SEQA 2019

# XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA

VALLADOLID 17, 18 Y 19 DE JULIO

# LIBRO DE RESÚMENES

SEQA
Sociedad Española de Química Analítica

UVa

# BIENVENIDA

Queridos participantes en la XXII Reunión de la Sociedad Española de Química Analítica:

Nos reunimos en Valladolid para celebrar la edición de 2019 del congreso bienal de nuestra Sociedad.

Para la Conferencia Plenaria inaugural contamos con la presencia de José Manuel Pingarrón Carrazón, actual Secretario General de Universidades, al que agradecemos el esfuerzo por estar con nosotros en esta Reunión. Igualmente, queremos agradecer a todos los demás conferenciantes plenarios e invitados que hayan aceptado la propuesta del comité científico. Con su colaboración y las comunicaciones de los participantes se ha podido configurar un programa científico que esperamos que resulte atractivo para todos los asistentes.

Aunque para muchos de nosotros la asistencia a reuniones científicas no resulta fácil, en esta edición de la Reunión de la SEQA la participación vuelve a ser numerosa. Las contribuciones en forma de cartel, presentaciones orales y *flash* dan una idea del estado de la investigación analítica que se está llevando a cabo en España. En esta ocasión se puede observar un cierto incremento de comunicaciones en los campos relacionados con nano-bio-ómicas y técnicas de separación y se mantienen las comunicaciones que podrían encuadrarse en campos relacionados con alimentación y medio ambiente. El área de "Técnicas analíticas" ha sido la que más autores han elegido para encuadrar su comunicación.

Otro aspecto que cabe destacar es la amplia presencia de jóvenes investigadores, casi la mitad de los asistentes. La SEQA ha hecho un esfuerzo, como es habitual, para becar a un número elevado de sus socios adheridos; en esta edición de la Reunión las becas cubren, además, los gastos de alojamiento. Esperamos que, en un futuro no muy lejano, se produzca la transformación de estos jóvenes en socios numerarios y sean el relevo que mantenga viva nuestra Sociedad.

La Reunión de la SEQA ha sido siempre un marco adecuado para volver a vernos en persona y para iniciar o mantener relaciones científicas provechosas. Además, la asistencia a la Asamblea general es importante para tener información directa de la situación de la Sociedad y para plantear inquietudes que podrían orientar la labor de la Junta Directiva.

El programa es, como suele ocurrir en nuestras reuniones, denso. A pesar de ello, esperamos que los participantes encuentren el tiempo para poder conocer un poco la ciudad de Valladolid y disfrutar de la excursión a Peñafiel y de la cena de la Reunión que seguirá a la ceremonia de clausura en la que tendrá lugar la entrega de premios.

Gracias por vuestra presencia.

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**José Luis Pérez Pavón**
Presidente de la SEQA

**Enrique Barrado Esteban**
Presidente del Comité Organizador

# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*
# *Valladolid 17-19 julio 2019*

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Sociedad Española de Química Analítica (SEQA)

Departamento de Química Analítica. Universidad de Valladolid

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# PROGRAMA CIENTÍFICO

| Programa Científico XXII SEQA | | | | |
|---|---|---|---|---|
| **Miércoles 17/07** | | **Jueves 18/07** | | **Viernes 19/07** |
| | 8:30-9:00 | Recogida documentación / Colocación posters | 8:30-9:00 | Colocación de posters |
| | 9:00-9:30 | Ceremonia apertura | 9:00-9:50 | Conferencia plenaria: Fernando RULL |
| | 9:30-10:20 | Conferencia inaugural: José Manuel PINGARRÓN | | |
| | 10:30-11:30 | Comunicaciones orales (S1 y S2) | 9:55-10:45 | Comunicaciones orales (S7 y S8) |
| | 11:30-12:00 | Café /Pósters/Exposición comercial | 10:45-11:30 | Café /Pósters/Exposición comercial |
| | 12:00-12:40 | Conferencia Invitada: Susana Campuzano | 11:30-12:10 | Conferencia Invitada: Encarna Moyano |
| | 12:45-13:30 | Comunicaciones orales (S3 y S4) | 12:15-13:30 | Comunicaciones orales (S9 y S10) |
| | 13:30-15:30 | Comida / Pósters | 13:30-15:30 | Comida / Pósters |
| | 15:30-16:20 | Conferencia plenaria: CORAL BARBAS | 15:30-16:20 | Conferencia clausura: Aldo Roda |
| | 16:20-17:05 | Comunicaciones orales (S5 y S6) | 16:20-17:00 | Seminario-Mesa redonda : ABC |
| | 17:05-17:30 | Café /Pósters/Exposición comercial | | |
| | 17:30-18:10 | Conferencia Invitada: Marisol Cárdenas | 18:00 | Salida hacia Museo del Vino |
| Clausura Jornada Especiación y | 18:10-19:00 | "Flash Communications" (10) | | Entrega de premios y ceremonia clausura |
| | 19:00-20:00 | Asamblea SEQA | | CENA CONGRESO |
| Cóctel de bienvenida XXII SEQA | 19:00-20:00 | EYCN, the power of networking | | |
| Recogida documentación | | | | |

Especiación

## Día 17 de julio de 2019

**RETOS DE LA ESPECIACIÓN QUÍMICA EN EL SIGLO XXI:**
**ESPECIACIÓN QUÍMICA Y ÓMICAS**

| Hora | PROGRAMA CIENTÍFICO |
|---|---|
| 10:30-11:00 | Recogida de documentación |
| 11:00-11:15 | Presentación de la jornada |
| 11:15-12:00 | Conferencia invitada: **Bernard Michalke**<br>**COMBINED SPECIATION TECHNIQUES PROOF CHANGES IN THE METALLOME AND METABOLOME AS A CAUSE FOR TRANSITION-METAL RELATED NEURODEGENERATION**<br>*Moderadora: Tamara García Barrera y Fermín López* |
| 12:00-12:45 | Conferencia invitada: **José Luis Luque**<br>**ABORDANDO LA COMPLEJIDAD DE LA INTERACCIÓN DE METALES Y NANOPARTÍCULAS METÁLICAS CON SISTEMAS VIVOS MEDIANTE TÉCNICAS -OMICAS**<br>*Moderadores: Yolanda Madrid y Francisco Laborda* |
| 12:45-13:30 | Mesa redonda: The role of speciation in clinical analysis<br>*Moderadores: Tamara García Barrera y Yolanda Madrid*<br>*Invitados: Bernard Michalke y José Luis Luque* |
| 13:30-15:15 | Almuerzo |
| 15:15-15:45 | Conferencia invitada: **Helmut Ernstberge** |
| | **"INNOVATION IN SPECIATION AND NANOMETERIAL ANALYSIS USING ICP-MS"** |
| | *Moderadores: Fermín López y Francisco Laborda* |
| 15:45-17:15 | Sesión de comunicaciones orales<br>Moderadores: Jorge Ruiz y Mª Carmen Barciela |
| 17:15-18:15 | Sesión de posters y café |
| 18:15-18:30 | Entrega del Premio a la mejor comunicación |
| 18:30-19:00 | Asamblea del Grupo de Especiación de la SEQA |

| DÍA 18 DE JULIO DE 2019 | |
|---|---|
| 8:30-9:00 | **Recogida documentación / Colocación pósters** |
| 9:00-9:30 | **Ceremonia apertura: Sala Paraninfo** |
| 9:30-10:20 | **Moderadores: José Luis Pérez Pavón, Elena Domínguez**<br>**Conferencia inaugural: JOSÉ MANUEL PINGARRÓN**<br>**ACTUACIONES REALIZADAS EN EL MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES SOBRE LAS REFORMAS EN EL SISTEMA UNIVERSITARIO ESPAÑOL** |
| 10:30-11:30 | **Comunicaciones orales. Sala Paraninfo**<br>**Moderadores: Feliciano Priego, Rafael Pardo**<br>**Comunicaciones: NBO-O01; NBO-O02; NBO-O03; NBO-O04** |
| 10:30-11:30 | **Comunicaciones orales: Sala Claudio Moyano**<br>**Moderadores: Carlos Bendicho, Laura Toribio**<br>**Comunicaciones TAN-O01; TAN-O02; TAN-O03; TAN-O04** |
| 11:30-12:00 | Café /Pósters/Exposición comercial |
| 12:00-12:40 | **Sala Paraninfo**<br>**Moderadores: Paloma Yáñez, Bernardo Moreno Cordero**<br>**Conferencia Invitada: SUSANA CAMPUZANO**<br>**BIOSENSORES PARA EPIGENÉTICA Y METÁSTASIS EN CÁNCER** |
| 12:45-13:30 | **Comunicaciones orales. Sala Paraninfo**<br>**Moderadores: Juan Ramón Castillo, Yolanda Castrillejo**<br>**Comunicaciones: NBO-O05; NBO-O06; NBO-O07** |
| 12:45-13:30 | **Comunicaciones orales: Sala Claudio Moyano**<br>**Moderadores: Ramón J. Barrio, José Bernal**<br>**Comunicaciones TAN-O05; TAN-O06 y FyO-O01** |
| 13:30-15:30 | Comida / Pósters |
| 15:30-16:20 | **Sala Paraninfo**<br>**Moderadores: Alberto Escarpa, Mª Teresa Tena**<br>**Conferencia plenaria: CORAL BARBAS**<br>**ANALYTICAL DEVELOPMENTS IN METABOLOMICS WORKFLOW** |
| 16:20-17:05 | **Comunicaciones orales. Sala Paraninfo**<br>**Moderadores: Alberto Chisvert, Juan José Jiménez**<br>**Comunicaciones: NBO-O08; NBO-O09; casa comercial** |
| 16:20-17:05 | **Comunicaciones orales: Sala Claudio Moyano**<br>**Moderadores: Santiago Maspoch, María del Álamo**<br>**Comunicaciones TSE-O01; TSE-O02; TSE-O03** |
| 17:05-17:30 | Café /Pósters/Exposición comercial |
| 17:30-18:10 | **Sala Paraninfo**<br>**Moderadores: Pilar Campins, Marisol Vega**<br>**Conferencia Invitada: MARISOL CÁRDENAS**<br>**FASES SORBENTES CON NANOMATERIALES PREPARADAS SOBRE PAPEL: DO IT YOURSELF!** |
| 18:10-19:00 | **Sala Paraninfo**<br>**Moderadores: E. Alonso; J. J. Santana; Isabel Durán**<br>**"Flash Communications"** |
| | **RETIRADA DE PÓSTERS** |
| 19:00-20:00 | **Sala Claudio Moyano: Sesión jóvenes investigadores**<br>**Moderadores: Diego García Gómez, Noelia Caballero Casero**<br>**EYCN, the power of networking**<br>**Antonio M. Rodríguez García** |
| 19:10-20:00 | **Sala Paraninfo: Asamblea SEQA** |

**VIERNES 19 DE JULIO DE 2019**

| | |
|---|---|
| 8:30-9:00 | Colocación de pósteres |
| 9:00-9:50 | **Sala Paraninfo**<br>**Moderadores: Enrique Barrado, José Miguel Vadillo**<br>**Conferencia plenaria: FERNANDO RULL**<br>**LOS NUEVOS RETOS DE LA ESPECTROSCOPÍA RAMAN: DE LA EXPLORACIÓN DE MARTE A NUESTRO PASADO HISTÓRICO** |
| 9:55-10:45 | **Comunicaciones orales. Sala Paraninfo**<br>**Moderadores: José Luis Gómez Ariza , Manuel Hernández Córdoba**<br>**Comunicaciones: MAB-O01; MAB-O02; MAB-O03; MAB-O04** |
| 9:55-10:45 | **Comunicaciones orales: Sala Claudio Moyano**<br>**Moderadores: Luis Cuadros, M. Teresa Martín**<br>**Comunicaciones NBO-O10; NBO-O11; ALI-O01, ALI-O02** |
| 10:45-11:30 | **Café /pósteres/Exposición comercial** |
| 11:30-12:10 | **Sala Paraninfo**<br>**Moderadores: Félix Hernández, J. Ignacio García Alonso**<br>**Conferencia Invitada: ENCARNA MOYANO**<br>**AMBIENT IONIZATION-MASS SPECTROMETRY: MAKING REAL THE DIRECT ANALYSIS BY MASS SPECTROMETRY** |
| 12:15-13:30 | **Comunicaciones orales. Sala Paraninfo**<br>**Moderadores: Antonio Molina, M. Celia García Álvarez-Coque**<br>**Comunicaciones: MAB-O05; MAB-O06; MAB-O07; MAB-O08** |
| 12:15-13:30 | **Comunicaciones orales: Sala Claudio Moyano**<br>**Moderadores: Manuel Miró, Elena M. Peña Vázquez**<br>**Comunicaciones PMU-O01; PMU-O02, PMU-O03; PMU-O04** |
| 13:30-15:30 | Comida / pósteres |
| 15:30-16:20 | **Sala Paraninfo**<br>**Moderadores: M. Cruz Moreno, Alfredo Sanz-Medel**<br>**Conferencia clausura: ALDO RODA**<br>**NATURE INSPIRED BIOLUMINESCENCE : TOWARD NEW GENERATION CELL-BASED AND REAGENT-LESS CHEMICAL LUMINESCENCE BIOSENSORS** |
| **16:20-17:00** | **Seminario-Mesa redonda : ABC**<br>**Aldo Roda, Alfredo Sanz-Medel, M. Cruz Moreno-Bondi**<br>**Susana Campuzano** |
| | **RETIRADA DE PÓSTERES** |
| **18:00** | **Salida hacia Museo del Vino (Peñafiel)**<br>**Entrega de premios y ceremonia clausura**<br>**CENA DEL CONGRESO (Peñafiel)** |

**SESIONES DE PÓSTERES**

| DÍA 18 DE JULIO | Pósteres | |
|---|---|---|
| DOCENCIA | DOC | P01-P11 |
| FARMACIA Y OTRAS | FyO | P01-P22 |
| NANO-BIO-OMICAS | NBO | P01-P24 |
| TÉCNICAS DE ANÁLISIS | TAN | P01-P53 |

| DÍA 19 DE JULIO | Pósteres | |
|---|---|---|
| ALIMENTACIÓN | ALI | P01-P31 |
| MEDIO AMBIENTE | MAB | P01-P36 |
| PREPARACIÓN DE MUESTRAS | PMU | P01-P18 |
| TÉCNICAS DE SEPARACIÓN | TSE | P01-P17 |
| "LAST MINUTE" | LAST | P01-P03 |

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***JORNADA DE ESPECIACIÓN***
***Retos de la especiación química en el siglo XXI:***
***"Especiación química y ómicas"***

***Valladolid 17 julio 2019***

**EsCP1**

## COMBINED SPECIATION TECHNIQUES PROOF CHANGES IN THE METALLOME AND METABOLOME AS A CAUSE FOR TRANSITION-METAL RELATED NEURODEGENERATION

**Bernhard Michalke**

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**Introduction**
The etiology of neurodegenerative disorders such as Mn-dependent Parkinsonian like disease or idiopathic Parkinson´s disease (PD) are incomplete understood, but occupational and environmental exposure to redox-active-metals (Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn)), and to some of their specific binding forms (metal-species) influence the risk of disease progression.

