al equilibrio con disoluciones de cromato potásico que contienen hasta 100 mgL^{-1} de Cr. La desorción se llevó a cabo con $NaNO_3$ 0,02 M.

La adsorción de cromato es relativamente baja (entre 18 y 97% de la cantidad añadida en el Andosol vítrico y entre 23 y 56% en el Andosol silándico). El cromato es más adsorbido por el Andosol vítrico en el rango de bajas concentraciones, pero a elevadas concentraciones es el Andosol silándico el que más adsorbe. Esta mayor adsorción parece indicar el papel principal de los óxidos de Al y Fe no cristalinos. La adsorción se ajusta en ambos suelos a la isoterma de Freundlich, no existiendo un máximo predecible para dicha adsorción. La desorción de Cr es inferior al 45% del adsorbido en ambos suelos, correspondiendo la mayor liberación al Andosol vítrico, particularmente a concentraciones altas. Las isotermas de adsorción y desorción son coincidentes en el Andosol vítrico, indicando la reversibilidad de la adsorción. Por el contrario, en el Andosol silándico la isoterma de desorción está por encima de la de adsorción: hay una cierta irreversibilidad de la adsorción. Los resultados indican una alta movilidad del cromato en el ambiente y una baja eficacia de los suelos como protectores de otros compartimentos ambientales frente a la contaminación por este anión.

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ADSORCIÓN Y DESORCIÓN DE ARSENIATO POR DOS SUELOS VOLCÁNICOS DEL ÁFRICA ECUATORIAL

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La adsorción de oxianiones por superficies minerales ha recibido considerable atención, debido al importante papel de aquellos como nutrientes o contaminantes [1]. La presencia de formas activas de aluminio y hierro, como óxidos, oxihidróxidos, silicatos de bajo grado de orden (alofano, imogolita), complejos Al(Fe)-humus, es una característica de los suelos volcánicos. Estos materiales confieren a los suelos la capacidad de adsorber fosfato y otros aniones.
El arsénico es un elemento ubicuo en la corteza terrestre, formando parte de rocas y minerales. Su concentración media en la corteza terrestre es de 1,8 mg kg^{-1} [2]. La presencia de As en el ambiente puede tener carácter litogénico o antropogénico. En condiciones oxidantes y a los pHs habituales en los suelos, el As se encuentra en estado de oxidación +5, como $H_2AsO_4^-$ y $HAsO_4^{2-}$. Los minerales de arsénico son fácilmente solubles, pero la movilidad de este elemento está limitada por su adsorción por los coloides del suelo, en particular los óxidos e hidróxidos de hierro y aluminio. Debido a su carácter tóxico para hombres, animales y plantas, la retención del arsénico por el suelo es crucial.

Se estudia la adsorción/desorción de arseniato por dos horizontes A de suelos representativos de áreas volcánicas del África ecuatorial: un Andosol vítrico [3] desarrollado sobre materiales basálticos en Santo Tomé y Príncipe (0º20'00"N, 6º39'00"E) y un Andosol silándico desarrollado sobre cenizas volcánicas en Ruanda (1º36'32"S, 29º32'57"E). Los suelos son ligera o moderadamente ácidos, con una textura franco limosa el Andosol vítrico y franco arenosa el Andosol silándico. Las concentraciones de Fe y Al extraíbles con ditionito-citrato (Fe_d, Al_d) y con oxalato ácido (Fe_o, Al_o) se presentan en la Tabla 1.

	Andosol vítrico	Andosol silándico
Fe_o, %	1,11	3,46
Al_o, %	0,40	2,48
Fe_d, %	7,84	4,60
Al_d, %	1,74	1,81

Se determinaron las isotermas de adsorción de As por los dos suelos, llevándolos al equilibrio con disoluciones que contienen hasta 100 mg L^{-1} de As en forma de arseniato. La desorción se llevó a cabo con $NaNO_3$ 0,02 M.

Las adsorciones de arsénico son elevadísimas en los dos suelos, aproximándose al 100% de la cantidad añadida para concentraciones iniciales de hasta 40 mg L^{-1} en el Andosol silándico y para todo el intervalo estudiado en el Andosol vítrico. La mayor adsorción en el Andosol vítrico indica el papel predominante de los óxidos de hierro cristalinos en la adsorción de arseniato. La adsorción se ajusta en ambos suelos a la isoterma de Temkin, no existiendo un máximo predecible para dicha adsorción. La desorción de As es inferior al 5% del adsorbido en ambos suelos. La mayor liberación corresponde al Andosol silándico. Las isotermas de adsorción y desorción son coincidentes, indicando la reversibilidad del proceso. Ambos suelos, particularmente el Andosol vítrico, pueden jugar eficazmente un papel protector de otros compartimentos ambientales frente a la contaminación por arsénico.

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EVALUACIÓN TEMPORAL DE LA PRESENCIA DE PRINCIPIOS ACTIVOS FARMACOLÓGICOS EN MUESTRAS DE PROCAMBARUS CLARKII Y AGUA EN EL PARQUE NACIONAL DE DOÑANA (2011-2014)

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El objetivo del presente trabajo es la determinación de principios activos farmacológicos de en muestras de musculo abdominal y vísceras de la especie animal *Procambarus Clarkii (P.Clarkii)* y aguas, procedentes del Parque Nacional de Doñana (PND). Nuestro grupo de investigación lleva participando numerosos años en proyectos de investigación [CTM2006-08960-C02-02, CTM2009-12858-C02-01 y CTM2012-38720-C03-01] que evalúan la presencia de contaminantes en los organismos vivos del Parque Nacional de Doñana a fin de encontrar bioindicadores que muestren de forma inequívoca situaciones de estrés ambiental en dicho entorno producidos por efectos de la contaminación de diferentes fuentes, entre estos contaminantes, se encuentran los principios activos farmacológicos que se incluyen dentro del grupo de los llamados contaminantes emergentes. De todos los contaminantes emergentes, los principios activos farmacológicos han generado una gran preocupación científica e impacto social en los últimos años, debido principalmente a su uso cotidiano y a la falta de preocupación social sobre su uso durante muchos años.

En este estudio, se ha desarrollado y optimizado un procedimiento analítico para la determinación de principios activos farmacológicos pertenecientes a tetraciclinas (oxitetraciclina y clortetraciclina), penicilinas (amoxicilina), trimetropin, sulfonamidas (sulfadiazina, sulfametazina, sulfamerazina y sulfametoxazol), fluoroquinolonas (marbofloxacina, danofloxacina, enrofloxacina, flumequina, gatifloxacina, grepafloxacina, norfloxacina y ciprofloxacina), anfenicoles (cloranfenicol, tianfenicol y fluorfenicol), anti-inflamatorios (ácido salicílico e ibuprofeno), antiepileptico (carbamazepina) y beta-bloqueante (atenolol) mediante HPLC-MS/MS en muestras de agua, musculo abdominal y vísceras de *P. Clarkii*..

La separación cromatográfica se diseñó para obtener una eficiente separación de los analitos a fin de obtener la máxima sensibilidad con la menor cantidad de tamaño muestral. Se llevó a cabo en una columna Eclipse XDB- C_{18} (150 x 3 mm I.D., de 3.5 µm tamaño partícula) y una fase móvil constituida por ácido fórmico al 0.1 % (A) y acetonitrilo (B) en modo gradiente durante 21 minutos.

El estudio se realizó sobre muestras de agua y *P. Clarkii* capturados de forma general en primavera (mayo-junio) de 2011-2014 en seis puntos de muestreo distribuidos en el propio parque, así como el entorno del PND, siendo estos: Arroyo de la Rocina curso alto (Coordenadas UTM= X= 178653, Y=4119937), Arroyo de la Rocina curso bajo (Coordenadas UTM= X= 187036, Y=4116086), Arroyo el Partido (Coordenadas UTM= X= 191173, Y=412977), Arroyo El Ajolí (Coordenadas UTM= X= 192352, Y= 4115565), Lucio del Palacio (Coordenadas UTM= X= 193800,Y=4099515) y Matochal (Coordenadas UTM= X= 208681, Y=4102207). Las muestras de agua fueron determinadas de forma directa previa microfiltración. El tratamiento de muestra de *P. Clarkii* se realizó sobre tejido liofilizado, usando como extractante 10 mL de una mezcla acetonitrilo:agua 50:50 (v/v) a la que se añadió 50µL de proteinasa-k y 5 µL de ácido fórmico puro, mediante aplicación de energía microondas (5 min, 40 W potencia), posteriormente se centrifugaron a 7000 rpm durante 10 min, se evaporaron hasta casi sequedad bajo una corriente de N_2 y se reconstituyeron con 1 mL de ácido fórmico al 0.1%.

Entre los resultados más relevantes cabe destacar la presencia de flumequina en el músculo abdominal de *P. Clarkii* en el punto de Ajolí, así como sulfametoxazol y carbamazepina en el Matochal. Asimismo cabe destacar la presencia de ácido salicílico e ibuprofeno en el Lucio de Palacio, punto que originalmente se eligió como ausente de contaminación por su situación.

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BIOMARCADORES DE ESTRÉS OXIDATIVO COMO INDICADORES DE TOXICIDAD METÁLICA EN DORADAS (*Sparus aurata,* L.) EXPUESTAS A COBRE Y PLOMO

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El efecto de la acumulación y contaminación de metales pesados, como el Cu y Pb, en peces de interés comercial tiene una gran importancia debido a los riesgos que para la salud puede tener su ingesta. Dado que el estrés oxidativo parece estar producido por la toxicidad de los contaminantes, se han realizado bioensayos de toxicidad donde se ha evaluado la inducción y respuesta antioxidante en diversos órganos de ejemplares de dorada (*Sparus aurata, L.*) debida a la exposición a ambos metales. Para ello, se realizaron ensayos en el laboratorio con doradas en fase juvenil que fueron expuestas a concentraciones nominales de Cu y Pb de 0 (Controles), 0,01; 0,1; 1; y 10 ppm. Las concentraciones de los metales en el agua de los ensayos fueron analizadas mediante ICP-AES o DPASV y la de los tejidos mediante ICP-MS. Se evaluaron como biomarcador de daño oxidativo el nivel de hidroperóxidos (ROOH) y como respuesta antioxidante se evaluaron tanto la actividad de la superóxido dismutasa (SOD) y la catalasa (CAT). Todos estos biomarcadores se estudiaron en tejidos de branquia, músculo, cerebro e hígado, así como los niveles metálicos acumulados.

La concentración más alta de Cu (10 ppm) y en el período más largo (96 h) resultó en una mortalidad total. En el caso del Pb, la tasa de mortalidad fue nula para todas las concentraciones estudiadas.

En general, los niveles de ROOH se incrementan con la concentración de Cu y la duración de la exposición en todos los órganos. Por su parte, la actividad SOD muestra un comportamiento bimodal. Así, a las 24 h tiende a inhibirse a concentraciones altas de Cu en las branquias, el hígado y el cerebro, pero es estimulada a las 96 h en estos mismos órganos, especialmente en las branquias. En el músculo no se producen variaciones respecto al control. Finalmente, se observa que la actividad CAT se inhibe en todos los órganos.

En el caso del Pb, los niveles de ROOH crecen en todos los órganos para concentraciones altas de metal tras 96 h de exposición; y así mismo tras 24 h en las branquias y el hígado. Ello coincide con un aumento importante en la actividad CAT en estos mismos órganos, a las 24 y 96 h en hígado y cerebro, y sólo a las 96 h en las branquias. No se detectó alteración de los niveles de ROOH ni de la CAT en el músculo, pero si una reducción en la actividad SOD a las 24 h.

De estos estudios se puede concluir que los parámetros relacionados con el estrés oxidativo son biomarcadores de toxicidad metálica, eficaces en la mayoría de los casos para indicar niveles de contaminación en el medio.

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SYNTHESIS AND CHARACTERIZATION OF A NEW NANOSORBENT BASED ON FUNCTIONALIZED MAGNETIC NANOPARTICLES AND ITS USE IN THE DETERMINATION OF MERCURY BY FI-CV-ETAAS

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In this work, a new chelating sorbent which employs 1,5-bis(di-2-pyridil)methylene thiocarbohydrazide as the functional group and magnetic nanoparticles (MNPs) as its support (DPTH-MNP) was synthetized and characterized. The MNPs were prepared by coprecipitation of Fe^{+2} and Fe^{+3} with NH_3 and then coated with silica in order to easily bind the support and the functionalizing molecule. The aim of the synthesis of this material is applying it as a solid-phase extracting agent and evaluating its potential for the extraction and pre-concentration of trace amounts of analytes present in biological and environmental samples with on-line methods. The MNPs' magnetic core would allow overcoming the usual backpressure problems that happen in solid-phase extraction methods thanks to the possibility of immobilizing the MNPs by applying an external magnetic field. From the study of its adsorption capacity toward metal ions, mercury and antimony were the most retained. Thus, a flow injection solid phase extraction and cold vapor generation method for mercury determination based on the use of this new chelating nanosorbent was optimized. The greatest efforts were put into the reactor design to minimize compaction and loss of nanosorbent. The knotted reactor shown in Figure 1 was chosen as the best. Then, chemical and flow variables were optimized by Central composite designs (CCDs). The method developed has showed to be useful for the automatic pre-concentration and determination of mercury in environmental and biological samples. The determination was performed using electrothermal atomic absorption spectrometry (ETAAS). Under the optimum conditions, pH 5 and 120 s preconcentration time, the enrichment factor was 5.33; the detection limit (3σ) was 7.8 ng L^{-1}; the determination limit (10σ) was 99 ng L^{-1}; and the precisions (calculated for 10 replicate determinations at a 1 and 5 μg L^{-1} standards) were 1.7 and 1.9 % (RSD), respectively. Two linear calibration graphs were obtained, from the determination limits to 10 μg L^{-1} and from 10 to at least 50 μg L^{-1}. From the comparison with other similar methods found in the bibliography, the detection limit and precisions calculated with our method were better. In order to evaluate the accurate and applicability of the method, the analysis of five certified samples LGC 6016 estuarine water, TMDA 54.4 fortified lake water, SRM 2976 mussel tissue, TORT-1 lobster hepatopancreas and DOLT-1 dogfish liver by standard addition and external calibration, were addressed. The results showed good agreement between the certified values, or added amounts of mercury, and the found concentrations. The method was successfully applied to the determination of mercury in sea-water samples collected in the Málaga Bay.

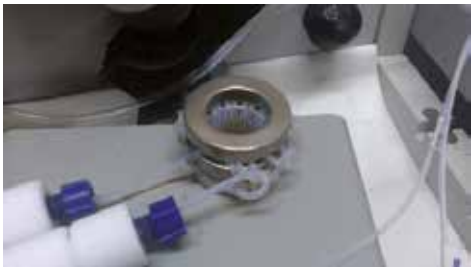

Figure 1. Knotted reactor to a Nd magnet as a sandwich between other two Nd magnets

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PESTICIDE RESIDUES IN BEE WAX SAMPLES BY MICROFLOW-LC-MS/MS AND GC-MS/MS METHODS

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The honey bee population in apiaries, where EU represents the second major global producer of honey producing 23% of the global production, and which is used to provide pollination system management, is facing growing threats from pests and diseases; and declines in bee colony numbers are still being registered as much in the EU as in other parts of the world [1]. Various factors have been identified in causing the reduction in bee and other pollinators including an expansion of pathogens, the incorrect use of phytosanitary products and/or environmental contaminants, along with other factors such as loss or fragmentation of habitat, invasive species and/or climate change [2]. In addition, an emerging problem for apiculture is caused by the fact that bee wax is widely recycled, thus leading to a progressive accumulation of pesticides in it [3].

The aim of this analytical study is to develop improved methodologies for pesticides multiresidues of high sensitivity and expanded scopes, and reports pesticide occurrence on bee wax. To provide new data and insights, bee wax samples were analyzed for 308 pesticide residues (including acaricides, insecticides, fungicides and herbicides).
Two multi-residue methods using microflow-liquid chromatography system coupled to a triple quadrupole mass spectrometer and gas chromatography coupled to a triple quadrupole mass spectrometer were the analytical tools for the quantitative analysis of pesticide residues. The samples were extracted using a QuEChERS based method, including a freezing-out clean up step in order to reduce the amount of matrix constituents.

Recoveries at concentration levels of 5 and 50 µg kg^{-1} were within the 70-120 % range with an associated precision RSD $\leq$ 20%. The LOQ values were mostly in the range of 0.1 and 10.0 µg kg^{-1} for the most target pesticides. The methods were applied to determine residue levels in 100 bee wax samples collected from apiaries of different regions of Spain. The total load of pesticide residues ranged from 114 to 9557 µg kg^{-1} for the collected bee wax samples. Over 48 % of the samples contained a total load of pesticide residues lower than 1000 µg kg^{-1}, 39 % in the range of 2000-5000 µg kg^{-1} and 13 % higher than 5000 µg kg^{-1}.

The most commonly detected pesticide residues were the acaricides used for Varroa mite control: Fluvalinate-Tau (100%), Coumaphos (79%) and Amitraz (17%); the organophosphate insecticide Chlorphenvinphos (94%) and the pyrethroid insecticide Acrinathrin (52%).

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MULTIDISCIPLINARY APPROACH TO MONITOR AND ASSESS THE EFFECTS ON MUSSELS EXPOSED TO HYDROPHOBIC ORGANIC MICRO-CONTAMINANTS

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In this work we have combined and fine-tuned the monitoring of aquatic contaminants and the bioanalytical assays to get a better understanding of the potential effects attributed to the controlled exposure of mussels to organic micro-contaminants.

The experiments were held in aquaria set-ups at controlled conditions at the PIE and *Mytilus galloprovincialis* mussels were chosen as target organisms. Up to three exposure experiments were carried out in different tanks with mussels exposed to a cocktail of alkylphenols, organophosphorous pesticides, organochlorine pesticides (DDT related), hexachlorocyclohexanes (HCH related), musk fragrances and phthalates, varying the concentrations from 10 to 1000 $ng \cdot l^{-1}$. Mussels were retrieved from the exposure and control tanks for the analysis after 2, 4, 7, 10 and 14 days. Simultaneously, the concentration of the target compounds in contaminated tanks was continuously monitored by both active and passive sampling measurements.