**Methods:**
In case-control-studies we investigated Mn-, Fe-, Cu- and Zn-speciation in cerebrospinal fluid (CSF) from PD patients and controls by SEC-ICP-MS and CE-ICP-MS. In parallel, we performed non-targeted metabolomics studies using FT-ICR-MS for elucidation affected molecular pathways. For underlining our findings from diseased humans we performed animal and cell culture experiments with respective metal exposure and analyzed metal speciation and performed non-targeted metabolomics studies. Statistics: Multivariate statistical analyses were used for differentiation. Among others Sparse Partial Least Squares-Discriminant Analysis (sPLS-DA) and Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) model yielded in good group separation.

**Results:**
For manganese we found Mn-citrate to be the important Mn-species, being accumulated beyond blood-cerebrospinal barrier of humans. In brains of exposed rats, we observed Mn-dependent shift of iron-redox (Fe(II)/(III)) balance and molecular markers of increased oxidative stress, lipid peroxidation and changes in manifold metabolic pathways. In neuroblastoma cells, Mn resulted in shift of iron-redox balance and decreased levels of APP and H-Ferritin, both known as neuroprotective iron homeostatic agents.
In human idiopathic PD-CSF-samples, we monitored significantly increased ratios of redox-active metals such as Zn, Fe or Mn as numerator vs. Cu – amino acid fraction as denominator. Overall 20 ratios with Cu-LMW (amino-acid) compounds as denominator showed significant increase in PD. Metabolomics analysis revealed 68 metabolites decreased and 152 metabolites increased in PD samples. Fifteen metabolites in CSF were highly significant shifted in PD, allowing clear and significant differentiation of PD patients from age-matched controls. Predominantly compounds from lipid metabolism (e.g. decanoic acid, 10-hydroxydecanoic-acid, arachidonic-acid, dihomo-γ-linolenic-acid) were affected in PD. Redox metabolism with Cu-amino-acids as key-player and lipid peroxidation appeared as major causes inducing oxidative stress in PD patients.

**Conclusion:**
Redox-active metal species and misbalanced metal ratios play an important role in neurodegeneration etiology. Manganese shifted iron redox balance and induced oxidative stress and lipid peroxidation. In CSF of idiopathic Parkinsonian patients Cu-amino-acid-fraction appeared to be crucial. We also found a set of metabolites changed in CSF of Parkinson´s patients when using non-targeted metabolomics technique FT-ICR-MS. Molecules of the glutathione and lipid metabolism were mainly affected.

**EsCP2**

## ABORDANDO LA COMPLEJIDAD DE LA INTERACCIÓN DE METALES Y NANOPARTÍCULAS METÁLICAS CON SISTEMAS VIVOS MEDIANTE TÉCNICAS-ÓMICAS

**José L. Luque García**

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La contaminación de los ecosistemas y la exposición a metales tóxicos es una de las principales preocupaciones en todo el mundo. Algunos de estos metales se encuentran ubicuamente en el medio ambiente debido a su liberación en cantidades sustanciales como consecuencia de actividades geológicas y/o impactos antropogénicos. A pesar de que algunos de estos metales son micronutrientes esenciales a bajas concentraciones, pueden causar una amplia gama de efectos nocivos en exceso. Además, en los últimos años, las nanopartículas (NPs) han recibido una gran atención por su uso y aplicabilidad en muchos nuevos productos. Una estimación reciente sugiere que actualmente hay en el mercado más de 1.000 productos de consumo que contienen NPs, muchas de las cuales son NPs metálicas, por lo que cada vez los niveles de exposición a estas NPs son más elevados.

La mayoría de los estudios realizados hasta ahora para evaluar la toxicidad de metales y NPs metálicas se han centrado en el desarrollo de métodos analíticos para la determinación de especies tóxicas en diferentes muestras, como suelos contaminados, aguas y tejidos vegetales y animales, utilizando principalmente técnicas de análisis elemental como la espectroscopia atómica de absorción y emisión; siendo hoy en día la espectrometría de masas con plasma acoplado inductivamente (ICP-MS) la técnica analítica más ampliamente usada para el análisis simultáneo y multielemental, así como con fines de especiación. Sin embargo, poco se ha hecho para comprender los mecanismos moleculares que subyacen a la toxicidad de los metales y la forma en que interactúan con sistemas vivos.

El conocer estos mecanismos de interacción es importante no sólo desde el punto de vista toxicológico, sino también desde el punto de vista biomédico. Las propiedades citotóxicas de metales y NPs metálicas se emplean en la actualidad, de forma controlada, para el tratamiento de determinadas enfermedades. Tal es el caso, por ejemplo, de compuestos de Pt que se usan en clínica como terapia antitumoral; o de las NPs de Ag, usadas como agente bactericida.

En esta conferencia se discutirá el papel de las tres grandes técnicas ómicas: genómica, proteómica y metabolómica, como complemento a otras técnicas analíticas para el estudio de la interacción de metales y NPs metálicas con sistemas vivos a nivel molecular [1]. Se pondrá de manifiesto la utilidad de estas técnicas para descifrar los mecanismos biomoleculares responsables de los efectos tóxicos observados [2], y las posibilidades para regularlos con fines terapéuticos [3]. Se prestará especial atención a las particularidades de los tres grupos de biomoléculas, y a cómo dichas diferencias condicionan la estrategia analítica a emplear, especialmente desde el punto de vista instrumental. Finalmente, se presentarán ejemplos seleccionados con objeto de demostrar la necesidad de estas técnicas para obtener una visión completa y global sobre la toxicidad y el potencial biomédico asociado a las especies metálicas.

**Referencias:**

[1] J.L. Luque-Garcia, P. Cabezas-Sanchez, C. Camara (2011) Treds Anal. Chem. 30, 703.
[2] M.N. Fernandez, R. Muñoz-Olivas, J.L. Luque-Garcia (2019) Nanotoxicology, doi:10.1080/17435390.2019.1579374.
[3] S. Montalvo-Quiros, G. Aragoneses-Cazorla, L. Garcia-Alcalde, M. Vallet-Regí, B. González, J.L. Luque-Garcia (2019) Nanoscale 11, 4531

**EsCP3**

## INNOVATION IN SPECIATION AND NANOMATERIAL ANALYSIS USING ICP-MS

**Helmut Ernstberger**

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Speciation is widely employed in water, food, environmental and clinical analysis for research and increasingly also to satisfy regulatory requirements put in place to protect human health. The expanding fields of speciation analysis require separation and detection systems that allow measurement without altering the species distribution or introducing contamination from one of the instrument components, whilst allowing maximum flexibility to adapt to the requirements of the particular analysis needs.
This year, PerkinElmer launched the NexSAR HPLC Speciation Analysis Ready system, featuring a metal free fluid path. The system features are briefly discussed in terms of their relevance to speciation analysis, and the performance achieved for arsenic speciation of apple juice [1] as an application example is presented.

With the increasing capabilities of ICP-MS for nanoparticulate analysis the characterization of nanomaterials and the study of their fate after application in various food or consumer products is continuing to gain momentum. ISO norm 19590 has been released in response to the demand associated with the more widespread use of single particle ICP-MS.

*Pictured: NexION 2000 with NexSAR HPLC system*

Innovations in instrument hardware and software in SP-ICP-MS are primarily driven by the need for data accuracy, measurement of lower particle sizes, and expanding the range of elements under study. Five key innovation areas will be discussed within this context: plasma generation, data acquisition speeds [2], the role of instrument sensitivity and background, interference removal capabilities, and signal integrations and processing. Performance achieved on the NexION 2000 ICP-MS will be presented for various elements.

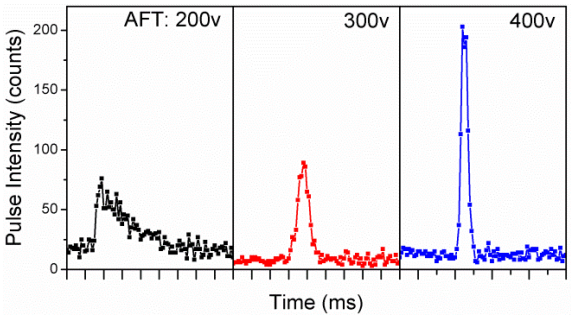


*Fig.: Application of Axial Field Technology for interference removal at the analysis of Fe nanoparticles.*

**References:**

[1] E. M. Kroukamp, "Characterization of Arsenic Species in Apple Juice using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution," *PerkinElmer Appl. Note*, 2019.

[2] A. Hineman and C. Stephan, "Effect of dwell time on single particle inductively coupled plasma mass spectrometry data acquisition quality," *J. Anal. At. Spectrom.*, vol. 29, no. 7, pp. 1252–1257, 2014.

**ESP-O01**

# ESPECIACIÓN DE ADITIVOS ALIMENTARIOS METÁLICOS EN PROCESOS DIGESTIVOS *IN VITRO*: DETECCIÓN DE NANOPARTÍCULAS Y FORMAS DISUELTAS

**Celia Trujillo[1], Ana C. Gimenez-Ingalaturre[1], Josefina Perez-Arantegui[1], Juan R. Castillo[1], Francisco Laborda[1]**

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El uso y la aparición de nanopartículas en productos alimenticios está generando serias preocupaciones acerca de sus riesgos potenciales. A este respecto, la Autoridad Europea de Seguridad Alimentaria (EFSA, por sus siglas en inglés) ha publicado recientemente una guía sobre los aspectos de la salud humana y animal en la evaluación de riesgos de las aplicaciones de la nanociencia y la nanotecnología en la cadena alimentaria [1]. Una de las principales conclusiones de la guía es la falta de métodos estandarizados para la caracterización analítica y la evaluación de riesgos.

La lista actual de aditivos alimentarios aprobados por la EFSA incluye tres metales: aluminio (E173), oro (E175) y plata (E174). Los tres están autorizados para ser utilizados en el revestimiento externo de los productos de confitería, aunque la información disponible sobre estos aditivos es aún insuficiente para evaluar su seguridad alimentaria.

En este trabajo se ha estudiado la liberación de plata y aluminio a partir de los aditivos E173 y E174 presentes en alimentos, así como las transformaciones que sufren ambos metales, tanto en el proceso de liberación en medios acuosos como a lo largo del proceso digestivo. Para ello se ha hecho uso de técnicas de ultrafiltración en combinación con ICP-MS, al igual que de métodos directos basados en la detección de partículas individuales mediante ICP-MS (SP-ICP-MS), además de microscopía electrónica. Mediante los dos primeros tipos de métodos se ha conseguido diferenciar la presencia de especies disueltas de plata (I) y de formas nanoparticuladas de plata, confirmándose la presencia de estas últimas mediante microscopía. Los alimentos que contenían los aditivos citados se sometieron a lixiviación en agua, así como a digestión gastrointestinal *in vitro*, analizándose los lixiviados y las etapas oral, gástrica e intestinal para determinar la fracción de elemento total liberado, además de las distintas formas físico-químicas en que lo hacía. Aunque una proporción importante de los metales se liberó en forma de especies oxidadas, Ag(I) y Al(III), mediante SP-ICPMS se detectó la presencia de formas nanoparticuladas de plata y aluminio, tanto en lixiviados acuosos como en las distintas etapas del proceso digestivo.

Los resultados de este trabajo ponen de manifiesto la presencia de formas nanoparticuladas de los aditivos alimentarios E173 y E174 al final del proceso digestivo, lo cual justifica su subsiguiente evaluación toxicológica para evaluar sus riesgos en la salud humana.

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**Agradecimientos:**
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**ESP-O02**

# ESPECIACIÓN DE NANOPARTÍCULAS DE PLATA EN AGUA MEDIANTE EXTRACCIÓN EN PUNTO DE NUBE Y ETAAS

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Las nanopartículas de plata son ampliamente empleadas en la industria debido a sus propiedades bactericidas y fungicidas. El uso masivo de las nanopartículas está generando un problema de contaminación a escala mundial, siendo tóxicas para los organismos vivos.

El objetivo de este estudio es el desarrollo de un método analítico para la preconcentración y separación de nanopartículas de Ag en muestras de agua, empleando la técnica de extracción en punto de nube (CPE). La concentración de Ag en forma de nanopartículas en los extractos se determina mediante Espectroscopía de Absorción Atómica con Atomización Electrotérmica (ETAAS). El tamaño de las nanopartículas se analiza mediante el modo Single-Particle de la técnica Plasma de Acoplamiento Inductivo con detector de Espectrometría de Masas (sp-ICP-MS)

El objetivo del estudio fue la selección de las condiciones experimentales en CPE que permitan la separación selectiva de las nanopartículas de plata en una muestra que contenga también plata en forma iónica. La selección de las condiciones óptimas para realizar el proceso de extracción se realizó mediante un diseño de experimentos siendo las variables estudiadas, pH, concentración de Tritón X-114, agente complejante (AEDT, $Na_2S_2O_3$), tiempo de calentamiento, y temperatura. Los mejores resultados fueron obtenidos utilizando AEDT como agente complejante, pH 7 (tampón ácido acético/acetato sódico), calentamiento a 60ºC durante 20 min, seguido de centrifugación a 7000 rpm durante 20 min a 4ºC. A continuación, se separan las dos fases, realizando la determinación de Ag iónica en la fase acuosa y nanopartículas de plata en la fase surfactante.

Para la determinación de Ag en la fase surfactante mediante ETAAS, se realizó la calibración en un intervalo de concentraciones de 0-20 µg $L^{-1}$, preparada con nanopartículas de plata, sometidas al proceso de extracción en punto de nube. Se evaluaron las características analíticas del método obteniendo límites de detección y cuantificación de 0,04 y 0,13 µg $L^{-1}$, presentando una buena precisión (RSD% <10%) y porcentajes de recuperación 103 ± 4%.

Finalmente, el método propuesto fue aplicado para la determinación de nanopartículas de plata en muestras de agua.

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**ESP-O03**

# SELECTIVE SOLID PHASE EXTRACTION OF TRACE INORGANIC ARSENIC (III & V) USING AN ION IMPRINTED POLYMER (IIP)

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Arsenic is present in foods as a species of different toxicity. The European Food Safety Authority has established that more analytical data on inorganic arsenic (the most toxic species) is required, in particular in fish and seafood, and in products that provide a significant contribution to the dietary exposure (e.g. rice and wheat-based products) [1]. That is one of the reasons why is important to develop fast and reliable methods of arsenic speciation in foodstuffs.

A novel metal ion-imprinted polymer (IIP) was synthesized by ionic imprinted technique for selective Solid Phase Extraction (SPE) of trace inorganic arsenic [(As (III) and As (V)] in water/methanol extracts from fish samples. In the first step, sodium (meta) arsenite ($NaAsO_2$) formed a coordination linkage with 1-vinyl imidazole as a functional monomer. Then, the complex was copolymerized with the divinylbenzene (DVB) crosslinker in the presence of 2,2'-azobisisobutyronitrile (AIBN) as initiator. Subsequently, the As template was completely removed by leaching the dried and powdered material particles with 2.0 M $HNO_3$. The synthesized As (III) -based IIP and the NIP were characterized by Fourier Transform Infrared Spectrometry (FTIR) and Scanning Electron Microscopy (SEM). The obtained IIPs particles exhibited excellent selectivity for the target ions. The As-IIP was packed in cartridges that, after the optimization of the operation conditions, were used for As-III & V separation from fish matrixes with good extraction efficiency, distribution ratio, and selectivity coefficients. The prepared As-IIP showed to be promising for SPE combined with High-Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) for the determination of trace As III & V in fish samples, with low detection limits of 0.0632 and 0.0788 mg/kg (w/w), respectively.

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**ESP-O04**

# GC-ICP-MS/MS INSTRUMENTAL SET-UP FOR TOTAL AND SPECIATION SULFUR ANALYSIS IN GASOLINES USING GENERIC STANDARDS

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Crude oil consists of a complex matrix containing a large variety of organic molecules, including S- and N- containing compounds. In the particular case of sulfur, its content in crude oil can represent up to 8%. Sulfur compounds include thiols, sulfides, and aromatic sulfur heterocycles. their determination is important both for assessing the product quality of crude oil derivates but also for the characterization of future environmental emissions derived from their use.

Considering the wide variety of S-containing compounds present in gasolines, generic quantification without specific standards would be desirable. To the best of our knowledge compound independent calibration (CIC) for S compounds in petroleum derivates has only been achieved so far by post-column isotope dilution and GC-ICP-MS.[1] However, this method requires the synthesis and the continuous addition of a 34S-labelled gaseous compound flow which hampers it use in routine analysis.

In this communication, we describe the instrumental modifications [2] required in a commercial GC-ICP-MS/MS instrument, aiming to turn it into a compound-independent quantitative technique for both total and speciation sulfur analysis in gasolines using a simple and certified generic S-containing standard. Additionally, carbon-derived matrix effects were evaluated and were made negligible for both direct and fast total and speciation S analysis, making the use of relatively complex isotope dilution strategies not necessary anymore.

Under optimized working conditions, detection limits as low as 0.3 pg S was obtained (absolute LOD), which is to the best of our knowledge more than one order of magnitude below the ones reported for other sulfur GC selective detectors. Total analysis was performed by flow injection analysis through a transfer line and external calibration, whereas speciation analysis was carried out by chromatographic separation and both, with internal standardization in order to correct differences in the injected volume (manual injection was used).

The proposed method [3] was successfully applied to total and speciation sulfur analysis of a commercial gasoline sample and validated with a certified reference material ERM-EF213 (“sulfur free gasoline” with 9 ppm of total sulfur content). Robustness of the method was tested and confirmed after a successive 50 injections of the gasoline samples in 3 hours without any instrumental drift.

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**ESP-O05**

# A CRITICAL ASSESSMENT OF THE ICP-MS SPECIES-INDEPENDENT RESPONSE FOR PROTEINS AND NANOPARTICLES

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Inductively coupled plasma mass spectrometry (ICP-MS) has become the reference technique for elemental analysis. Moreover, in recent years, there is a rising awareness of the potential ICP-MS capabilities in the field of proteomics and nanotechnology.[1,2] One of the most powerful features of this technique is its quantitative character, which may result in species-independent signals and thus quantification without the need for specific standards.[3]

However, different studies have put in doubt the species-independent signal provided by this technique using conventional nebulizers, for the quantitative analysis of complex structures such as nanoparticles or proteins. Although the low efficiency of conventional nebulizers have been already stressed, there is not explicit evidence of this effect affecting their potential to provide species-independent quantification. Many different explanations such as transport nebulization or ionization effects have been considered to be the cause behind this fact although the specific reasons remain unknown. [4]

Herein, we present the first critical and statistical comparison of the ICP-MS response factors obtained for the analysis of very different intact nanoparticles (polymer coated CdSe-ZnS quantum dots and silver nanoparticles) and proteins (Bovine serum albumin, β-casein, cytochrome C and intact monoclonal antibody) and their corresponding dissolved elemental constituents (inorganic elemental standard) using two regular flow nebulizers, a concentric nebulizer and a cross flow nebulizer. For regular flow analysis, it is necessary to assure that there is no difference in nebulization efficiency at different nebulizer gas flows. The results show that the difference in ICP-MS response factor observed for each analyte (nanoparticles and proteins) stays constant with nebulizer gas flow. From the statistical comparison between inorganic standards and intact nanoparticles and protein (the nebulizer gas flow most commonly used for each nebulizer has been chosen) differences in the response factor up to 21% and 39% in the case of nanoparticles and proteins, respectively, have been observed.

In order to demonstrate that such significant difference in the signals obtained for inorganic standards and intact complex bio(structures) was not related to the ICP ionization process, a strategy based on the use a total consumption nebulizer was evaluated. The results obtained show that this nebulizer corrects any previous difference observed with regular flow nebulizers providing statistical indistinguishable response factor for both the inorganic and intact analytes (nanoparticles and proteins).

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**ESP-O06**

# ESTUDIO DE ESTABILIDAD DE ESPECIES DE ARSÉNICO, ANTIMONIO Y HIERRO EN ELECTROLITO DE COBRE

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La producción industrial de cobre mediante electrorrefino se basa en un proceso de electrólisis mediante el cual a partir de un ánodo de cobre de baja pureza se produce cobre catódico del 99.99%. Durante la etapa de electrólisis, algunas de las impurezas presentes en el ánodo (e.g. As, Sb, Fe, Bi) bien se disuelven en el electrolito, o bien precipitan formando los llamados lodos anódicos.

Aquellos metales que son más nobles que el cobre son los que precipitan, mientras que los que son menos nobles permanecen en disolución. Estos últimos tienen que permanecer en unos límites determinados de concentración para evitar que afecten negativamente a la calidad del cobre catódico debido por ejemplo a la formación de lodos flotantes, los cuales dependen en parte del estado de oxidación de las impurezas.[1]

En este estudio se ha desarrollado un procedimiento de especiación de As, Sb y Fe en muestras industriales de electrolito de cobre de la refinería de Atlantic Copper. Para As y Sb se ha empleado un acopamiento instrumental de cromatografía líquida, generación de hidruros y espectroscopia de fluorescencia atómica (HPLC-HG-AFS), que permite la determinación de As(III), As(V), Sb(III), Sb(V) en el rango de µg $l^{-1}$. Para la especiación de Fe se ha empleado un método colorimétrico basado en la formación de un complejo coloreado de Fe(II)-fenantrolina, que permite la determinación de Fe(II) y $Fe_{total}$ en el rango de mg $l^{-1}$.

La matriz de las muestras de electrolito de cobre es compleja debido a su gran acidez (ca. 180 g/l de $H_2SO_4$) y su alto contenido metálico (ca. 45 g Cu $l^{-1}$, 11 g Ni $l^{-1}$ y 9 g As $l^{-1}$). Por ello, es necesario estudiar la estabilidad del electrolito desde la toma de muestra hasta el análisis de especiación, con el fin de garantizar una determinación adecuada de los distintos estados de oxidación de cada elemento estudiado.

Para ello, se ha realizado un estudio de estabilidad de las especies de As, Sb y Fe durante un mes considerando varios parámetros, como son la dilución de la muestra y su acidificación por adición de HCl (procedimiento habitual para la determinación del contenido total de estos elementos mediante ICP-OES). Otro parámetro a tener en cuenta fue el control de temperatura (65 °C, temperatura ambiente y refrigeración a 4º C).

Los resultados de especiación de las muestras de electrolito indicaron que el As(V) es la especie mayoritaria de As, Fe(II) la de Fe, y Sb(III) la de Sb. La dilución de la muestra resultó en un periodo corto de estabilidad de 1 día para las distintas especies de Fe y Sb. La acidificación con HCl provocó además una oxidación parcial del Sb(III) a Sb(V) y de Fe(II) a Fe(III). La estabilidad no se vio afectada por el control de la temperatura. Las mejores condiciones de estabilidad se obtuvieron conservando las muestras de electrolito sin diluir a temperatura ambiente, siendo el periodo de estabilidad de al menos de 2 semanas para As y Sb, mientras que para el Fe su estabilidad siguió siendo de solo 1 día.

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**ESP-O07**

# AN ANALYTICAL STRATEGY BASED ON SEPARATION TECHNIQUES COUPLED TO ICP-MS AS A VALUABLE CONTRIBUTION FOR SPECIATION ANALYSIS OF GOLD NANOPARTICLES IN *IN VITRO* TOXICOLOGICAL ASSAYS

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Gold nanoparticles (AuNPs) exhibit excellent physical, chemical and biological properties leading to applications in many different fields, such as biomedicine [1]. Due to these numerous applications, it is expected that the exposure to AuNPs will increase substantially over the next years with unknown impacts on the environment and human health. Therefore, toxicological studies are necessary. The first approach for these studies is the *in vitro* assays with cells grown in cell culture medium. During these studies the AuNPs can suffer different transformations that should be followed to get a correct interpretation of the toxicological results [2]. One of these transformations is the release of the gold ion from the AuNPs and speciation methods are needed for the simultaneous determination of both the NPs and their corresponding ions in complex samples. However, classical microscopic and spectroscopic techniques used for NPs analysis cannot provide this information and new analytical approaches are required. Among them, hyphenated systems are promising options recently proposed and still under development for complex samples.

Thus, the aim of this study has been the optimization of systems based on separation techniques (reversed-phase liquid chromatography, RP-LC, and asymmetric flow field flow fractionation, AF4) hyphenated to inductively coupled plasma mass spectrometry (ICP-MS), and its application for the speciation analysis of AuNPs and dissolved gold species ($Au^{3+}$) in cells and cell culture medium used in toxicological assays.

The separation and acquisition conditions of the RP-LC-ICP-MS and AF4-ICP-MS systems have been optimized. HeLa cells (a human cervical adenocarcinoma cell line) have been incubated in cell culture medium (Dulbecco's Modified Eagle Medium, DMEM, containing fetal bovine serum, FBS, and antibiotics) with AuNPs and $Au^{3+}$. Different experimental variables related both to AuNPs characteristics (size, concentration, and stabilizing medium) and cell culture conditions (incubation time, and percentage of FBS), have been evaluated and will be discussed. In general, the oxidation process was more important for small size and long times of incubation. These results can contribute to a better understanding of the mechanisms of toxicity of the AuNPs.

**Acknowledgments:**

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**ESP-P01**

## SPECIATION OF GOLD NANOPARTICLES AND TOTAL GOLD BY GRAPHITE FURNACE-ATOMIC ABSORPTION SPECTROMETRY USING MAGNETIC MICRO-SOLID PHASE EXTRACTION

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The widespread use of gold nanoparticles (AuNPs) in a variety of applications, *i.e.* nanomedicine, biosensing, catalysis, etc., and their further release into the environment has become an issue of much concern [1]. As a result of the different toxicity and bioavailability of nanoparticles and their corresponding metal ions, a speciation analysis is required. Recently, nanomaterials have been proposed as efficient tools for trace element speciation taking advantage of their sorptive, catalytic or optical properties [2].