In this study we have included the following four steps. First, in the case of passive sampling, polydimethylsiloxane (PDMS stir bars) and polyethersulfone tubes (PES) were used. The sampling rates (R, $ml \cdot day^{-1}$) of these polymers were obtained in parallel experiments and PDMS and PES materials were analysed respectively by thermal desorption-gas chromatography mass spectrometry and large volume injection – programmable temperature vaporizer – gas chromatography – mass spectrometry, after the solvent stripping of PES tubes. Then, the uptake of contaminants was determined in pools of freeze-dried mussels by matrix solid-phase dispersion coupled to gas chromatography mass spectrometry. Additionally, foot muscle, gonad and hemolymph were extracted from the exposed and non-exposed mussels (at least 10 organisms per tank) to build the 1H-Nuclear Magnetic Resonance (1H-NMR) metabolomic profiles and to proceed with the discriminant analysis. The tissues were snap frozen in $N_2(l)$ and homogenized before the methanol/chloroform/water extraction, and both the polar and non-polar fractions were analysed (1H NMR and JRES in a 500 MHz instrument). The multivariate analysis of all collected spectra was carried out by means of the PLS_Toolbox (Matlab). Finally, the histological analysis included the digestive glands and gonads. In this case, the tissues were paraffin embedded, cut on microtome and stained with haematoxylin-eosin.

According to the obtained results, stir-bars offer more robust results since the sampling rates are statistically equivalents at all salinities, and therefore, we can provide freely available concentrations instead of the total ones. Besides, the biological inner variability (environment, genetic, sex, age, or feeding) and the ability of the metabolome to change so readily masks partially the effects of chemical stressors in the metabolic differences between healthy and stressed mussels. However, metabolomics results confirm the class clustering based on the biochemical profiles in the studied tissues and biofluid. Moreover, the most significant and visible biological effect was the induction of a premature spawning (a meaningful change in the reproductive cycle of the mussels) due to the subjected stress for the exposure to the contaminants cocktail.

As a final remark, and taking into account that the impact of anthropogenic stressors and to assess the marine ecosystem health is a very complex issue, it is worth mentioning that we have assured the suitability of the methodology for future experiments.

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UPTAKE OF TONALIDE, GALAXOLIDE AND BISPHENOL-A BY CARROT AND COMPARISON WITH CONCENTRATIONS DETERMINED BY MEANS OF POLYMERIC MATERIALS

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Agricultural application of sewage sludge has become the most widespread method for disposal since it is the most economical outlet for sludge. However, concern about the risk of organic pollutants present in the sludge and their possible translocation or accumulation in crops cultivated in soil amended with compost is gaining attention. Therefore, uptake experiments in soils contaminated or fortified with organic pollutants have increased in the literature [1-3].
In this work, a known amount of compost (according to the experiment set) was weighed, covered with acetone, fortified with the corresponding analytes (tonalide, AHTN, galaxolide, HHCB and bisphenol-A, BPA) at 5000 ng g^{-1} concentration (high level) and stirred for 24 h. After that, it was placed under a fume hood for solvent evaporation and the sample was aged for one week. The compost was always thoroughly manually mixed with the soil. Besides, a (95:5) soil: compost mixtures containing AHTN and HHCB at an average concentration of 33-21 ng g^{-1} and BPA fortified at 500 ng g^{-1} were also used (low level). Carrots were cultivated in (95: 5, w/w) soil: fortified compost mixture under controlled environmental conditions in a greenhouse (temperature was set to 25 ºC during the day and at 18 ºC during the night with a 14-h day length and a relative humidity of 50 % during the day and 60 % overnight) and they were regularly watered with Hoagland nutritive solution. In all the experiments polymeric materials such as silicone rod (SR), polyethersulfone (PES) and polyoxymethylene (POM) were also inserted in the amended soil during the cultivation period (12-15 weeks) in order to test whether they were useful to predict the current bioavailability of the target compounds. Carrot plants were harvested, cleaned with Milli-Q water and divided in different compartments such as peel, core and leaves, freeze-dried and storage at - 20°C before analysis. AHTN and HHCB determination was performed by means of gas chromatography-mass spectrometry (GC-MS) and BPA was measured using liquid chromatography-triple quadrupole-tandem MS (LC-MS/MS).

Concerning musk fragrances (HHCB and AHTN), no degradation was observed during the cultivation period, but the nominal concentration differed from the detected compounds concentrations before the initial of the experiments which could be related with losses during the fortification step. The same behaviour was observed by Macherius et al. [3] during the uptake of HHCB, AHTN and triclosan by carrot, barley and meadow fescue. In the case of BPA, however, a dramatically degradation was observed in both cases, low and high concentration levels after the harvesting period. Due to the degradation, BPA was not observed in the different carrot compartments. The studied fragrances were detected in all the compartments of the carrot plants except at the low level and values lower than MDLs were determined for core compartment in the case of all the experiments. The highest concentration was measured in root peel and similar values were obtained when compost was fortified at high level for core and leaves. On the other hand, despite similar structural properties and log K_{ow} values of AHTN (5.7) and HHCB (5.9), their concentrations measured in root peel differed significantly. HHCB tended to accumulate more than AHTN in all the plant compartments. The same tendency was observed by Macherius et al. [3]. Regarding to the tested polymeric materials, although the highest bioconcentration factors (BCFs) were determined for SR, the best correlation was obtained between BCF_{peel} and BCF_{PES} for the fragances.

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APPLICATION OF MERCURY STABLE ISOTOPE FRACTIONATION TO EXPLORE SOURCES AND FATE OF POLLUTION IN A MINING AREA

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Mercury (Hg) is a global pollutant with a complex biogeochemical cycle. This element has seven stable isotopes and can undergo isotopic fractionation processes, both mass-dependent and mass-independent. In the environment, Hg can be fractionated during natural or industrial processes and the application of Hg isotopes as tracers in environmental systems has emerged in the past decade. Previous studies have demonstrated that stable isotopes of Hg are particularly efficient to trace Hg pollution sources and transformations undergone by Hg in the environment. In this context, analytical advances (mainly involving the use of multicollector-ICPMS) have been required to increase the sensitivity of traditional metal isotope methods since the precise determination of the variations in Hg isotope compositions represents a promising research area.

The Almadén area (Spain) is the largest Hg mining district and one of the most impacted Hg areas worldwide becoming an exceptional case study for Hg risk assessment. Past investigations in this mining district have focused on the study of Hg contamination, while little attention has been paid to Hg isotope signatures in samples from this area until now. Hence, the aim of this work has been to explore the variations in Hg isotope compositions in environmental samples (sediments and lichens) from an area directly impacted by Almadén mining activities and its surroundings to identify and track Hg pollution sources.

A large variability in mass dependent isotope fractionation (MDF) was recorded in both types of samples. Sediments presented δ^{202}Hg values ranging from -1.86±0.21‰ to 0.01±0.15‰, whereas lichens displayed δ^{202}Hg values from -1.95 to -0.40‰ (2SD=0.15‰). In contrast, no remarkable mass independent fractionation (MIF) was observed in sediments from this study, while more negative Δ^{201}Hg values for lichens ranged between -0.12 to -0.21‰ (2SD=0.06‰). Both MDF and MIF were generally in agreement with values previously reported for sediments and lichens from other sites close to contamination sources and/or for background sites.

A dilution of Hg contamination and Hg isotope signatures downstream the mines was found in sediment and lichens suggesting that Hg isotope compositions in these samples are providing a direct assessment of Hg inputs and exposure from the mining district.

Therefore, this study confirms the applicability of Hg isotope signatures of environmental samples such as sediments and lichens as an effective and complementary tool for tracing aquatic and atmospheric Hg contamination sources and better understanding the transfer of Hg from mining activities to the environment.

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POST-RUN TARGET SCREENING STRATEGY FOR PESTICIDE METABOLITES IN AMBIENT AIR OF VALENCIA REGION (SPAIN)

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Pesticide metabolites are a matter of concern, as they may have intrinsic properties comparable to the active parent substance in terms of certain toxicological properties that make necessary control of their presence in air. In this study, a methodology for the systematic post-run screening of pesticide metabolites was created, using ultra high performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry (UHPLC-HRMS).

An accurate mass-database was constructed consisting of 238 pesticide metabolites using experimental and theoretical data available from the literature [1, 2]. For each substance, the screening database included the elemental composition (molecular formula) and the theoretical accurate mass of the monitored (quasi)ion. Furthermore, information about fragments were included when available in the literature [2-4], mainly from HRMS (exact mass) and QqQ (nominal mass) studies.

Samples (PM10) were collected in the atmosphere of Valencia Region (Spain), using a large-volume sampler from Digitel (Madrid) and quartz filters of 150 mm of diameter, supplied by Munktell filter AB (Falun, Sweden). A sampling flow of 30 m^3h^{-1} that provides a total volume of around 760 m^3 was employed. Samples were collected from May to June 2014 in Burriana site. Burriana is a rural station situated in Valencia region and surrounded by many citrus crops.
A generic extraction method using ethyl acetate was employed. [5]. Pesticide metabolites were analyzed by UHPLC-HRMS.

The proposed strategy approach was tested on 12 ambient air samples from the Burriana site (Spain). After performing post-target analysis, 25 pesticide metabolites were tentatively identified, using the defined criteria for identification. For 10 of these metabolites, standards solutions were available to confirm. All 10 metabolites were confirmed. Terburthylazine-2-OH, methiocarb-sulfoxide and ethiofencarb-sulfoxide were the pesticide metabolites more detected, with frequencies of detection of 100 %.

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MERCURY (II) AND METHYLMERCURY DETERMINATION IN WATER BY LIQUID CHROMATOGRAPHY HYPHENATED TO COLD VAPOUR ATOMIC FLUORESCENCE SPECTROMETRY AFTER ONLINE SHORT-COLUMN PRECONCENTRATION

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Mercury is present in the environment in several inorganic and organic forms of varying toxic character. Inorganic forms can be deposited on land and water where biomethylation processes can cause species transformation to the methylated more toxic species. Methylmercury can be accumulated through the aquatic food chain in fish, but also it can be accumulated in soils and different types of crops such as vegetables and cereals. Furthermore, even if mercury and its compounds are included in lists of priority and hazardous substances, for the assessment of for example, water quality, Water Directive considers only total mercury concentration. Thus species-specific regulations must be enforceable, and this fact would require validated routine control methods and suitable reference materials.