In this work, naked magnetite nanoparticles ($Fe_3O_4$NPs) have been used in combination with graphite furnace-atomic absorption spectrometry (GFAAS) for the determination of AuNPs and total Au [*i.e.*, AuNPs+Au(III)] in natural waters. Ascorbic acid enables the quantitative co-extraction of both species, AuNPs and Au(III). Thiosulfate is able to reduce Au(III) to Au(I), which subsequently forms the non-extractable complex $[Au(S_2O_3)_2]^{3-}$, thus achieving the selective extraction of AuNPs. The experimental variables affecting the extraction process, namely, mass of $Fe_3O_4$NPs, L-ascorbic acid concentration, pH, extraction time, sample volume, sodium thiosulfate concentration and re-dispersion volume, are evaluated. Once the extraction process is accomplished, the magnetic solid phase is directly injected into the graphite furnace, so that analyte elution or digestion of the extractant phase can be omitted. This simplifies the method and improves the enrichment factors for both species, which are higher than 196 under optimal conditions, corresponding to extraction efficiencies over 98%. The proposed method yielded detection limits of 19.5 and 19.7 ng $L^{-1}$ for AuNPs and Au(III), respectively. The intra-day repeatability and inter-day reproducibility are lower than 5.3% (n=6) and 7.6% (n=4), respectively for both species. It should be highlighted that non-significant effects of AuNPs size (8-98 nm), capping agent (citrate, sucrose, CTAB and $NaBH_4$) and morphology (nanospheres and nanorods) over the extraction efficiency are observed. Finally, the method is applied for AuNPs/Total Au speciation in environmental waters with good recoveries. The reported method represents a suitable alternative to other methodologies for quantification of AuNPs and total Au at ultratrace level [3].

**Acknowledgements:**

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**ESP-P02**

## COMPARISON OF DIFFERENT MASS BIAS CORRECTION PROCEDURES FOR THE MEASUREMENT OF Hg SPECIES-SPECIFIC ISOTOPE RATIOS BY GAS CHROMATOGRAPHY COUPLED TO MULTICOLLECTOR ICP-MS

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Mercury is a global pollutant released to the atmosphere by natural and anthropogenic processes, and occurs in different chemical forms and/or oxidation states in terrestrial, atmospheric and aquatic ecosystems. On one hand, the determination of the different Hg species in a sample (speciation analysis) can be very helpful to understand Hg biogeochemical cycle as Hg reactivity, mobility and bioaccumulation depend on its chemical form. On the other hand, the accurate and precise measurement of Hg isotopic composition in real samples is a valuable tool to understand Hg pathway in the environment and to fingerprint contamination sources. Therefore, the coupling of chromatographic techniques to multicollector instruments to measure compound-specific Hg isotopic compositions may lead to new insights into the biogeochemical behaviour of mercury species in the environment.

Optimum GC separations provide very short transient signals (typically 2-5 s at the peak base). Therefore, the number of acquisition points during the chromatographic peak is not enough to obtain comparable levels of accuracy and precision in the isotope ratio measurements than those obtained when measuring continuous signals. The accuracy and precision of compound-specific isotope ratios was improved by calculating the isotopic ratios from the slope of a linear regression between isotopic signals. This strategy was initially developed by Fietzke et al [1] for transient signals obtained by Laser Ablation coupled to MC-ICP-MS and lately applied by Epov et al [2].

When calculating delta values GC-adapted standard-sample-standard bracketing approaches have been shown to provide external reproducibilities lower than 0.5 ‰ [2] expressed as 2SD. The standard-sample-standard bracketing approach corrects for most of the mass discrimination effects but the elution of the sample matrix from the GC column may induce plasma instabilities affecting mass bias during the chromatographic peak profile of Hg compounds. Mass bias correction is typically carried out with the measurement of the $^{205}Tl/^{203}Tl$ isotope ratio in a nebulised Tl solution mixed with the Ar flow transporting the eluted Hg compounds from the GC column. However, the data treatment for mass bias correction when measuring compound-specific isotope ratios from the slope of a linear regression between isotopic signals has not been deeply investigated yet. This work reports the effect of different mass bias correction procedures on the accuracy and precision of Hg(II)-specific isotope ratios by GC-MC-ICP-MS.

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**ESP-P03**

## BIOACCESSIBILITY OF ARSENIC SPECIES FROM EDIBLE MUSHROOMS SAMPLES BY IN VITRO PBET METHOD

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Arsenic is mobile in the environment causing a wide distribution throughout the metabolism of plant, fungi and animal kingdoms. However, the toxicity of this trace element depends on its species and it is generally recognized that the soluble inorganic arsenicals are more toxic than organic ones. Aware of this situation, the European Food Safety Authority (EFSA) pointed out the need to produce reliable speciation data for different food commodities to estimate the health risk associated with arsenic exposure. Moreover, it is also necessary to assess arsenic species that the digestive system can absorb. There are several tools to determine the bioavailability and all of them try to simulate the conditions presented in a human stomach. Most of these approaches simulate the enzymatic process, the acidic medium and other conditions to reproduce the digestion processes.

The aim of this work is to study the bioaccessibility of arsenic species in mushrooms depending on their preparation, fresh, boiled and grilled, using the PBET physiological extraction method. Shitake (*Lentinula edodes*), oyster (*Pleurotus ostreatus*) and champignon (*Agaricus bisporus*) mushrooms were selected because they are among the most consumed worldwide. Total and arsenic species were determined in fresh samples as well as in all the extracts by ICP-MS and by LC-ICP-MS respectively.

The results show that the fresh samples analysed have total arsenic content below 1.5 mg/kg, being higher in the case of the shitake sample and lower for oyster and champignon ones. Regarding arsenic species, the presence of inorganic arsenic forms is observed in all cases, being the major species in most samples. However, the presence of methylated forms is also observed, and in the case of the champignon mushroom arsenobetaine has been also detected and quantified. When considering the different mushroom preparations, boiled samples lose between 50 to 70% of the total arsenic compared to fresh samples, while grilled samples lose around 10%, and the speciation patterns remain quite similar to those observed for fresh samples. Finally, the application of the PBET method shows that in this type of food almost 100% of the arsenic present in the samples is bioaccessible already in the gastric stage, regardless of the treatment of the sample or the chemical species present. However, due to the low concentration present in the samples, their consumption, in normal quantities, would not represent a health risk.

**ESP-P04**

## ROLE OF SELENIUM SPECIES IN NEURODEGENERATIVE DISEASES

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Nowadays, around 50 million people worldwide suffer from dementia, discovering 10 million cases more every year, and nearly 70% of them are affected by Alzheimer's disease (AD). This number is predicted to reach 152 million by 2050. However, at the present time, there is no treatment to cure neurodegenerative disorders [1]. AD is characterised by the aggregation and deposition of β-amyloid protein (Aβ) plaques and the hyperphosphorilation of tau protein in the brain. Therefore, most of the treatments under development are based on the inhibition or reversion of the Aβ aggregation [2].

The metal ion hypothesis suggests that oxidative damage produced by metals is the main cause of this dementia, especially iron, copper, zinc and aluminum. Thus, new treatments based on metal chelators are being developed [3]. Selenium is an essential trace element which has been reported to play an important role in AD, due to its tendency to bind metals and its antioxidative properties [4]. However, selenium benefits depend on its chemical form, being the organic ones, SeMet, $(SeCys)_2$, SeMetSeCys and selenonanoparticles (SeNPs), the most beneficial for human health.

The main aim of this work was to study the effect of selenium species in the $A\beta_{1-42}$ aggregation, in presence and absence of iron (II) and copper (II). In order to achieve this objective, UV-Vis and Transmission Electron Microscopy (TEM) measurements were performed. For this purpose, spectra and derivative spectra were achieved by UV-Vis, to show if selenium compounds can act as metal chelators. In order to enhance differences among spectra, to resolve overlapping bands and to reduce the effects of other absorbing compounds, derivative spectroscopy was applied. Therefore, once UV-Vis spectra were registered, mathematical methods were used to generate the first-order derivative spectra.

Concerning to $A\beta_{1-42}$ aggregation studies, the Aβ was pretreated with hexafluoroisopropanol (HFIP), divided into aliquotes and followed by the evaporation of the solvent in a SpeedVac system. Obtained aliquotes were stored under the dark at -20 ºC until use. Treated $A\beta_{1-42}$ samples were then incubated during 48 h at 37 ºC in HCl 10 mM, and measured by using TEM.

UV-Vis results evidenced that some selenium species, including selenium nanoparticles, were able to chelate iron and copper, with a probable stoichiometry of 1:1.

On the other hand, TEM images indicated that iron (II) and copper (II) indeed increased length, number and aggregation of Aβ fibrils, when compared to Aβ alone.

Therefore, selenium species could reduce the Aβ fibrils aggregation, by complexing iron and copper cations and inhibiting their oxidative effect. Thus, the studied selenium species could be used as promising chelating agents in order to prevent AD.

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**ESP-P05**

# DESARROLLO DE METODOLOGÍA PARA LA ESPECIACIÓN DE NANOPARTÍCULAS DE PLATA Y PLATA IÓNICA MEDIANTE CROMATOGRAFÍA HIDRODINÁMICA ACOPLADA A ICP-MS

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Los continuos avances en nanociencia han contribuido al aumento en el uso de nanopartículas (ENPs) en un amplio número de productos de consumo habitual. Este hecho requiere la adaptación de las técnicas y métodos existentes, o el desarrollo de nuevos para monitorizar la aparición y la transformación de ENPs en los diferentes escenarios. Se han estudiado diferentes técnicas y enfoques metodológicos para la caracterización y cuantificación de ENPs y sus derivados en muestras complejas [1]. Para entender el impacto medioambiental o los mecanismos toxicológicos de las ENPs inorgánicas, es clave discriminar entre formas disueltas y partículas del elemento involucrado.

Es habitual que las técnicas de Fraccionamiento en flujo con campo de flujo asimétrico (AF4) y cromatografía hidrodinámica (HDC) se acoplen a ICP-MS como detector de un elemento específico para la separación y determinación de ENPs inorgánicas en una amplia variedad de muestras. Si se compara AF4 con HDC [2], HDC es una técnica de separación robusta y versátil, sin embargo su poder resolutivo es mucho menor que el de AF4. Por otra parte, la recuperación obtenida con HDC es mejor que con AF4. Otra ventaja del HDC frente al AF4 es el tiempo de análisis, que puede ser reducido a menos de 10 minutos en comparación con los 30-45 minutos del AF4. Además con HDC las especies con bajo peso molecular no se pierden como sucede con el AF4 debido a las membranas de ultrafiltración usadas en el canal de separación. En consecuencia, HDC-ICP-MS a diferencia del AF4 puede dar información simultánea sobre especies disueltas y partículas de un elemento en menos de 10 minutos.

El principal objetivo de este estudio es la investigación y evaluación de la influencia de los principales parámetros de HDC (fase móvil y flujo) para evaluar el comportamiento del HDC-ICP-MS para la determinación simultánea de especies disueltas y nanopartículas de plata (AgNPs). Se ha prestado especial atención al valor de resolución obtenido entre especies disueltas y NPs y su recuperación. Para la optimización de las condiciones de separación se han utilizado AgNPs comerciales estabilizadas en citrato. Los resultados de la investigación se han aplicado para llevar a cabo la especiación de AgNPs y Ag iónica en diferentes complementos alimenticios. Los resultados cuantitativos obtenidos por HDC-ICP-MS para las distintas especies se han comparado con los obtenidos por ultrafiltración y determinación por Espectroscopía de Absorción Atómica con llama; la caracterización de tamaños se ha comparado con los resultados obtenidos por Microscopía Electrónica.

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**ESP-P06**

## EFFECTS OF CO-ADMINISTERED CHITOSAN-STABILIZED SELENIUM NANOPARTICLES IN CELLS EXPOSED TO CISPLATIN: VIABILITY AND SPECIATION STUDIES

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Cisplatin is one of the most worldwide employed chemotherapeutic agents, although it may produce important side-effects such as nephrotoxicity [1]. Therefore, extensive research for the development of renoprotective strategies is still carried out to improve the efficacy of this drug. In the last years, the use of selenium nanoparticles (SeNPs) has been investigated for various biomedical applications [2]. In this regard, SeNPs could be assessed as a potential nephroprotective agent for cisplatin treatments due to the intrinsic antioxidant properties of selenium, which may minimize the oxidative damage in renal cells. However, SeNPs should not decrease the cytotoxic effect of cisplatin on tumor cells. With this aim, in the present work HeLa (cervical cancer) cells were exposed to 9 mg/L cisplatin and/or chitosan-stabilized 5 mg/L SeNPs (Ch-SeNPs), for 24 and 40 h, and also control cultures were prepared for comparative purposes. In all the cases cell viability was determined by MTT assay. The intracellular Se and Pt contents of adherent cells were quantified by ICP-MS after acid digestion. Moreover, anion exchange (AEC) and size exclusion chromatography (SEC) coupled with UV/VIS and ICP-MS were employed for Se and Pt speciation studies of cell cytosols.

Results showed that HeLa cells did not suffer significant changes in terms of cell viability and morphology when they were incubated with Ch-SeNPs for up to 40 h. In contrast, as expected, significant damage was observed in cisplatin-cultured cells, especially after 40 h of incubation (33% of viability), this being more acute when mixing Ch-SeNPs with cisplatin (18%). On the other hand, the amount of cisplatin internalized by cells did not decrease when the drug was co-administered with Ch-SeNPs, and the chromatographic profile of cytosolic Pt was also similar, showing an increase in the platination degree in the higher mass protein fraction. The Pt content in adherent cells was lower at 40 hours than at 24 hours, and this was observed for all the cultures treated with cisplatin, both alone (48 and 20 fg Pt/cell) and in the presence of Ch-SeNPs (52 and 24 fg Pt/cell), which would be explained by detoxification mechanisms of cells. These results suggest that not only Ch-SeNPs do not impair the antitumoral activity of cisplatin, but instead they may even intensify it.