There are many proposals for the determination of mercury species; however, especially for the investigation to low contaminated matrices but with concentrations above the tolerated levels, LODs need to be decreased. One strategy to overcome this problem is to use solid-phase preconcentration methods both in batch and online coupled systems. For low polluted samples (fish, sediments and drinking water), the use of batch preconcentration systems have been proposed for improving detection limits. However, there are few reports dealing with the use of such preconcentration methods on-line. Thus, the development of coupling systems including on-line preconcentration and LC-AFS would be suitable for control laboratories working with mercury speciation.

This work reports a method developed for the simultaneous determination of methylmercury ($MeHg^+$) and mercury(II) (Hg^{2+}) species in water by liquid chromatography coupled to online UV irradiation and cold vapour atomic fluorescence spectrometry (LC-UV-CV-AFS) after online short-column preconcentration. It is focused on systematic studies of several variables to establish the maximum species recoveries, preconcentration factors and good reproducibility. The optimum results obtained were the following: 0.07 mmol L^{-1} 2-mercaptoethanol as a complexing agent, precolumn conditioning with the mobile phase: a mixture of 80% of methanol (MeOH) and 20% of the following buffer: 0.0015 mol L^{-1} ammonium pyrrolidine dithiocarbamate (APDC) and 0.01 mol L^{-1} ammonium acetate (NH_4CH_3COO) at pH 5.5, 2 cm precolumn length and 2 mL min^{-1} sample flow.

This method was applied to three water samples with different mineralisation contents. Various tests, based on spikes, were performed on each sample. A breakthrough volume of 4 mL was found. The recovery values of 72 ± 3% and 81 ± 5% for $MeHg^+$ and Hg^{2+}, respectively, were obtained regardless of the matrix composition, and the PF values were 30 and 32 for $MeHg^+$ and Hg^{2+}, respectively. The accuracy of the preconcentration method was verified by analysing a certified reference material. The detection limits (LDs) obtained were 10 ng L^{-1} for $MeHg^+$ and 2 ng L^{-1} for Hg^{2+}. The quantification limits (LQs) were 50 ng L^{-1} for both species. The established analytical online preconcentration method is suitable for the quantification of mercury species in a wide range of environmental waters.

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NUEVAS METODOLOGÍAS ANALÍTICAS PARA EL CONTROL DE PLAGAS EN MUESTRAS DE INTERÉS AGROALIMENTARIO

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La producción de alimentos seguros y saludables es una prioridad en la Unión Europea (UE) y en todo el mundo. Las plagas de insectos son las principales competidoras de los seres humanos por los recursos generados por la agricultura [1], teniendo un profundo impacto económico y ambiental. Además de las pérdidas económicas causadas por la acción directa de las plagas de insectos, las medidas adoptadas para su control también pueden causar pérdidas indirectas debidas a tanto a la adquisición y aplicación de insecticidas como a los daños causados por la contaminación ambiental. En la mayoría de los casos, los insecticidas han abordado este problema de forma eficaz. Sin embargo, su uso en la agricultura tiene una percepción negativa debido a sus efectos nocivos sobre los seres humanos y su riesgo ambiental [2,3]. Además, su amplio uso favorece el desarrollo de plagas resistentes a estos, perjudica a los insectos beneficiosos, y en ocasiones pueden ocasionar incluso la aparición de plagas secundarias [4]. Por todo lo anteriormente expuesto, el uso único de pesticidas no es la solución ideal. Por fortuna, van apareciendo otras alternativas como el control biológico y genético de las plagas, uso de hormonas y feromonas, la radiación, entre otras. El uso combinado de estas técnicas complementado cuando sea necesario con la utilización moderada de pesticidas, recibe el nombre de Control Integrado de Plagas, programa que está siendo impulsado por la FAO como método idóneo para llevar a cabo una agricultura sostenible. Todos estos métodos tienen una etapa clave: el control de la población de la plaga con objeto de la detección temprana y del estudio de la evolución de las mismas. En la actualidad, este control se basa en la evaluación física de los cultivos y en el recuento exhaustivo de capturas en trampas feromonadas. Por ello es conveniente disponer de alternativas analíticas que permitan la detección temprana de la plaga sobre la base de indicadores más objetivos.

En esta comunicación, se propone el diseño de nuevas metodologías para el control de plagas en muestras de interés agroalimentario, usando los componentes y característicos de sus feromonas sexuales como indicadores basadas en la combinación de espacio de cabeza (HS) y cromatografía de gases con detección por espectrometría de masas (GC/MS). Las metodologías desarrolladas permiten la determinación de los diferentes componentes específicos de la plaga de la Tuta Absoluta en tomates [5], de la mosca del olivo en aceitunas [6] y de la polilla de la vid en uvas. El análisis de muestras reales determinó la presencia de las diversas plagas. Estos resultados se confirmaron con la información publicada por la RAIF (Red de Alerta e Información Fitosanitaria de la Junta de Andalucía). Los métodos desarrollados se caracterizan por su simplicidad, automatización y robustez, siguen los principios de la química analítica verde y suponen una alternativa fiable a los métodos actualmente disponibles y que no permiten la detección temprana de las plagas.

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EVALUACIÓN DE LA DISPONIBILIDAD METÁLICA MEDIANTE ESPECIACIÓN CINÉTICA DE SEDIMENTOS DE LA ENSENADA DE PORT-REITZ-KILINDINI (MOMBASA, KENIA)

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La ensenada de Port-Reitz-Kilindini es una de las dos más importantes que existen en Mombasa (Kenia) rodeada de manglares, con una profundidad irregular. Se encuentra en uno de los laterales de la isla de Mombasa, que está separada del continente por dos arroyos; Tudor Creek y Kilindini Harbour, donde se encuentra el principal puerto de Mombasa, el cual se ha dragado ampliamente para profundizar el canal y activar el puerto. Todo ello ha alterado la naturaleza de los fondos, que ya presentan de por sí una gran influencia antropogénica, por lo que resulta de gran interés conocer el potencial tóxico de tipo metálico que estos fondos poseen después de estos procesos de dragado.

Por ello, se realizó un muestreo en diciembre de 2013 de sedimentos superficiales. Los sedimentos fueron caracterizados y se determinaron los contenidos totales de diversos metales pesados tales como Co, Ni, Pb, Cr, Cu, Zn, Cd, V, entre otros. Se encontraron concentraciones mayores en aquellos puntos con elevado contenido en materia orgánica, superando en algunos puntos los niveles guía de calidad de sedimentos para Cr, Ni y Pb.

Con objeto de definir la fracción metálica más disponible y potencialmente más tóxica presente en estos sedimentos se realizaron estudios de especiación cinética, los cuales proporcionan una buena aproximación del comportamiento de las especies en el medio natural. Estos resultados fueron comparados con aquellos que suministra la primera fase del método de extracción secuencial BCR, que proporciona la fracción de metal lábil intercambiable. Para ello, se seleccionó el sedimento de uno de los puntos de muestreo, situado en la zona central de la cuenca y bastante afectado por la actividad industrial y urbana de esta región. Se emplearon dos extractantes para estos estudios cinéticos: el ácido etileldiaminotetraacético (AEDT) y el ácido acético. El primero es muy común su uso en estudios edafológicos y el segundo es el empleado en la 1ª fase del método BCR de especiación. Se obtuvieron los extractos a diferentes tiempos (tiempos cortos de 0; 4; 6; 8; 10; 20 y 60 minutos; y tiempos largos de 1; 5; 16 y 36 horas), los cuales fueron analizados por Espectroscopía de Emisión Atómica con Plasma Acoplado Inductivamente (ICP-AES).

A partir de los resultados obtenidos, se modelizó la cinética de disponibilidad metálica lábil, observándose un comportamiento muy similar para la mayoría de los metales estudiados en presencia de AEDT, aunque destacando con valores ligeramente superiores el Zn, Pb y Cu. Estos resultados indican que se hacen necesario estudios integrados sobre el impacto de esta contaminación sobre la biota de la zona.

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RELATIONSHIP BETWEEN METAL CONCENTRATIONS IN SEDIMENTS AND CLAMS FROM THE MOROCCAN MEDITERRANEAN COAST

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It is well known that sediments act as a reservoir of metals in aquatic environment; therefore they present higher metal concentrations than water. As a consequence, organisms living in contact with sediments, mainly filter-feeding bivalves, are directly exposed to these high metal concentrations and they can accumulate these elements in their tissues without metabolizing them noticeably. For this reason, bivalves are considered as excellent bioindicators. They have long been employed in the monitoring of toxic substances in aquatic environment, as metals, due to they have the ability to accumulate different chemicals and present long lifespan, high density and sessile life style.

In this work, metals concentration in two different clam species from Moroccan Mediterranean coast, *Calista chione* and *Acanthocardia tube*, has been evaluated and compared with metals concentration in sediments from the same area.

Organisms, as well sediments, were taken in two different sampling campaigns, October 2007 and October 2008. A compose sample was obtained for each sampling station removing the soft tissue from the shell and after lyophilization and homogenization of different individuals. Then, a microwave acid digestion was performed following the method EPA 3052 and metals were analyzed by atomic absorption spectrometry. The effect of some variables on the extraction efficiency was evaluated using a certified material. For some elements, addition of hydrogen peroxide was necessary to obtain acceptable recoveries.
Finally, spatial and temporal variability in metal concentrations for each species was evaluated. In addition, a comparison of bioaccumulation ability of the two clam species was also performed. The relationship between metals concentration in the tissues of the organisms and the sediment samples was also evaluated using the Pearson correlation ($p < 0.05$).

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BIOTRANSFORMATION AND BIOACCUMULATION OF ANTIDEPRESSANTS IN ZEBRAFISH LARVAE BY GC-MS AND LC-MS/MS

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Antidepressants as selective serotonin reuptake inhibitors (SSRI) are drugs widely used to treat different psychiatric disorders in patients with severe depression or eating disorders including anorexia and bulimia nervosa [1-3]. As a consequence of high use of SSRIs, they have been detected in aquatic environment along to analgesics, antibiotics, antiepileptic and hormones, due to their occurrence through to wastewater treatment plants which could lead to physiological and behavioral effects on aquatic organisms and chronic environmental toxic effects [4]. This fact has carried out to a public, regulatory and scientific concern about their environmental impacts. European legislation (REACH) required evaluation of the ecotoxicity in which parameters such as bioaccumulation and persistence are studied. OECD Guideline 305 describes experimental methods to determine bioconcentration factors (BCFs) of chemical compounds using adult fishes but it is a complex procedure that requires highly costs and uses more than one hundred animals [5]. For this reason, with main aims of reducing animal suffering and high cost, we propose an alternative methodology using zebrafish larvae as model organism to calculate BCFs.

The bioconcentration assay consists of two steps: exposure (uptake, the larvae were exposed in a contaminated solution during 48 hours) and post-exposure (depuration which the larvae were exposed in a clean solution during 24 hours). To determine the bioaccumulation factors, a methodology was developed assessing different solvents and sorbents for clean-up in larvae samples. Acetonitrile with ultrasonic probe were chosen as extraction procedure followed by a dispersive SPE using C18 sorbent for clean-up. The determination of SSRIs in larvae samples were carried out with gas chromatography and liquid chromatography coupled to mass spectrometry (GC-MS and LC-MS/MS, respectively). The recoveries obtained were between 73-108% for all the analytes studied. Also, a methodology for determination of antidepressants SSRIs in culture media were optimized using a liquid-liquid extraction with toluene for extraction of selected compounds which allowed to obtain recoveries up to 80%.

The possible biotransformation of parent compounds in larvae samples can be studied in bioaccumulation assays at different times. This study will allow to know the metabolism of SSRI in Zebrafish larvae.

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CHARACTERIZATION OF SOLID WASTES FROM THE TAILING-PROCCESSING OF SILVER AMALGAMATION RESIDUES (JALES) FROM ZACATECAS (MEXICO)

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The residues (*jales*) from the colonial silver production by the amalgamation technique, extensively used in Zacatecas (Mexico) until 1820, were dispersed by the rivers of the region and deposited in low-lying areas used for crops and livestock farming. From 1920 onwards, there have been tailing-processing activities to recover precious metals from these soils, based upon their lixiviation with calcium thiosulfate and the subsequent recovery of silver and mercury in the lixiviate by reduction with metallic copper. Because of the low efficiency of the initial lixiviation step, the processed solids are expected to still contain relatively high concentrations of mercury, silver, lead and other dangerous chemical elements. These wastes are stored in mounds within the treatment plant, usually without further control, so there is a non-negligible environmental risk to the population of the nearby towns. In this communication, the results of a survey carried out to assess the chemical stability and environmental safety of the residues from the storing mound of a treatment plant located in Tacoaleche (valley of Zacatecas, Mexico), are presented. Samples were collected in two cuts of the mound at different depths, ranging from 1 to 10 m, in 1-meter intervals, and closely corresponding to the activity of the 10 last years. The 19 samples were initially characterized by X-Ray Diffractometry (XRD), finding in all cases a uniform mineralogical composition, based upon the majority presence of quartz and plagioclases.

The investigated elements were Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn. The total concentration of Hg was determined with a MILESTONE DMA-80 Direct Mercury Analyzer, whereas the other elements were measured by X-Ray Fluorescence (XRF). The contents of Ag, Cd and Hg ranged between 20-50 mg kg^{-1}; As, Cr and Ni between 150-250 mg kg^{-1}; Cu between 600-700 mg kg^{-1} whereas Pb and Zn were around 2000-4000 mg kg^{-1}. The actual environmental risk of a solid residue polluted with toxic elements depends not only on the total metal concentrations, but also on their mobility or availability, usually evaluated by chemical fractionation. The total available (or pseudo-total) concentrations of the elements can be estimated after a single extraction of the residue with concentrated HNO_3/HCl (US-EPA 3051a norm) followed by their determination in the extracts by ICP-OES. The found percentages of pseudo-total elements ranged between 40-80 % for all metals, with exception of Cd (100%) and Hg (not evaluated). The analytical results were interpreted by multivariate statistical techniques: Hierarchical Clustering and Principal Component Analysis (PCA), which allowed to separate the information due to the samples from that corresponding to the chemical elements. Whereas no definite pattern was found for the samples, three different element associations were found for both total and pseudo-total concentrations: Ag-Cu-Pb, As-Cd-Zn and Cr-Ni. Lastly, the BCR sequential fractionation procedure was applied to the three more shallow samples at mound cut. This procedure allows distributing the total available concentrations into three fractions with decreasing availability, corresponding to elements bonded to (i) exchangeable and carbonate phases, (ii) iron/manganese oxide and hydroxide phases, (iii) organic and sulphide phases and a fourth and almost non-movilizable fraction named residual. By using multivariate techniques as above, it was found that elements Cd-Cu-Pb-Zn were associated to iron/manganese oxide and hydroxide phases whereas Ag- As- Cr-Ni were bonded to the residual fraction. The use of n-way PCA techniques confirmed these associations through a Tucker3 [2 2 1] model, that also showed that the samples have an almost common behavior with regards to the fractionation of these elements, unrelated to their spatial location in the mound.

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DISTRIBUTION OF TRACE ELEMENTS IN SEDIMENTS FROM A RIVER OF THE MIDWESTERN ARGENTINA

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Heavy and trace elements are a very hazardous group of pollutants because of their toxic and accumulative characteristics. They are non-biodegradable and undergo global eco-biological cycles, so their circulation amongst the different environmental compartments is a main concern. River sediments can act as temporary reservoirs from which those elements could be released back, but the true environmental risk must consider the total concentrations as well as their mobility/availability. Chemical fractionation, using single or sequential extractions, is the usual approach to evaluate metal mobility in sediments and several leaching/extraction tests have been developed and applied for these purposes.

Single extraction of metals with a strong acid such as concentrated HNO_3 (US-EPA 3051 norm) allow a rapid evaluation of the pseudo-total content, which is an estimation of the total available metals. On the other hand, sequential extraction procedures provide more information about the different metal-solid phase associations. The BCR sequential procedure, now SM&TP, is the standard approach for these studies and uses three reagents: acetic acid, hydroxylamine hydrochloride and hydrogen peroxide, yielding three fractions of decreasing mobility/availability associated with metals bonded to (i) exchangeable and carbonate phases, (ii) iron/manganese oxide and hydroxide phases, and (iii) organic and sulphide phases. A fourth fraction, labeled Residual, can be calculated by difference from the 'pseudo-total' contents or by digestion of the last residue. The total concentrations can be determined after total digestion of the sediment, or by a non-destructive technique such as X-Ray Fluorescence (XRF).

The Río Quinto (Argentina), an endorheic fluvial system is the main source of superficial water in the province of San Luis and its sediments have not be studied so far with regards to toxic metals. Sediment samples were collected at 10 different locations and characterized by X-Ray Diffractometry (XRD) to ensure their mineral homogeneity. The total contents of As, Cd, Cr, Cu, Ni, Pb and Zn in the sediment samples were determined by XRF, whereas the pseudo-total ones were measured by ICP-OES in the extracts obtained after application of US-EPA 3051 norm. Chemical fractionation was carried out by means of the BCR SMT&P sequential extractions procedure, determining the metal contents in each fraction by ICP-MS. All the procedures were validated with the appropriated Certified Reference Materials.

Pseudo-total contents (around 10 mg/kg for Cr and Zn and 1 mg kg^{-1} for the other elements) are below the reference values for environmental risk. The chemical fractionation results showed that the metals were mainly present in the Residual fraction. Results have been interpreted by multivariate statistical techniques: Hierarchical Clustering and normal and n-way Principal Component Analysis (PCA). In general, As, Pb and Zn showed a similar behavior, but different from that of Cd, Cr, Cu and Ni. It was also shown that Cu tends to concentrate in Fraction 3, while As and Pb tend to do it in Fraction 2. The best n-PCA model was the Tucker3 [2 2 1] with two significant interactions or factors, explaining the 66,1% of the information and confirming the above considerations.

Therefore, it can be concluded that the studied toxic metals present in the sediments of the Río Quinto do not pose any environmental risk.

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AMBIENT CONCENTRATIONS OF PM10, PM10-BOUND POLYCYCLIC AROMATIC HYDROCARBONS IN TWO URBAN SITES OF THE SOUTH OF GALICIA

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Polycyclic Aromatic Hydrocarbons (PAH) can be released to the atmosphere by natural and anthropogenic sources, being the human activity responsible of more than 90% of their atmospheric levels [1]. The human health risk associated to PAHs is higher in the urban atmospheres, considering the density of population, increasing vehicular traffic and scarce dispersion of the atmospheric pollutants [2]. One of the best characterised and most toxic PAH compound is benzo(a)pyrene (BaP), which is generally used as the indicator PAH.