An increase in Se content occurred in cisplatin-incubated cells (22 fg Se/cell at 40 h) compared to control cells (9 fg Se/cell), along with some changes in the chromatographic profile of cytosolic Se, which could be due to an overexpression of selenoproteins involved in the cellular defense against oxidative stress. On the other hand, the highest levels of intracellular Se were found in samples incubated with Ch-SeNPs, which clearly demonstrated their internalization by cells. Moreover, AEC chromatograms revealed the presence of different selenium compounds after enzymatic digestion of cells exposed to Ch-SeNPs, as selenocysteine, selenite and selenate. In the presence of cisplatin, the quantity of uptaken Ch-SeNPs was much lower (50% lower after 24 h, and five-fold smaller after 40 h), suggesting altered cellular mechanisms of transport and metabolism of selenium as a consequence of the Pt-drug cytotoxicity.

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**ESP-P07**

# DISTRIBUCIÓN ELEMENTAL EN SUERO, ORINA Y LAVADO BRONCOALVEOLAR DE PACIENTES CON CÁNCER DE PULMÓN. IMPORTANCIA DE LAS SELENOPROTEÍNAS EN LA CARCINOGÉNESIS.

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El estudio sobre el papel de los metales en la aparición y progresión del cáncer, así como en el proceso de metástasis[1–3] ha permitido establecer dos posibles hipótesis generales. La primera relaciona la presencia y la evolución de la enfermedad con la perturbación de los niveles de los elementos esenciales del metabolismo, y la segunda asocia el proceso carcinogénico con una alta exposición a los metales. Por otra parte, los oligoelementos juegan un papel muy importante en el proceso carcinogénico activando e inhibiendo reacciones enzimáticas fundamentales en las que suelen participar como cofactores. En este estudio se ha desarrollado una metodología de análisis multielemental basada en ICP-QQQ-MS y se ha aplicado a muestras biológicas de pacientes con cáncer de pulmón (CP). Concretamente, se analizaron once elementos (V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Cd y Pb) en muestras de suero, orina y, por primera vez, en lavado broncoalveolar (LBA) de pacientes con CP. Además la optimización de un método de fraccionamiento basado en la precipitación de proteínas en condiciones no desnaturalizantes (NDPP) permitió el análisis de metales en la fracción de alta y baja masa molecular por ICP-QQQ-MS en las muestras de suero. Tanto las concentraciones de elementos totales en suero, orina y LBA, como sus fracciones asociadas a moléculas de alta y baja masa molecular de pacientes CP y controles, se analizaron estadísticamente con el fin de identificar las variables más significativas en la enfermedad. Además de las concentraciones totales, también se estudiaron los ratios de los elementos para comprobar la posible interrelación entre los mismos para mantener el equilibrio homeostático en CP y su posible uso como biomarcadores. En particular, el selenio es uno de los elementos más estudiados en el cáncer debido a su carácter quimiopreventivo, asociado a la función antioxidante que desempeñan las selenoproteínas[4]. Existen varios estudios que relacionan la alteración en la expresión de las selenoproteínas con el riesgo de padecer cáncer, como la eGPx [5] y la SELENOP [6]. Así pues, adicionalmente, se ha desarrollado una metodología basada en la técnica de dilución isotópica y un acoplamiento de columnas de exclusiónd e tamaños y de afinidad al ICP-MS (SEC-AF-HPLC-SUID-ICP-QQQ-MS) para la cuantificación absoluta de selenoproteínas (eGPx, SELENOP, SeAlb), y del contenido total de selenometabolitos, en muestras de suero de pacientes CP. Los resultados mostraron que eGPx, SELENOP, SeAlb y el contenido total de los selenometabolitos son mayores en el grupo CP, habiendo diferencias significativas en las concentraciones de eGPx y SeAlb entre pacientes CP y controles sanos.

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**ESP-P08**

# DESARROLLO DE UN NUEVO MÉTODO PARA LA DETERMINACIÓN DE SELENOBIOMOLÉCULAS EN LECHE MATERNA, SUERO DEL CORDÓN y LECHE DE FÓRMULA MEDIANTE "COLUMN SWITCHING" E ICP-QqQ-MS

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El selenio se regula en el organismo para mantener las funciones vitales de las selenoproteínas y, al mismo tiempo, evitar la toxicidad. El mecanismo de regulación de la glándula mamaria controla la síntesis y la secreción de seleno-compuestos durante la lactancia, con un alto nivel total de Se en el calostro que disminuye a medida que ésta avanza. El Se aparece en la leche materna como un componente de selenoproteínas específicas y seleno-aminoácidos que son bien tolerados por los lactantes alimentados con leche materna, incluso en grandes cantidades. El Se es ingerido en su mayor proporción en su forma orgánica, como selenometionina (SeMet) y selenocisteína (SeCys), siendo ésta última la forma en la que el Se se encuentra en las selenoproteínas y la de mayor biodisponibilidad. También se ingieren, aunque en menor cantidad como selenito y selenato [1,2].

En este trabajo, se ha desarrollado un nuevo método para la especiación simultánea de selenoproteínas y selenometabolitos y se ha aplicado a leche materna, suero del cordón y leche de fórmula basado en la separación mediante cromatografía líquida bidimensional (cromatografía de exclusión de tamaño y de afinidad) y detección por espectrometría de masas por plasma de acoplamiento inductivo de triple cuadrupolo (ICP-QQQ-MS). El método permite el análisis cuantitativo simultáneo de selenoproteína P (SePP), glutatión peroxidasa extracelular (eGPx), y selenometabolitos en suero humano y leche materna mediante dilución isotópica de especies no específicas (SUID) [3]. Este método analítico se ha aplicado sueros de cordón umbilical y maternos, obtenidos en el momento del nacimiento y leche calostro (7 madres). Se llevaron a cabo mediciones adicionales de la concentración de SePP en suero y de la actividad de la enzima GPx usando ELISA. La concentración total de Se fue significativamente mayor en el suero materno que en el suero del cordón umbilical. La concentración de SeAlb fue significativamente mayor en los recién nacidos, mientras que las concentraciones de SePP y GPx fueron significativamente mayores en las madres. Además, las selenoproteínas y los selenometabolitos se han identificado y cuantificado en la leche materna humana, estando ausentes en leche de fórmula. En la leche calostro, el selenio está en forma de glutatión peroxidasa (4-32% del Se total)> selenocistamina> selenocistina> selenometionina a 26, 18, 15 y 17 μg $L^{-1}$ en calostro (0-5 d), leche de transición (6-21) d), leche madura (1-3 meses) y lactancia tardía (> 5 meses), respectivamente.

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**ESP-P09**

# CHEMICAL COMPOSITION OF PM10 AND ORIGIN OF ARSENIC DERIVED FROM FUGITIVE AND CHANNELIZED INDUSTRIAL EMISSIONS (HUELVA, SW SPAIN)

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The present work focuses on identifying the sources contribution of the atmospheric particulate matter (PM10) in a complex industrial area of SW Spain (near the city of Huelva), which is under the influence of fugitive emissions generated by the cargo handling sector in its harbour. To this aim, chemical composition of PM10 between 2015-2017 with their subsequent source contribution, were carried out at La Rabida monitoring station (urban-industrial background). Concurrently, pollutant gases and PM10 levels series were studied in the area during the period 1996-2017. The evolution of these pollutants according to the wind direction helped to discriminate possible origin sources. Finally, arsenic speciation was performed to estimate the origin of this metalloid.

La Rabida station is equipped with automatic instrumentation which enables monitoring hourly data. PM10 sampling was performed using quartz fiber filters and MCV high volume captors (30 m3) at the monitoring site. Gravimetric PM10 levels and their chemical composition were determined following the method described by Querol et al. 2001 [1]. Arsenic speciation was achieved by HPLC-HG-AFS after liquid extraction of PM10 samples.

Negative trends were observed for PM10 and gaseous pollutants levels in the whole period as a result of the emission abatement strategies developed by port authorities and the economic recession since 2008. It was inferred a strong industrial contribution of $SO_2$. $NO_2$ levels were derived from both traffic and industrial emissions. The high resuspension from the bulk material handled in the harbour enhances high PM10 concentrations, especially crustal components.

Arsenic was found to be the main geochemical anomaly in PM10. Factorial analysis of the chemical composition of PM10 (Fig. 1) and arsenic speciation analysis allowed to identify two As sources: channelized Cu-smelter emissions and fugitive emissions from the bulk polymetallic sulfides handled in the harbour. Therefore, the results obtained in this study helped to identify for the first time two different sources contributing to the high As concentrations in a complex industrial area.

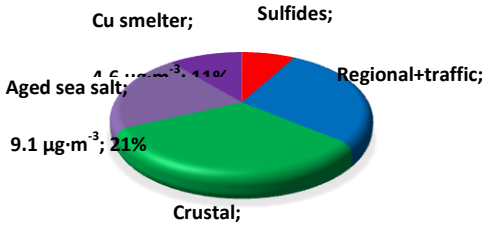


Figure 1: Source apportionment at La Rabida monitoring station during the period 2015-2017.

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**ESP-P10**

# CARACTERIZACIÓN Y CUANTIFICACIÓN DE PARTÍCULAS DE ÓXIDO DE TITANIO EN DERIVADOS DE PESCADO MEDIANTE DIGESTIÓN ALCALINA Y AF4-DLS-ICPMS

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El dióxido de titanio ($TiO_2$) es comúnmente empleado como aditivo en gran cantidad de productos de consumo. Debido a sus propiedades específicas a escala nanométrica, se utiliza en una amplia gama de sectores, tales como el energético, farmacéutico, medioambiental, cosmético o el alimentario. En este último, el $TiO_2$ se suele incorporar como aditivo, regulado por la Unión Europea (E171), con la función de pigmento blanqueador de los productos finales.

El aumento progresivo de su utilización evidencia la necesidad del desarrollo de metodologías analíticas que permitan obtener información adicional sobre este tipo de nanomaterial en los medios en los que se encuentre presente, y así poder evaluar los efectos que potencialmente pueda ocasionar tanto en la salud humana como en el medio ambiente.

Con este fin, en este trabajo se plantea el uso de la técnica de Fraccionamiento en Flujo mediante Campo de Flujo Asimétrico (AF4) para productos que contienen el aditivo E171, que posibilita la separación de las nanopartículas de $TiO_2$ en función de su tamaño, acoplada a diversos sistemas de detección que permiten su caracterización, como absorción molecular en ultravioleta-visible (UV-Vis) y dispersión de luz dinámica (DLS); y su cuantificación, a través de espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS).

Las muestras analizadas consistieron en productos congelados derivados de pescado (surimi), sometidas a digestión alcalina con hidróxido de tetrametilamonio (TMAH). Los resultados obtenidos mediante AF4-UV-Vis-DLS-ICPMS indican que las muestras contenían partículas de óxido de titanio con diámetros hidrodinámicos comprendidos en un rango de 150-325 nm, con concentraciones que variaron entre 3 y 15 $\mu g \cdot g^{-1}$, en función de la muestra analizada. Los valores de recuperación obtenidos fueron de entre el 70 y el 110%. Estos resultados fueron validados a través de la determinación de contenidos totales en los extractos mediante espectrometría de emisión óptica con plasma de acoplamiento inducido (ICP-OES).

**Agradecimientos:**

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***XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA***
***Valladolid 18-19 julio 2019***

## *CONFERENCIAS PLENARIAS*

**CP1**

## ACTUACIONES REALIZADAS EN EL MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES SOBRE LAS REFORMAS EN EL SISTEMA UNIVERSITARIO ESPAÑOL

**J. Manuel Pingarrón**
Secretario General de Universidades del Ministerio de Ciencia, Innovación y Universidades

Prof. **JOSÉ MANUEL PINGARRÓN CARRAZÓN,** nombrado Secretario General de Universidades del Ministerio de Ciencia, Innovación y Universidades en Julio de 2018. Doctor por la Universidad Complutense de Madrid (1981). Estancia posdoctoral en l' École Nationale Supérieure de Chimie de Paris (1982-83). Catedrático de Química Analítica en la UCM desde 1994. Profesor Visitante en la Universidad de Cornell, USA (1997).

Medalla de la Facultad de Química. Premio de investigación en Química Analítica de la Real Sociedad Española de Química (2012). Fellow de la International Society of Electrochemistry (2017). Premio Investigación Científica 2018 del Grupo de Electroquímica de la Real Sociedad Española de Química.

Líneas de investigación en electroquímica analítica, interfases electroquímicas nanoestructuradas, nanomateriales y sensores y biosensores electroquímicos. Autor o coautor de más de 390 artículos científicos en revistas internacionales, 32 capítulos de libro, 2 libros de texto y 10 patentes de invención. La producción científica puede consultarse en: ORCID: 0000-0003-2271-1383; Google Scholar: José M. Pingarrón (public profile); Web of Science: Pingarron J* + Carrazon J*. Editor asociado para Europa de la revista científica Electroanalysis (Wiley-VCH). Pertenece o ha pertenecido a los Comités Editoriales de revistas internacionales (Journal of Electroanalytical Chemistry, Talanta, Analyst, Chemical Sensors and ChemElectroChem). Investigador Principal de proyectos competitivos regionales, nacionales, internacionales y de colaboración con empresas.

Fue Presidente de la Sociedad Española de Química Analítica, SEQA de 1998-2001 y es Vicepresidente de la Real Sociedad Española de Química y su representante en la División de Química Analítica de la Asociación Europea de Ciencias Químicas y Moleculares. Miembro de la Junta Directiva de la Confederación de Sociedades Científicas de España (tesorero). Vicepresidente de la División I (Electroquímica Analítica) de la Sociedad Internacional de Electroquímica (2015-2017). Miembro del Comité de la División de Química Analítica de la Unión Internacional de Química Pura y Aplicada (2007-2014). Gestión de investigación: Miembro del equipo de gestión del subprograma de Química Básica (BQU) del Plan Nacional de Investigación del Ministerio de Economía e Innovación (2008-2015). Presidente del Consejo Asesor de Ciencia, Tecnología e Innovación (MINEICO) desde 2017.