An assessment of the air quality of Pontevedra and Vigo (South of Galicia) was performed by determining the ambient concentrations of PM10 and, PM10-bound polycyclic aromatic hydrocarbons (PAHs) in the period of May-July of 2009. The filters used are made of quartz fiber and subjected to pre-treatment at 400 ºC for 12h. Before and after sampling, they are maintained and kept in a conditioned room at 20 °C and 50% relative humidity as described in the standard EN 12341 (1998) for measuring gravimetric PM10. The samples were stored at -18 °C until analysis. We collected a total of 56 samples of PM10 for 24 hours (on a weekly basis) using a high-volume sampler Digitel DHA-80 in the stations of Mollabao (Pontevedra) and Coia (Vigo) which belong to the Galician Network Monitoring Air Quality. The filters were extracted using a microwave assisted solvent extraction with a mixture of hexane and acetone (1:1) at 105ºC. The extracts filtered were concentrated to dryness, redisolved in hexane and fractionated in silica gel column (partially deactivated). After an initial washing with hexane, PAHs were eluted with a mixture of DCM/hexane (30:70). This extracts, concentrated with rotary vacuum evaporator and to dryness with nitrogen, were identified by gas chromatograph coupled to a mass spectrometry detector (GC-MS/MS) using isotopic dilution with perylene-d^{12} to quantify the PAHs. Recoveries of PAHs ranged from 70% to 109%, while recoveries of naphthalene were very low (<60%) in some cases. The PM10 concentrations ranged from 12 to 35 $\mu g/m^3$ with the mean value of 20 $\mu g/m^3$ in Mollabao (Pontevedra) and from 12 to 134 $\mu g/m^3$ with the mean value of 34 $\mu g/m^3$ in Coia (Vigo). The total PAH concentrations ranged from 0,48 to 2,57 ng/m^3 in Pontevedra and from 0,98 to 9,23 ng/m^3 in Vigo, which were predominated by intermediate and high molecular weight PAHs. On average, the concentration of BaP was 0,11 ng/m^3 in Vigo whereas in Pontevedra all the samples, except one, presented concentrations below detection limit for this compound. The comparison of the PM10 and BaP determined with other Spanish and European urban sites and the limit or target values for health protection has revealed that the air quality of Pontevedra and Vigo for the above pollutants generally corresponds to the EU average. In this work, the carcinogenic character of the airborne PM10 of Pontevedra and Vigo was studied using different parameters: BaP equivalents, etc. [3]. Diagnostic ratios were used to discern regarding the main pollution sources in these cities in which the prevailing emission sources were related to traffic emissions. These Galician cities have excellent air quality with respect to this contaminants.

Acknowledgements
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DESARROLLO Y APLICACIÓN DE METODOLOGÍAS ANALÍTICAS PARA LA EVALUACIÓN DE LA CONTAMINACIÓN POR ELEMENTOS TRAZA Y RADIONÚCLIDOS EN SISTEMAS FLUVIALES

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La investigación sobre el efecto de las actividades humanas en los sistemas fluviales es un tema de gran interés y actualidad, debido a la presión excesiva que el ser humano está ejerciendo sobre estos entornos naturales. En este trabajo, se ha abordado un estudio analítico sobre diferentes parámetros físico-químicos (temperatura, conductividad, perfil de elementos y radionúclidos) en el entorno fluvial de la Central Nuclear de Almaraz ubicada en las proximidades del río Tajo, con el fin de evaluar si algunos de estos parámetros pudieran estar alterados como consecuencia de la actividad de dicha instalación industrial. Además de las medidas "in situ" de conductividad y temperatura se han aplicado dos metodologías de laboratorio: determinación de elementos traza por ICP-MS en muestras de aguas y determinación de radionúclidos en muestras de agua y sedimentos por espectroscopía gamma de alta resolución.

Se trata de una investigación preliminar, especialmente original en lo que se refiere al perfil completo de elementos que no se encuentra descrito en la bibliografía ni en los informes oficiales de la confederación hidrográfica para este tramo del río Tajo. Los resultados experimentales han puesto de manifiesto que la actividad de la central genera efectivamente una alteración de los niveles de algunos de los parámetros medidos, respecto al estado natural del río. En el caso de la temperatura, la conductividad y el perfil de elementos en aguas, se han relacionado los resultados experimentales con la actividad del sistema de refrigeración de la central, concretamente con el tratamiento de las aguas para su uso industrial y con la corrosión de los conductos de dicho sistema. Se trata en todo caso de alteraciones de escasa relevancia ambiental, que no producen superaciones de los valores guía o límite marcados por la legislación de protección de las aguas naturales, pero que han podido detectarse gracias a la elevada sensibilidad de la técnica ICP-MS empleada en la detección de elementos traza. En cuanto a los parámetros de radiactividad en aguas y sedimentos, se ha detectado la presencia de diferentes radionúclidos artificiales pero en concentraciones muy inferiores a las permitidas por la legislación, en concordancia con lo observado regularmente por los sistemas de vigilancia radiológica que operan en la zona.

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IDENTIFICATION OF ANTIMYCOTIC DRUGS TRANSFORMATION PRODUCTS GENERATED UNDER UV EXPOSURE BY HIGH RESOLUTION TANDEM MASS SPECTROMETRY

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Clotrimazole, ketoconazole and miconazole are three widespread antimycotic drugs that tend to be sorbed in activated sludge, during water treatment, at sewage treatment plants. Since they cannot be completely degraded [1, 2], the fraction that becomes a part of active sludge may end in agricultural soil as part of fertilizer preparations [3]. In the present work, the stability of these three pharmaceuticals under ultraviolet radiation (254 nm and 365 nm) has been tested.

For this purpose, the degradation kinetics of the compounds have been followed and the generated transformation products have been identified by liquid chromatography quadrupole time-of-flight mass spectrometry. Clotrimazole, ketoconazole and miconazole could be largely degraded, ranging half-lives between 8 and 16 minutes under 254 nm ultraviolet radiation and between 9 and 93 hours under 365 nm ultraviolet radiation. At the latter wavelength, clotrimazole resulted by far more stable than the other two antimycotics. Generated transformation products arose from the studied pharmaceuticals, which had been previously loaded in bulk silicone supports before exposing this body to the radiation, and were retained by this material. The chemical structures of these new compounds were characterized from high resolution full scan mass spectra and tandem mass spectra acquired by the quadrupole time-of-flight mass spectrometer, being de-chlorination, cleavage, intra-molecular cyclization and oxidation the most common degradation processes that happened during the experiments.

In further experiments carried out on sand, agricultural soil and water, most of the depicted transformation products were detected, indicating the suitability of this low-cost material to follow degradation processes of organic compounds. Degradation rates were matrix dependant, with increased stabilities when passing from silicone supports to sand and soil.

Finally, the eco-toxicity of the transformation products and their precursors was compared, estimating their 50% lethal concentration in the 48-h Daphnia Magna test with a toxicity estimation software. Two transformation products generated from the clotrimazole and another one from the miconazole were estimated to be more toxic than their precursors, the three species were generated through intra-molecular cyclization following a de-chlorination step. The rest of TPs, including all the substances generated from ketoconazole, were less toxic than their precursors.

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NOVEL METHOD FOR QUANTIFICATION OF Hg AND Se BIOMOLECULES IN MARINE TISSUES BY ICP-MS/MS (ICP-QQQ-MS)

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Monomethylmercury (MeHg) is by far the most toxic and the most commonly occurring organo-mercury compound, and is recognized as a major environmental pollution issue because it is biomagnificated through the aquatic trophic chain and, as a result, the average proportion of MeHg over total Hg in fish tissues can be up to 95% [2]. Contaminated seafood is the major route of exposure for humans to MeHg. Therefore, high number of studies of Hg speciation in fish and marine mammals have been carried out. Most of these studies have been focused on the edible part (muscle) and to the quantification of Hg species (inorganic Hg and MeHg) but little is known about the mechanisms that enables Hg uptake and subsequent bioaccumulation. It has been extensively discussed the Hg binding to sulphur and selenium containing biomolecules. This is an essential information for the assessment of its metabolic pathways and toxicological impacts. However, the exact nature of most Hg binding molecules is unknown and the identification and quantification of biomolecules containing Hg is a challenge.

The determination of Hg metabolic pathways is of particular interest in marine mammals since these animals exhibit the highest Hg levels in marine ecosystems and their elevated Hg tolerance is very intriguing. Therefore, the main aim of this work was to develop an analytical method to extract and quantify Hg and Se binding biomolecules in the water-soluble fraction of dolphin liver. The analysis have been carried out by inductively coupled plasma equipped with triple quadrupole mass spectrometry detector (ICP-QQQ-MS). This configuration allows to operate an ICP-MS instrument in MS/MS mode and this technology drastically improved detection limits for interfered elements, such as S and Se, and helps to improve the accurate quantification at trace levels in complex matrix, such as marine animal tissues. These capabilities have been exploited in this work for the analysis of dolphin liver extracts. The extractions have been carried out using soft conditions by homogenization with Tris-HCl buffer (0.1 M pH=7.4). Size exclusion chromatography (SEC) have been used for the separation of biomolecules. Different Hg and Se biomolecules standards have been evaluated and a quantification method was developed and used for the quantitative evaluation of different extraction conditions.