El Secretario General de Universidades nos hablará sobre la previsión del Ministerio de un decreto por el que se establece la ordenación de las enseñanzas universitarias oficiales, con "novedades importantes" como la posibilidad de formación dual, de itinerarios abiertos y de proyectos académicos integrados.

**CP2**

## ANALYTICAL DEVELOPMENTS IN METABOLOMICS WORKFLOW

**Coral Barbas**

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We are living a shift in research paradigm from a reductionist research to “omics” technologies. Among them, Metabolomics is the one with a stronger link to Analytical Chemistry, because the broad range of physicochemical properties of metabolites, their range of concentrations and the influence of the matrix make their measurement, even in a semiquantitative way, a real challenge.

The final success of non-targeted metabolomics depends on applying the principles of the Analytical Process to every step in the workflow:

-Clear definition of the **objective** of the analysis.

-Sampling and sample storage to guarantee a **representative** and homogeneous sample

-Sample pre-treatment to obtain a sample as **complete and non-biased** as possible while being compatible with the instrumental techniques.

-Analytical methods with a proper **quality control**

-Use of appropriate **statistics** and data treatment

-**Identification** of statistically significant metabolites

-**Interpretation** of results

Most of the colleagues in the analytical field will identify those terms even if they are not familiar with the metabolomics concepts.

Our group has been working in improving the methodology in most of these areas while at the same time applying our developments in real world studies because the path is made by walking.

Examples of different solutions such as sample treatments for small amounts of sample aiming a broad metabolite coverage (1,2); normalization strategies; CEU Mass Mediator platform (CMM) (3), a knowledge-based metabolite annotation tool, among others, will be presented.

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**CP3**

## LOS NUEVOS RETOS DE LA ESPECTROSCOPIA RAMAN: DE LA EXPLORACIÓN DE MARTE A NUESTRO PASADO HISTÓRICO

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Exomars es una misión de la Agencia Espacial Europea (ESA) que va a jugar un papel clave en la exploración robotizada de Marte. Dicha misión, con fecha de lanzamiento prevista en Julio de 2020, tiene como objetivos científicos prioritarios la posible detección de signos de vida pasados o presentes en el planeta rojo y la mejor caracterización mineralógica y geoquímica de los procesos relacionados con la actividad del agua para indagar sobre su pasado similar al de la Tierra.
Para ello, el vehículo, llamado Rosalind Franklin (Figura 1)

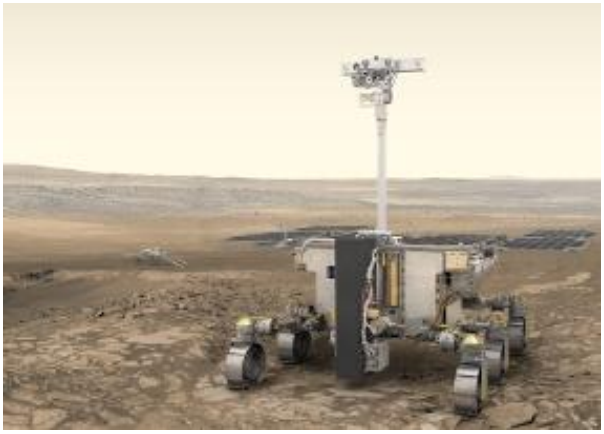

*Figura 1. Vista general del "rover" de Exomars 2020, Rosalind Franklin. Movido por paneles solares lleva al frente un perforador capaz de obtener muestras hasta 2 metsros debajo de la superficie marciana*

(http://exploration.esa.int/mars/61114-esas-mars-rover-has-a-name-rosalind-franklin/), cuenta con una herramienta única hasta el presente en la exploración marciana: un perforador capaz de obtener muestras hasta 2 metros de profundidad debajo de la superficie. La razón es que estas muestras pueden preservar de manera más eficiente compuestos orgánicos al estar mejor apantalladas de los severos efectos de la radiación espacial que las muestras superficiales donde estos compuestos son rápidamente degradados.
También cuenta, en su interior, con un laboratorio analítico con tres instrumentos esenciales (http://exploration.esa.int/mars/45103-rover-instruments/), uno de los cuales, es un espectrómetro Raman. Las muestras obtenidas por el perforador serán procesadas bajo la forma de polvo cristalino y presentadas debajo de los instrumentos analíticos por un carrusel en un pequeño contenedor rellenable (Figura 2).

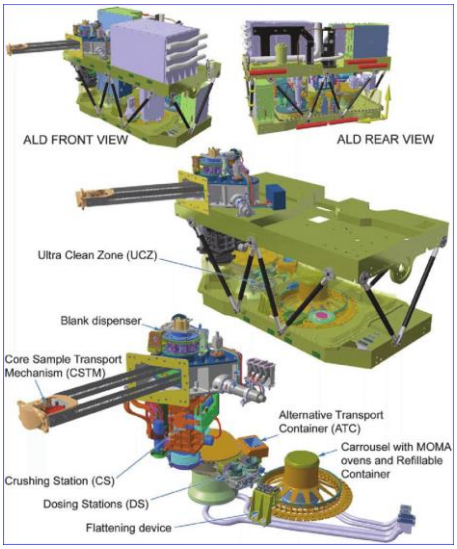


*Figura 2. Vista general del laboratorio analítico (ALD) incluido dentro del "rover"*

La espectroscopia Raman se basa en el proceso de dispersión inelástica de la luz por la materia. Cuando una luz monocromática, en general un láser, ilumina una muestra, una ínfima cantidad de la luz dispersada tiene una longitud de onda diferente a la de excitación. Esta fracción, contiene la información atómico-molecular y estructural del material iluminado. En este proceso, no hay contacto físico entre el instrumento y la muestra, resulta no destructivo y se puede realizar en múltiples configuraciones (macro o micro) a proximidad o a distancia y no es necesario ningún tipo de preparación de la muestra. Debido a que las bandas Raman son en general muy estrechas permiten una precisa identificación de los compuestos a analizar.

En el caso de Exomars, los objetivos científicos del instrumento Raman se derivan directamente de los objetivos principales de la misión: análisis mineral y detección de orgánicos y este trabajo se

centra en la descripción del desarrollo, la fabricación y los test funcionales y de prestaciones científicas del instrumento Raman (RLS) para la misión Exomars 2020. (Figura 3).
Este instrumento, es el primero de la historia, usando esta técnica, totalmente calificado, verificado y listo para volar al planeta rojo. Está liderado por un equipo español de la UVA y de INTA coordinando a un consorcio internacional en el que participan Francia, UK y Alemania.

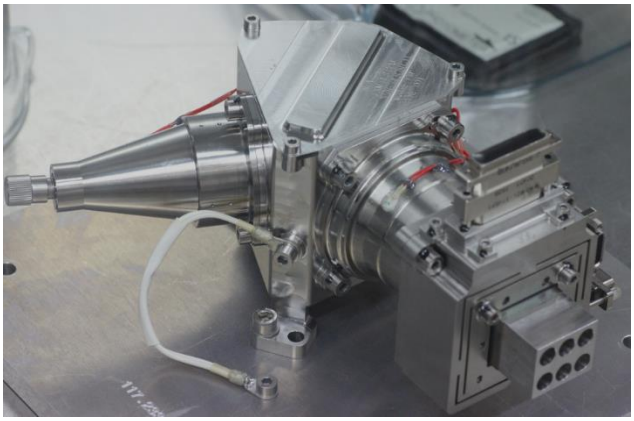

*Figura 3. El espectrómetro Raman desarrollado para la misión Exomars de ESA y totalmente calificado para su vuelo a Marte. Su masa es de 840g.*

Se van a detallar, además de los aspectos técnicos relacionados con los modelos esenciales de desarrollo, el modelo de calificación y el modelo de vuelo, aspectos relacionados con la operación. Estos aspectos son de gran trascendencia para conseguir optimizar la respuesta espectral, ya que el espectrómetro ha de trabajar en modo completamente automático.

En misiones dirigidas por intereses científicos, no solo hay que prestar atención al desarrollo tecnológico, hay también que desarrollar la ciencia asociada, la cual, en este caso, se basa en varias fuentes: el estudio de los meteoritos, la experiencia previa de misiones en Marte y sobre todo, el estudio de posibles análogos terrestres a los procesos geológicos y mineralógicos marcianos, sobre todo, usando prototipos adaptados al trabajo en campo. A este último aspecto se dará particular importancia. (Figura 4).


*Figura 4. El instrumento Raman trabajando en el Ártico en coordinación con un nuevo prototipo de "rover" de NASA para recogida de muestras automática en futuras misiones de retorno a Marte.*

Finalmente, como consecuencia del desarrollo de estos prototipos de bajo peso y gran robustez asociados al desarrollo de los modelos de vuelo, se abren interesantes aplicaciones para estudio in-situ de diversos problemas en tierra. Aquí se destacarán algunos ejemplos relevantes, relacionados con el medio ambiente y el patrimonio histórico y artístico. (Figura 5).

*Figura 5. Análisis in-situ por espectroscopia Raman de los pigmentos de las figuras de la cueva de Altamira.*

**CP4**

## NATURE INSPIRED BIOLUMINESCENCE : TOWARD NEW GENERATION CELL-BASED AND REAGENT-LESS CHEMICAL LUMINESCENCE BIOSENSORS

**Aldo Roda**

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Despite being widespread in nature, the phenomenon of bioluminescence (BL) is not yet fully understood. Many questions are still unanswered. How is the “living light” processed and which physiological and behavioral consequences does it evoke in different species? Furthermore, many BL organisms are still unexplored, or they have just been discovered, such as BL mushrooms. For analytical applications, naturally BL firefly represents a muse for the design of biosensors and actuators, both for the chemistry of the BL process and for the high-performing nanotechnology involved in BL emission, including the anatomy of nanostructured photophores and materials optimized for efficient light emission. The in vivo regulation of the light color, the neural network involved in light emission trigger, the regulation of flash frequency, used not only for courtship but also for rational movement in the dark: these all represent unique sources for the inspiration of synthetic biology, that can lead to high performance new analytical devices including biosensors, actuators and robots. Despite their diversity, BL species, from fireflies to marine bacteria, share a common chemistry for light production. It relies on the radiative decay of an intermediate dioxetanone analogue in its singlet excited state, obtained by the luciferase-catalyzed oxidation of a given luciferin substrate. Although the luciferin/luciferase systems display completely different chemical structures from one luminous species to another, they all are designed and optimized to efficiently produce light via this mechanism. This observation has indeed inspired the design of several chemiluminescent (CL) probes, such as dioxetane analogues, acridinium esters and aryl-oxalates, which however display quantum yields lower than natural BL. As light emission can be simply measured employing a CCD or a CMOS, with no need for any optics nor additional light sources, portable devices based on smartphone have been designed for BL/CL-based biosensors. The firefly lantern anatomical structure has inspired the design of an efficient photophore, able to maximize the light output exploiting nano-assemblies of different materials. Among the various CL reactions, the luminol/HRP/H2O2 is the most exploited in biosensing, using a variety of luminol analogues or inorganic nanostructured catalysts replacing HRP to improve analytical performance. The most versatile an simple device format is the lateral flow (LFIA), which has been successfully widely used by us achieving high detectability even when using simple smartphone-based portable devices. Recently, we developed for the International Space Station a portable device used in space by astronauts, in which the LFIA is assisted by a microfluidic-based chip able to deliver the needed reagents even in microgravity. More recently, we proposed the use of thermochemiluminescence (TCL) as a reagent-less system, in which the emitting excited species are generated by thermal decomposition of an acridine 1,2-dioxetane, rather than by a chemical trigger. We obtained TCL Pdots by doping fluorescent cyano-polyphenylene vinylene (CN-PPV) with such a TCL derivative and employed them as a label in immunoassay, showing that a broad

panel of ultrabright nanosystems can be designed for a variety of bioscience applications, taking advantage of the efficient energy transfer. Exploiting nucleic acids to design allosterically regulated structure-switching biomolecules, we recently developed BL/CL biosensors based on stem-loop DNA nanoswitch and proximity nanoBit luciferase recomplementation. Recently, a new split luciferase-based biosensor was developed for rapid and sensitive drug discovery and cancer diagnostics. These new re-complementation assays provide new analytical tools for fast and sensitive “cut and sew” technologies. Living cells can be also reprogrammed to perform a desired function by reengineering and rewiring of natural and synthetic genetic circuits. By genetically engineering cells with a BL reporter gene fused to a regulatory DNA sequence, cells can be exploited as “living sensors”, a sort of animal friendly evolution of the old coal miner’s canary concept. These biosensors have proved to be valuable for predicting the physiological response to drugs and chemicals in complex matrices. Furthermore, by combining luciferases with different features (e.g., emission wavelength, kinetics, half-life), cell-based BL assays in multiplex formats can be developed, based on chemical, spatial, temporal and spectral resolution. Natural BRET occurring in jellyfish inspired nanohybrid biotic-abiotic systems consisting in nanorods-luciferase hybrids having highly efficient energy transfer. Pushing the boundaries of in vitro sensing systems, mammalian 3D cell-culture models, i.e. "spheroids" were also obtained to faithfully mimic in vivo tissue physiology, thus providing highly predictive data for toxicity and bioavailability studies. All these systems were implemented into field-deployable biosensors employing a smartphone as a light detector and bioinspired supporting materials to confine and preserve cells. Moreover, we implemented an additional capability into our biosensing systems, i.e., magnetic actuation, by bioengineering magnetotactic bacteria, able to align according to the geomagnetic field thanks to genetically encoded nanostructured magnetosomes, with BL reporters and used them as toxicity sensors. Proof-of-principle applications of these biosensors will be presented together with main limitations, such as those related to the limited shelf-life of cells, and current challenges to turn them into marketable biosensors


# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*
## *Valladolid 18-19 julio 2019*

## *CONFERENCIAS INVITADAS*

**Ci1**

## BIOSENSORES PARA EPIGENÉTICA Y METÁSTASIS EN CÁNCER

**Susana Campuzano, María Pedrero, Paloma Yánez-Sedeño, José M. Pingarrón**

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El constante progreso en la identificación de biomarcadores a diferentes niveles moleculares en muestras de diversa naturaleza y la necesidad de llevar a cabo análisis rutinarios en entornos descentralizados empleando protocolos simples y cortos son sólo algunas de las demandas actuales de la clínica, no satisfechas con las técnicas convencionales disponibles. En este contexto, las características únicas que presentan los biosensores electroquímicos en términos de coste asequible, monitorización en tiempo real, empleo de pocos reactivos, manejo sencillo, versatilidad y compatibilidad con diseños portátiles y multiplexados, los hacen especialmente interesantes para cumplir con los requisitos cada vez más exigentes que requieren los diagnósticos y pronósticos en el punto de atención.

En esta conferencia se discutirán las principales características y oportunidades que ofrecen las plataformas biosensoras electroquímicas desarrolladas recientemente en nuestro grupo de investigación para la determinación individual o multiplexada de biomarcadores de relevancia emergente en diagnóstico y pronóstico de cáncer a nivel genético, regulatorio y funcional en muestras clínicas de elevada complejidad [1].

Se prestará especial atención a la determinación de biomarcadores proteicos, tanto para diagnóstico precoz (autoanticuerpos séricos frente a antígenos asociados a tumores) como para detección de procesos metastásicos (FGFR4, E-cadherina, CDH-17 e IL-13sR2), y de biomarcadores epigenéticos (miRNAs y presencia de bases metiladas en ácidos nucleicos), en muestras clínicas complejas de pacientes oncológicos.

Las metodologías desarrolladas están basadas en el acoplamiento inteligente de atractivos biorreceptores comerciales y formatos de bioensayo y se han implementado tanto en microsoportes magnéticos funcionalizados como en electrodos desechables acoplados al empleo de químicas superficiales atractivas como la química de las sales de diazonio y de diferentes nanomateriales: nanopartículas de oro como modificadores electródicos [2] y nanomateriales híbridos de nanotubos de carbono de pared múltiple y puntos cuánticos de grafeno como como portadores de elementos de señalización [3]. Estas bioplataformas han demostrado, de forma pionera, su utilidad práctica para la determinación fiable de los analitos diana en pequeñas cantidades de muestras complejas mínimamente tratadas que incluyen suero escasamente diluido, células enteras y tejidos embebidos en parafina, muestras que han sido muy poco exploradas hasta la fecha con técnicas convencionales y biosensado electroquímico. A diferencia de los resultados semicuantitativos, y a veces de interpretación subjetiva y compleja, que proporcionan las metodologías convencionales utilizadas en las rutinas hospitalarias, estas bioherramientas electroquímicas de fácil manejo, para determinaciones simples o multiplexadas, se pueden adaptar fácilmente a la determinación de otros biomarcadores, proporcionan resultados cuantitativos objetivos con menor coste y empleando cantidades de muestra inferiores y tiempos de ensayo más cortos. Estas interesantes características las convierten en alternativas adecuadas para su implementación en dispositivos fáciles de usar y de coste asequible, especialmente atractivos para su uso en atención hospitalaria, ambulatoria e incluso domiciliaria y que contribuirían tanto a mejorar las estadísticas del cáncer y la calidad de vida de los pacientes como a aliviar la carga financiera que soportan los sistemas nacionales de salud.

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**Ci2**

## AMBIENT IONIZATION-MASS SPECTROMETRY: MAKING REAL THE DIRECT ANALYSIS BY MASS SPECTROMETRY

**Encarnación Moyano**

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Mass spectrometry (MS) is nowadays a powerful and wide-range technique used in many analytical and bioanalytical laboratories. Nevertheless, the analysis of complex mixtures is not straightforward, and this handicap makes difficult the direct analysis by MS of organic compounds in environmental, food and biological samples. Over the past 50 years, the important developments in mass spectrometric instrumentation (sources and analyzers) have played a significant role in reducing the complexity of mass spectrometry. The hyphenation of MS with common separation techniques, such as gas chromatography (GC) and liquid chromatography (LC) increased the dimensionality of analytical determinations and leaded to highly sensitive and efficient approaches for dealing with complex mixtures. However, extensive sample preparations are often required to get clean extracts to be used for the chromatographic separation, which became the bottleneck of many routine laboratories and constrained the analytical efficiency of MS-based methods.

Nowadays, the direct analysis of complex samples by mass spectrometry has become a reality mainly due to three instrumental capabilities: soft ambient ionization techniques, high-resolution mass analyzers and hybrid instruments. The development of ionization sources with the ability to produce soft ionization minimizes fragmentation during ion formation and makes possible the assignment of each *m/z* signal to one compound in the complex sample. Moreover, high-resolution mass analyzers and/or hybrid instruments, able to perform tandem mass spectrometry experiments, provide the selectivity and sensitivity required to identify unequivocally analytes within complex mixtures.

Ambient Ionization Mass Spectrometry (Ambient MS) is a new group of ionization techniques introduced in the last decades, which allows the direct sampling and ionization of analytes, in the same process and in the open atmosphere with minimum or no sample preparation requirements. Ambient MS techniques enable high-throughput analysis by placing the sample directly between the ionization source and the mass spectrometer inlet, thus reducing the total analysis time to less than a couple of minutes. This group of techniques is easy to interface to most types of mass spectrometers by simply replacing the original atmospheric pressure ionization (API) source by the Ambient MS device such as DESI (desorption electrospray ionization) or DART (direct analysis in real time), which are already commercially available.

Ambient MS techniques can offer advantageous characteristics to analytical laboratories. Real-time and in situ analysis, low sample requirements with little sample invasion, fast and high-throughput analysis, minimal or no sample prior preparation, small or no use of organic solvents, and relatively low matrix effects are some of the ambient MS characteristics that can be attractive for food, environmental and forensic applications. These features allow facing some requirements such as workload, turnaround time, and cost per sample frequently demanded by modern analytical laboratories.

In this presentation, an overview on the fundamentals lying on Ambient Ionization Mass Spectrometry Techniques is presented. Several examples in the fields of environmental, food and forensic analysis will help to illustrate the applicability of these new emerging techniques for the screening of a wide-range of compounds in complex samples. Limitations regarding quantitative analysis and some of the difficulties found to avoid sample manipulation are also discussed.

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**Ci3**

## FASES SORBENTES CON NANOMATERIALES PREPARADAS SOBRE PAPEL: DO IT YOURSELF!

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La aparición de las técnicas de microextracción, tanto en fase sólida como en fase líquida, en el ámbito del tratamiento de la muestra ha supuesto un cambio de paradigma en esta etapa del proceso químico de medida. Este cambio, inicialmente, estuvo orientado a la sustitución de las técnicas clásicas (especialmente la extracción líquido-líquido) por otras aproximaciones miniaturizadas que redujeran los aspectos negativos respecto al consumo de muestras y reactivos, tiempos de análisis y escasas consideraciones medioambientales.

Con el tiempo, este ha sido un campo de investigación abordado por muchos grupos en los que se ha innovado tanto en los formatos de microextracción como en los materiales (sorbentes o disolventes) que se emplean como fase extractante. En este sentido, la reducción de dimensiones inherente a la miniaturización de la técnica de extracción requiere el empleo de medios de extracción más eficientes que los empleados en las técnicas convencionales.

La Química Analítica ha sido capaz de incorporar en diversas facetas y de forma muy satisfactoria las ventajas que aportan los nanomateriales. Las dimensiones de estos sólidos, situados en la nanoescala (inferiores a 100 mn), hacen que se incrementen de forma excepcional muchas de las propiedades que presentan, incluyendo la capacidad sorbente. Los nanomateriales de carbono (fullerenos, nanotubos, nanocuernos), las nanopartículas metálicas (oro y plata) y los óxidos metálicos, incluyendo aquellos que presentan propiedades magnéticas, son ejemplos paradigmáticos en este contexto. Sin embargo, no se puede obviar el hecho de que, para que esas propiedades excepcionales se exploten es necesario conservar las dimensiones nanométricas durante la aplicación de estos materiales.

En los últimos años, se ha explorado también con notables resultados la combinación sinérgica de los nanomateriales con otras fases sorbentes como las poliméricas dando lugar a los *nanocomposites poliméricos*. En ellos, las nanopartículas quedan embebidas generalmente en la red polimérica, aumentando la superficie activa del polímero, pudiendo además conferirles nuevas propiedades (como el magnetismo) o aportar mecanismos adicionales de interacción con los analitos.

Estas nuevas fases extractantes pueden emplearse en la modalidad de microextracción dispersiva o bien pueden inmovilizarse en soportes inertes de diferente geometría. En este sentido, los soportes planos aparecen como una alternativa muy competitiva a las fibras puesto que mejoran la cinética del proceso gracias a la relación superficie-volumen más favorable. Además, pueden acoplarse fácilmente con técnicas instrumentales como las espectroscópicas y la espectrometría de masas abriendo la puerta al desarrollo de metodologías rápidas de análisis.

El empleo de fases sorbentes preparadas sobre papel constituyen una aportación muy relevante en este sentido. Entre las características más notables destacan su elevada superficie específica, en relación con otros formatos de (micro)extracción, su alta porosidad y su gran versatilidad. La unión entre la fase sorbente y el papel puede hacerse mediante: i) la formación de enlaces covalentes; o ii) la inmersión del papel en una disolución/dispersión de la fase sorbente. Si bien la primera aproximación resulta en uniones más estables desde el punto de vista mecánico, la segunda opción es mucho más simple y permite obtener unidades de microextracción suficientemente estables para su aplicación analítica. La rapidez de la síntesis y el bajo coste asociado a todo el proceso posibilita que estas unidades sean desechables, lo que es especialmente recomendable en algunos campos de aplicación, como el de los análisis clínicos y/o toxicológicos.

Esta aproximación la hemos empleado en el grupo de investigación para preparar una amplia variedad de fases sorbentes a partir de polímeros, nanopartículas y la combinación de ambas. Esta comunicación se centrará principalmente en presentar y discutir ideas relacionadas con la síntesis, los tipos de recubrimientos, los dispositivos de microextracción o la versatilidad de los materiales, más que en la descripción detallada de los procesos de medida en los que se han empleado. Se pretende resaltar de esta manera el potencial de estos dispositivos y su síntesis, así como la posibilidad de prepararlos en los laboratorios analíticos de forma similar a como hemos incorporado en otros ámbitos la filosofía de la cultura *do it yourself*


***XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA***
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## *COMUNICACIONES ORALES*

**ALI-O01**

## A MULTI-OMICS APPROACH REVEALS THE BIOMOLECULAR MECHANISMS ASSOCIATED TO THE TOXICITY OF A COMMON SURFACTANT USED IN FOOD PACKAGING

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Surfynol is a non-ionic surfactant present in many adhesives used either for building multilayer materials or to glue paper and plastic in food packaging. Although the potential toxicity of this compound has not been studied, it has been demonstrated that it migrates from the multilayer structure to both solids and liquids in contact with the packaging [1-2]. When this multilayer plastic was used for containing seminal doses for artificial insemination, it was found that fertility was seriously damaged in terms of motility, acrosome integrity, mitochondrial activity and penetration capacity in the cells, thus affecting male fertility [3].

Based on the above, we designed a multi-omics approach with the aim of unvealing the biomolecular mechanisms associated to the toxicity exerted by Surfynol at different molecular levels. As in-vitro model, we selected NTERA2 cells, consisting of germinal cells of testicular embryonal carcinoma since they are the precursors of the sperm.

On one hand, a SILAC-based quantitative proteomic approach allowed for the identification of more than 2000 proteins, from which 108 appeared de-regulated upon Surfynol exposure. These altered proteins were involved in the cytoskeleton assembly, the sperm motility, the energy machinery and the sperm defence mechanisms against oxidation [4].

On the other hand, untargeted and targeted mass spectrometry-based metabolomics were conducted on the same model. The untargeted metabolomics assay identified several altered metabolites in cells exposed to Surfynol. These metabolites participated in energy acquiring pathways, which are closely related with sperm motility. Since the energy metabolism seemed to be the main target for Surfynol toxicity, we further designed a targeted metabolomics approach for the determination of ATP, ADP, NADH and NAD in cells. The results validated the proposed mechanisms, since the levels of ATP, ADP and NAD were significantly lower in cells exposed to Surfynol.

This multi-omics approach has demonstrated the reprotoxicity associated to Surfynol through the de-regulation of key proteins and metabolites involved in the sperm fertilization capacity. This results open a door to further research in which male infertility caused by chemicals could be demonstrated.

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**ALI-O02**

# LA OCURRENCIA COMO PARÁMETRO SIGNIFICATIVO PARA LA VALIDACIÓN DE UN MÉTODO DE CLASIFICACIÓN MULTIVARIABLE – APLICACIÓN A LA AUTENTIFICACIÓN DE ACEITE DE OLIVA

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La aplicación de herramientas quimiométricas convencionales (partial least squares-discriminant analysis (PLS-DA), soft independent modelling by class analogy (SIMCA), etc.) y, con mayor frecuencia, algunas herramientas propias de la minería de datos (support vector machine (SVM), random forest (RF), etc.) está aumentando notablemente para desarrollar métodos de clasificación y/o cuantificación multivariable en el ámbito de la química analítica. Prueba de ello es el crecimiento significativo del número de publicaciones científicas en los últimos años dónde se describen el uso de éste tipo de métodos en diferentes aplicaciones analíticas.

El desarrollo de modelos multivariables consta de dos etapas fundamentalmente: (i) la etapa de entrenamiento donde se "enseña" al modelo con un conjunto de patrones representativos del producto de interés y de productos alternativos, y (ii) la etapa de validación donde el modelo predice la propiedad de interés de un conjunto de productos diferente a los usados en la etapa de entrenamiento.