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SPECIATION OF SELENIUM IN TESTES OF TERRESTRIAL MAMMALS FROM A POLLUTED AREA

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Selenium is known to take part in the defence mechanisms against pollutants through a number of species such as selenoaminoacids, selenoenzymes or selenoproteins. In the particular case of testis, the concentration of Se may be related to the reproductive function, but it is still unknown which species are responsible for the biological functions of Se in this tissue. This is why a Se species profile is necessary in terrestrial mammals.

Red deer were obtained from a polluted mining area and the total Se content in testes was found to be 1.2 ± 0.5 μg g^{-1} (d.w.). One of the difficulties that we can find to perform speciation in tissues is to get a quantitative extraction of species of Se. The enzymatic hydrolysis procedures give the best results for extraction of species [1, 2]. However, a previous step of removal of fats is necessary, in order to avoid interferences with the enzymatic treatment. According to Quijano et al. [3], the fat content of the samples were extracted with methanol:chloroform (2:1, v:v) under sonication for 5 min. The lipid free tissue was incubated at 37 ºC for 24 h with non-specific protease "Pronase E" in phosphate buffer at pH 7.5 in two successive steps. The organic species of Se were extracted enzymatically in mild conditions. The combined extract was cleaned up by solid phase extraction in a C18 cartridge and analysed for Se by ICP-MS.

The Se speciation analysis is currently being carried out by LC-ICP-MS using different stationary phases and also by liquid chromatographic system coupled to a quadrupole-time of flight mass spectrometer (LC/Q-TOF) for confirmation.

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DETERMINATION OF PHARMACEUTICALS COMPOUNDS IN WATER AND SLUDGE SAMPLES USING SOLID PHASE EXTRACTION AND MICROWAVE ASSISTED EXTRACTION COMBINED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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Because of thousands of tons of pharmaceutical compounds are used every year, the interest in having information about their presence in the environment is growing up. Some of these compounds are not completely eliminated in wastewater treatment plants. As a consequence exists a large number of researches about the presence of these kind of compounds in the environment [1]. However, the presence of these pollutants is a health and environmental problem which legal regulation is still unsatisfactory [2].

In this study, extraction and purification methods have been developed for the determination of four fluorescent pharmaceuticals, three anti-inflammatory (acetylsalicylic acid, naproxen and ibuprofen) and a lipid regulator (gemfibrozil) in water and sludge samples from different treatment plants of Gran Canaria island. Solid Phase Extraction (SPE) procedure is used in water samples and Microwave Assisted Extraction (MAE) procedure is used in sludge samples. Both procedures are combined with Ultra-High Performance Liquid Chromatography with Fluorescence Detection (UHPLC-FD).

An UHPLC system with fluorescence detector (excitation wavelength, 230 nm; emission wavelengths, 292 nm for ibuprofen and gemfibrozil, 344 nm for naproxen and 387 nm for acetylsalicylic acid) and an Acquity UHPLC BEH C_{18} column (1.7 µm, 2.1 mmx50 mm) were employed. The mobile phases consisted in water with 0.015% formic acid (phase A) and methanol (phase B) at flow of 0.3 $mL \cdot min^{-1}$ using the following gradient: starts at 70;30 (v/v, phase A/phase B). During 0.7 minutes, it changed to 10:90 (v/v). At 1.2 minutes it changed 30;70 (v/v) and then it grew up 90% of phase B. Finally, came back to initial conditions in 0.7 minutes, and stayed for 1.7 minutes.

In SPE, conditions were optimized, so conditioning cartridge (Oasis HLB, 200 mg) with 5 mL of methanol followed of 5 mL of mili-Q water. 250 mL of water sample were used. Before the elution, a clean-up step with 5 mL of mili-Q water was applied. Finally, the sample was eluted with 3 mL of methanol.

In MAE, 10 mg of sludge were taken and placed in an extraction tube with 5 mL of methanol. The extraction tube was put into a microwave unit and the power was set at 500 W. The suspension was irradiated for 6 minutes and was allowed to cool for 5 minutes.
Using the optimized method, levels of preconcentration of 83 in SPE and 50 in MAE were reached. Analytical parameters (LODs, LOQs, repeatability and accuracy) were determined in order to validate the proposed analytical method.
This method was applied in water and sludge samples from different treatment plants of Gran Canaria island with different treatment systems.

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METODOLOGÍA ANÁLITICA PARA LA DETERMINACIÓN DE FÁRMACOS ANTIHIPERTENSIVOS EN AGUAS SUPERFICIALESMEDIANTE LC/MS-MS CON ANALIZADOR DE TRIPLE CUADRUPOLO

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El consumo de principios activos farmacológicos se ha incrementado en los últimos años. Tras este consumo, los fármacos son descargados al medioambiente a través de las estaciones depuradoras de aguas residuales [1]. Muchos de estos compuestos son persistentes y, debido a su modo de actuación, pueden provocar efectos adversos en los organismos acuáticos y terrestres.

Entre los principios activos farmacológicos, tienen un especial interés aquellos empleados en el tratamiento de enfermedades crónicas, como los destinados al tratamiento de la hipertensión. Esto se debe, no solo a su consumo continuado, sino que además presentan un potente mecanismo de acción, lo que los hace especialmente peligrosos para el medioambiente [2].

El objetivo de este trabajo fue el desarrollo de un método analítico para la determinación de los tres principios activos farmacológicos más utilizados en el tratamiento de la hipertensión en aguas superficiales. Los fármacos estudiados fueron irbesartan, telmisartan y valsartan. El tratamiento de las muestras se realizó mediante extracción en fase sólida empleando cartuchos OASIS HLB y la determinación mediante cromatografía líquida acoplada a espectrometría de masas triple cuadrupolo. La optimización del proceso de extracción se realizó mediante el estudio de la influencia del pH de la muestra y el disolvente empleado en la elución. El método optimizado se validó en términos de efecto matriz, recuperación, precisión, linealidad, y límites de detección y cuantificación.

Las recuperaciones obtenidas se situaron entre el 84 y el 101 %. La precisión, medida en unidades de desviación estándar relativa, fue inferior al 10 % en todos los compuestos estudiados, y los límites de detección se situaron entre 0.24 y 2.01 ng L^{-1}.
La aplicabilidad del método se comprobó mediante el análisis de aguas superficiales. En dichas muestras se detectaron todos los compuestos estudiados.

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ANÁLISIS DE HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN HOJAS DE NARANJO AMARGO PARA SU APLICACIÓN COMO BIOMARCADORES DE LA CONTAMINACIÓN ATMOSFÉRICA

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Los hidrocarburos aromáticos policíclicos (PAHs) son contaminantes orgánicos originados como producto de la combustión de materiales como madera, carbón o derivados del petróleo, algunos de los cuales han sido identificados como carcinogénicos y mutagénicos [1]. Su presencia en el aire ha sido descrita en la mayoría de las grandes ciudades alrededor del mundo [2]. En estos estudios, el control analítico se realiza mediante el análisis de aire o partículas atmosféricas. Tales análisis presentan ciertos inconvenientes derivados principalmente de la complejidad de la toma de muestras y las bajas concentraciones a las que estas sustancias están presentes en la atmósfera. Por otra parte, estos contaminantes tienden a depositarse en las plantas, en las que se acumulan, por lo que sus hojas representan un muestreador pasivo de gran utilizada para la monitorización de contaminantes atmosférico en las grandes ciudades.

En este trabajo se ha desarrollado y validado un método analítico para la determinación de los 16 PAHs clasificados como contaminantes prioritarios por la Agencia Medioambiental Americana en hojas de naranjo amargo, para su uso como biomarcador de la contaminación en zonas urbanas. El análisis se basa en la extracción de los PAHs mediante extracción asistida por ultrasonidos, clean-up del extracto empleando extracción en fase sólida y determinación por cromatografía líquida de alta resolución con detectores de fila de diodos y de fluorescencia. Las recuperaciones obtenidas con el procedimiento de extracción se situaron entre el 48 y el 85 %, a excepción del benzo[ghi]perileno y benzo[a]antraceno, Los límites de detección y cuantificación fueron inferiores a 6 y 10 μg kg^{-1} de materia seca, respectivamente. La aplicabilidad del método propuesto se comprobó mediante el análisis de hojas de naranjos de la ciudad de Sevilla.

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EXPLORING THE USE OF DESI-HRMS FOR THE IDENTIFICATION OF UNKNOWNS: THE ADULTERATED PHYTOSANITARY PRODUCT CASE

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The analysis of unknowns remains a challenge in analytical chemistry. Specifically, organic unknowns in complex matrices are currently analyzed by mass spectrometry after an extensive sample manipulation that includes extraction and clean up and chromatographic separations. However, these sample treatments are frequently discriminant and as a result, some of the unknown compounds that could be of interest can be lost. In this context, the use of ambient mass spectrometry techniques that allow the direct mass spectrometric analysis from samples in their natural matrices opens the possibility to solve these problems. Desorption electrospray ionization (DESI) is an ambient technique that uses a standard electrospray charged liquid droplets to desorb and ionize analytes directly from the sample surface. DESI allows rapid (less than 10 s per sample) and in situ analysis with either minimal or without any sample pretreatment and it is applicable to both solid and liquid samples. The tangled DESI mass spectra obtained when analyzing complex samples requires the use of high resolution mass spectrometry (HRMS) since it provides accurate mass measurements for the correct assignment of elemental compositions and high quality isotope patterns free of interferences. Moreover, the combination of this mass spectral information with that obtained in tandem mass spectrometry performed at high resolution (MS/HRMS) can allow the reliable characterization of unknowns.

In this work, the applicability of DESI-HRMS (q-Orbitrap) for the analysis of a phytosanitary product suspected of being adulterated is explored. A fast and easy sample manipulation that consisted in impregnate a filter paper with the sample was used. The direct analysis of the filter paper by DESI-HRMS provided a full scan mass spectrum with the ions generated from the complex sample. A thorough examination of the data acquired in positive ion mode revealed signals with isotope pattern distribution features of metal ions. The accurate mass measurements, isotope pattern fits and structural information obtained by DESI-MS/HRMS allowed identifying the biocide as triphenyltin oxide. This compound has been extensively used for agricultural purposes and it is responsible for causing persistent and widespread pollution. Due to the well-known adverse effects of triphenyltins, maximum levels were established by the Commission of the European Communities under the Directive 2009/425/EC. The presence of Sn in the sample was confirmed by inductively coupled plasma atomic-emission spectrometry (ICP-AES) and the analysis of a standard of triphenyltin oxide by DESI-HRMS and DESI-MS/HRMS confirmed the presence of this compound in the sample. This is the first time that organotins are analyzed by DESI-HRMS.

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ID-ICP-MS TO ESTIMATE THE BIOAVAILABLE FRACTION OF CHROMIUM IN SEDIMENTS

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Isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) has been applied to determine the total metal content in a wide variety of samples. However, this absolute method of analysis was scarcely applied to the determination of the bioavailable fraction of metals in matrices as sediments, [1] where this information is of high interest since a equilibrium is established with the aquatic environment.

The present work describes the determination of the so-called bioavailable fraction of chromium in sediments using ID-ICP-MS and a unique extraction step. The spike was added prior to the extraction performed with diluted hydrochloric acid. The experimental parameters affecting the quality of the isotope ratio measurements were studied and the corresponding corrections performed.

The BCR-701 reference sediment, supplied by the Institute for Reference Materials and Measurements (IRMM), certified for the 3-step sequential extraction procedure, was analyzed. The results of the single-step procedure were compared with the sum of the certified contents for the three steps. The agreement is about 100%, which means that similar Cr fractions are extracted with both approaches. This bioavailable fraction represents about 58% of the total content calculated with the USEPA method 3052 (microwave assisted digestion of siliceous matrices). Three reference sediments, certified for total metal contents, PACS 2, PACS 3 and SRM 1944, were also analyzed with the proposed procedure obtaining recoveries around 16%, 18% and 53%. [2]

Samples from two Galician *rias* were analyzed to determine both the available and the total chromium contents.

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INFLUENCE OF MAJOR IONS AND PRESERVING REAGENTS ON THE STABILITY OF INORGANIC ARSENIC SPECIES IN GROUNDWATER

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The aqueous speciation of arsenic is a major factor controlling its biogeochemical cycling, bioavailability and toxicity. Meaningful analysis of arsenic speciation in waters requires the design and implementation of strategies that effectively preserve the in-situ speciation during sampling and storage [1]. Adsorption/desorption processes on metal oxyhydroxides or clay particles of As species, caused by shifts in redox potential or pH, and inter-conversion of inorganic species by redox reactions, are the main processes that can alter the chemical speciation of As in groundwater. A variety of preservation strategies employing addition of acids such as nitric, hydrochloric, sulphuric, phosphoric or acetic, and complexing agents such as EDTA, have been described in the literature to prevent inter-conversion of As species. However, there is no agreement as the results seem to be somehow dependent on the composition of the analytical matrix. On the other hand, the analytical technique used for As speciation also influences the selection of the preserving agent and its concentration, as it can cause interferences [2-4].

The occurrence of high concentrations of arsenic in aquifers located in the left margin of the Duero basin has prompted the determination and speciation of arsenic in groundwater to elucidate the sources, distribution and mobilization mechanisms of As in this region. Amongst the analytical techniques available for arsenic speciation, HPLC-ICP-MS has been selected as it combines the necessary sensitivity and accuracy. Prior measurements of As speciation in groundwater samples from areas of Valladolid, Segovia and Ávila showed that only inorganic As(III) and As(V) occur in these waters.

It has been investigated the effect of both major ionic constituents of groundwater and preserving agents recommended in the literature, assayed at different concentrations, on the speciation of inorganic As by HPLC-ICP-MS at As concentrations of 10 µg/L. It was observed the conversion of As(V) into As(III) in untreated samples within less than one week. Hydrochloric and acetic acids were rejected as preserving reagents as they caused interferences in the determination by HPLC-ICP-MS. The addition of nitric acid, sulphuric acid and EDTA preserved the distribution of inorganic arsenic species within one month, but high concentrations of sulphuric and EDTA interfered with the chromatographic separation. Finally, nitric acid at a concentration of 10-15 mM (0.1%) was selected as preserving agent as prevented the reduction of As(V) without oxidizing As(III). The effect on As speciation of ions Ca^{II}, Mg^{II}, SO_4^{2-} and HCO_3^-, major groundwater constituents in the region investigated, was assayed up to 1000 mg/L. Only interference of sulphate at the highest tested concentration was observed. The effect of potentially interfering ions such as Fe^{III} and phosphate was also assayed. It was concluded that Fe^{III} at concentrations above 1 mg/L causes oxidation of As^{III}, while the presence of phosphate above 0.5 mg/L hindered the determination of As^{V}, likely due to competitive effects in the separation column. Nevertheless, these interfering concentrations are much higher than the naturally occurring levels of these two ions.

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EXPOSICIÓN DE *PROCAMBARUS CLARKII* PROCEDENTES DEL PARQUE NACIONAL DE DOÑANA A PRINCIPIOS ACTIVOS FARMACOLÓGICOS. ACUMULACIÓN EN MUSCULO ABDOMINAL Y VÍSCERAS Y PRESENCIA DE METABOLITOS.

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El objetivo del presente trabajo es el estudio de la acumulación de principios activos farmacológicos en muestras de músculo abdominal y vísceras de la especie animal *Procambarus Clarkii* procedentes del Parque Nacional de Doñana.

La especie *Procambarus Clarkii* (*P. Clarkii* no autóctona) se encuentra integrada en el ecosistema del Parque Nacional de Doñana desde hace 40 años y se pretende utilizar como un bioindicador de estrés ambiental dado que las propiedades fisiológicas del mismo permiten la absorción de contaminantes en su organismo. Nuestro grupo de investigación participa en un proyecto de investigación [CTM2012-38720-C03-01] que evalúa la presencia de diversos contaminantes en organismos vivos del Parque Nacional de Doñana con el fin de encontrar bioindicadores que muestren de forma inequívoca situaciones de estrés ambiental producida por diferentes fuentes en dicho entorno, entre las que se encuentran los principios activos farmacológicos que se incluyen dentro del grupo de los llamados contaminantes emergentes.

En el presente trabajo, se han llevado a cabo una serie de ensayos de exposición sobre *P.Clarkii* en los cuales dichos organismos fueron expuestos a una concentración de 10 mg/L de mezclas binarias de ibuprofeno:flumequina e ibuprofeno:ciprofloxacina a fin de evaluar su acumulación en musculo abdominal y vísceras del mismo. Para ello, se desarrolló y optimizó un procedimiento analítico para la determinación de flumequina, ibuprofeno y ciprofloxacina mediante UPLC-Q-TOF-MS. La separación cromatográfica se llevo a cabo en una columna Acquity BEH C_{18} (50x 2,1 mm I.D., de 1,7 µm tamaño de partícula), termostatizada a 25 ºC y una fase móvil constituida por agua (A) y acetonitrilo (0,1% en ácido fórmico) (B) en modo gradiente durante 8 minutos. Dicho gradiente consistió en una composición inicial 90% A que varia hasta un 45% A en 4.5 minutos, manteniéndolo durante 1.5 minutos para volver posteriormente a condiciones iníciales y esperando 2 minutos entre inyecciones. Las muestras se mantuvieron termostatizadas a 10 ºC.

Los ensayos de exposición se llevaron a cabo sobre *P.Clarkii* capturados en el Parque Nacional de Doñana durante 40 días, de los cuales 30 fueron de exposición y 10 de depuración y aclimatación de los animales capturados. Para la captura, se utilizaron nasas cangrejeras estándar, en las que se introdujo un cebo apropiado para favorecer la entrada de los animales, colocadas en los cauces seleccionados para el muestreo. Transcurridas 24 horas, las nasas fueron retiradas y los cangrejos capturados fueros transportados hasta el laboratorio en neveras con hielo a fin de ralentizar su metabolismo. Los cangrejos fueron separados en grupos de 2 o 3 animales para la realización del ensayo. Transcurridos 10 días de aclimatación se procedió a realizar el ensayo de exposición. Para este fin, el agua de los estanques se sustituyó por agua fortificada con 10 mg/L en cada uno de los analitos de la mezcla objeto de estudio. El agua de los estanques fue renovada cada 4 días.

Se recogieron muestras del agua a fin de evaluar la concentración diaria presente en los estanques. Los cangrejos fueron retirados de los tanques y sacrificados transcurridos 30 días de exposición. Posteriormente, se diseccionaron obteniendo el músculo abdominal y las vísceras y tratadas siguiendo el siguiente procedimiento: las muestras fueron liofilizadas y extraídas mediante aplicación de energía microondas (5 min, 50 W potencia) utilizando para ello como extractante 10 mL de una mezcla acetonitrilo:agua 50:50 (v/v)a la que se añadió 50µL de proteinasa-k y 5 µL de ácido fórmico puro, posteriormente se centrifugaron a 7000 rpm durante 10 min, se evaporaron hasta casi sequedad bajo una corriente de N_2 y se reconstituyeron con 1 mL de ácido fórmico al 0.1%.

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