En la actualidad, en la mayoría de los trabajos encontrados en bibliografía, la etapa de validación es desarrollada usando la misma proporción de patrones del objeto de interés y patrones alternativos en el conjunto de validación. Sin embargo, esta metodología de trabajo no considera la ocurrencia como parámetro para seleccionar el número de las muestras representativas de cada uno de los patrones, y por tanto la aplicación de dicho modelo en un caso real puede conducir a conclusiones que no representan el comportamiento real del método en análisis de rutina.

La ocurrencia indica la fracción esperada de objetos que cumplen la propiedad de interés, con respecto a la población global. En la comunicación que se presenta se propone hacer uso de la ocurrencia para validar correctamente un método de clasificación multivariable, de forma que los patrones usados en esta etapa sean representativos de la probabilidad asociada a las muestras que realmente van a llegar a un laboratorio de análisis de rutina. En ese sentido ha sido aplicada en el desarrollo de diferentes modelos de clasificación para la detección de la adulteración de aceites de oliva con otros aceites vegetales, aplicando las herramientas SVM y RF a partir de datos obtenidos por cromatografía de líquidos acoplada a un detector de absorción molecular de fila de diodos (HPLC-DAD) y cromatografía de gases acoplada a un detector de ionización en llama (GC-FID).

**FYO-O01**

# USO INNOVADOR DE LAS REGRESIONES MULTIVARIANTES PARA LA DATACIÓN EN EL ÁMBITO FORENSE.

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¿Es posible saber en qué momento se ha firmado un testamento ológrafo? ¿Podemos determinar la fecha de creación de una obra de arte moderno? ¿Y el intervalo post-mortem (IPM) de unos restos óseos desenterrados? En el campo forense existe cada vez una mayor demanda por conocer con relativa exactitud el cuándo. La justificación puede estar soportada en cada uno de los casos por distintos motivos con importantes consecuencias sociales, judiciales y/o económicas. Hasta el momento actual, aunque se ha intentado dar respuesta a estas cuestiones mediante distintas metodologías analíticas y valoraciones de expertos [1-3], ninguna de ellas ha logrado asignar fechas concretas o intervalos de tiempo bien definidos. En estos últimos años nuestro equipo de investigación ha desarrollado varios trabajos de datación empleando regresiones multivariantes mediante mínimos cuadrados parciales (PLS, OPLS) [4-6] en distintos campos de aplicación forense (datación de documentos modernos, datación de obras de arte con pintura acrílica ó asignación del IPM en restos óseos humanos). La aplicación de estos modelos de regresión a las respuestas de los instrumentos analíticos más habituales (FTIR, Raman, UV-vis-NIR, Py-GC/MS) han logrado la asignación de fechas concretas con intervalos de error asociados entre un 4-30%. Aunque los resultados son más que aceptables, el uso de modelos multivariantes conlleva algunas decisiones críticas que deben ser revisadas con criterio como son: las técnicas instrumentales elegidas y sus distintos tratamientos, la selección de variables en la creación del modelo de regresión, la elección de rangos temporales o el uso de envejecimientos artificiales frente a naturales.
Por último, el estudio en profundidad de los modelos de regresión desarrollados, han servido a su vez para aportar información importante sobre los procesos físico-químicos que rigen los envejecimientos en cada uno de los casos prácticos. De este modo, se han podido deducir la secuencia de degradación de los distintos componentes de las tintas viscosas de bolígrafo (disolventes, colorantes y resinas), se han localizado las posibles interferencias por enfermedades óseo-degenerativas en la medida del IPM o se han observado las diferencias entre los procesos de escisión y entrecruzamiento de los polímeros constituyentes de las pinturas acrílicas en distintas marcas comerciales. Toda esta información complementaria podrá ser muy útil a la hora de desarrollar nuevos modelos de aplicación universal en el caso de la datación de documentos o pautas de conservación y restauración para el caso de las obras de arte.

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**MAB-O01**

# SÍNTESIS Y APLICACIÓN DE UN NUEVO DISOLVENTE SUPRAMOLECULAR BASADO EN 1,2-DECANODIOL PARA LA EXTRACCIÓN DE COMPUESTOS PERFLUORADOS EN AGUAS NATURALES

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El diseño y uso de disolventes funcionales ha sido un campo de creciente interés en los últimos años. Los disolventes supramoleculares (SUPRAS) son disolventes nanoestructurados cuya síntesis se basa en procesos de autoensamblaje y coacervación. Concretamente, se generan a partir de disoluciones coloidales acuosas u orgánicas de anfifilos, los cuales comienzan a formar agregados tridimensionales (micelas o vesículas, principalmente) a partir de una concentración de agregación crítica. Para la formación del SUPRAS como una nueva fase se añade un agente coacervante que induce un cambio ambiental (pH, temperatura, concentración de sal…), que disminuye la repulsión entre los agregados favoreciendo su ensamblaje.

En este trabajo, se sintetizó y caracterizó por primera vez un SUPRAS basado en un anfifilo de doble cabeza polar (1,2-decanodiol) en una disolución de tetrahidrofurano y agua. Con el fin de evaluar sus capacidades extractivas se aplicó, posteriormente, para la determinación de sustancias perfluoroalquiladas (PFASs), -concretamente, sulfonatos y ácidos con cadenas alquilo de longitudes comprendidas entre $C_4$-$C_{18}$-, en aguas ambientales de la provincia de Córdoba. Estos nuevos SUPRAS, en comparación con aquellos sintetizados a partir de surfactantes homólogos de una sola cabeza polar (decanol), presentan diferente estructura y composición, así como mayores capacidades extractivas para compuestos en un amplio intervalo de polaridad debido al incremento de las interacciones dipolo-dipolo y puente de hidrógeno. Estos SUPRAS son también más polares y presentan un mayor contenido en agua ya que se acomodan menos anfifilos de doble cabeza polar en un mismo agregado -debido a impedimentos estéricos- y se forman estructuras más abiertas. En esta nueva metodología desarrollada destacan el bajo consumo de disolvente (250 µL de SUPRAS por 36 mL de muestra) y la rapidez (30 min de agitación, 5 de centrifugación).

Tras la adición del agente coacervante (36 mL de muestra acuosa, 1 M NaCl) a una disolución de 4 mL de THF y 150 mg de 1,2-decanodiol, el nuevo SUPRAS se formó espontáneamente. Posteriormente, se inyectó directamente en el sistema LC-MS/MS sin etapas posteriores de limpieza o evaporación/reconstitución. Los límites de detección alcanzados fueron de entre 0.005 y 0.010 ng $L^{-1}$. En todas las aguas ambientales analizadas se encontraron PFAS en el intervalo 0.03-2.33 ng $L^{-1}$.

En conclusión, el uso y estudio de anfifilos con múltiples cabezas polares abre una nueva línea de investigación enfocada en el desarrollo y la síntesis de SUPRAS con propiedades de extracción mejoradas.

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**MAB-O02**

## ESTIMATION OF EXPOSURE TO PHTHALATE PLASTICIZERS OF THE SPANISH POPULATION USING WASTEWATER-BASED EPIDEMIOLOGY

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Phthalate diesters are high-production-volume chemicals that have been widely used in the manufacturing and processing of plastics for more than 80 years. Recently, they have been included in the priority lists of dangerous substances in most of the industrialized countries. Ingestion is considered the major route of exposure to phthalates, either by consuming contaminated food, accidental ingestion of contaminated dust and soil, or licking of products in which they are contained. Once in the human body, phthalates are hydrolysed to their corresponding monoesters and further oxidized or conjugated into glucuronide complexes and finally excreted.

Wastewater-based epidemiology (WBE) is a complementary approach to human biomonitoring to estimate the level of exposure to a substance through the analysis of its metabolic residues in urban wastewater [1], considering that raw wastewater is a highly diluted urine sample representing an entire community.

A sensitive analytical method was developed to quantitatively measure metabolites of 6 phthalate diesters in raw wastewater [2]. Thus, the objective of this study consisted of the application of the developed method to analyse wastewater samples collected in different locations in Spain and the evaluation of the exposure to phthalate diesters in the investigated cities. Raw wastewater from 17 wastewater treatment plants, serving a total population of 6.1 million inhabitants (13% of the Spanish population), was analysed. The results show that the highest population-weighted exposure loads were obtained for diethyl phthalate, followed by dimethyl phthalate and the isomers di-i-butyl phthalate and di-n-butyl phthalate.

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**MAB-O03**

## QUANTITATION CHALLENGES IN THE DETERMINATION OF PALYTOXIN AND OVATOXINS IN *Ostreopsis cf. ovata* MICROALGAE BY UHPLC-HRMS

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Some biological toxins (biotoxins) can be a threat to human health because they contaminate drinking water, are bioaccumulated in fish and seafood, or inhaled as part of marine aerosols. In this communication we focus on palytoxin (a polyhydroxylated complex compound) and analogues (ovatoxins) produced by the marine unicellular microalga *Ostreopsis*.

In the tropical latitudes palytoxins have been involved in serious food poisonings [1]. In the Mediterranean, although these biotoxins have been detected in some marine coastal fauna (sea urchins, fish, crabs), luckily, seafood poisonings have not been reported so far. However, since the 90s, *Ostreopsis* blooms in certain beaches have been related to mild respiratory disorders and certain skin injuries in people exposed to marine aerosols or having direct contact with seawater [2,3]. Some studies suggest that the palytoxin group could be responsible of these health impacts but the direct causal effect has not been clearly established yet. Uncertainties are due, in part, to the fact that the biotoxins have been rarely found in the aerosol [4], likely due to sampling and analytical limitations. Here we conducted a detailed evaluation of the mass spectrometry (MS) technique currently used to estimate these complex marine biotoxins in different types of samples (water, algae, sea urchins, fish, aerosols) in order to establish a fast, sensitive, selective and robust analytical procedure.

An ultra-high-performance liquid chromatography (UHPLC) system was coupled to a Q-Exactive Orbitrap Fourier-transform mass spectrometer (FTMS) equipped with a heated electrospray ionization source (HESI) operating in positive ion mode to analyse palytoxin and its ovatoxins analogues. The chromatographic separation was performed in a Hypersil GOLD$^{TM}$ C18 column, (100 mm x 2.1 mm id., 1.9 µm particle size) packed with totally-porous silica particles, under a gradient elution using an acetonitrile:water (0.1% formic acid) mobile phase.

Following optimization of the chromatographic conditions of different biotoxins (palytoxin and ovatoxins a-g), the separation was achieved in less than 10 min. These compounds have a similar behaviour under electrospray ionization conditions providing different multiple-charged adduct ions ($Ca^{2+}$, $Na^{+}$, $Mg^{2+}$). It has been observed that the absolute intensity of these adduct ions can change according to the maintenance of the instrument, the matrix type or even the salinity of sample extracts. For these reasons, the quantification of these compounds is not easy and in this work different quantitation strategies have been tested and compared. With this procedure, different palytoxin and ovatoxins were detected in seawater samples collected in particular sites of the Catalan coast affected by *Ostreopsis* blooms during the summer and fall seasons. In some cases, high toxin concentration in the water coincided with respiratory symptoms.

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**MAB-O04**

## MULTIRESIDUE DETERMINATION OF PESTICIDES IN GALICIAN VINEYARD SOILS BY UHPLC-MS/MS

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Economic sustainability of agricultural production depends on the use of phytosanitary compounds to cover the demand of the current society and to ensure the productivity of farms. However, the misuse of pesticides is becoming a worldwide environmental problem, particularly in the case of viticulture, which is recognized as the primary production activity with the highest application rate of certain groups of pesticides (fungicides) per unit of treated surface. Organic pesticides can remain in the upper layers of the soil during several production cycles, depending on their removal rates, which depend on their chemical structure and environmental conditions[1]. Moreover, pesticides can be leached from soil into groundwater[2] or be volatilized passing into the air. Conversely to environmental matrixes, such as water, there is no regulation about the concentration of these compounds in soil. Despite the lack of a specific guidance, the EU has recognized pollution of agriculture soils as a major challenging to face in coming years.[3] Thus, it is necessary to stablish methods to simultaneously determine phytosanitary residues at trace levels in soil.

The aim of this work is to optimize an accurate methodology to simultaneously determine a broad group of pesticides (fungicides and insecticides), from different chemical families, in vineyard soils. A second objective is to evaluate their persistence, or removal, during different production cycles, setting different removal rates in base of their chemical characteristics. With this purpose, a Pressurized Liquid Extraction (PLE) methodology has been optimized to attain quantitative extraction yields of pesticides from soil samples with different contents of organic matter, followed by Ultra-High-Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UHPLC-MS/MS). The above methodology has been validated and applied to different Galician vineyard soils in order to assess the residual concentrations of pesticides at the beginning of each production campaign. Furthermore, evolution of phytosanitary residues during different productive cycles has been studied to distinguish pesticides with different dissipation rates and persistence.

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**MAB-O05**

# Análisis multielemental de suelo antártico mediante espectroscopia de descomposición inducida por láser utilizando la metodología de calibración libre.

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La espectroscopía de descomposición inducida por láser (LIBS) es una técnica rápida que permite el análisis de todo tipo de muestras sin destruirlas y sin tener que llevar a cabo apenas tratamientos previos. Una de sus muchas aplicaciones es el estudio de muestras geológicas como son los suelos [1]. Debido a la complejidad de la matriz es de gran dificultad encontrar o fabricar patrones para este tipo de muestras por lo que una buena alternativa es hacer uso de una metodología muy reciente, llamada Calibración "Libre", donde en vez de utilizar patrones se estudian y se relacionan los parámetros físicos del plasma creado por interacción del láser con la muestra, con los elementos y especies que lo componen [2].

Se sigue esta metodología para realizar un análisis multielemental de una muestra de suelo procedente de la Antártida que fue tomada en la Base Antártica Gabriel de Castilla en la Isla Decepción.

Para hacer las medidas se empleó un láser de Nd:YAG que emite a una longitud de onda de 1024 nm y con una potencia máxima de 50 mJ por pulso. Todas las medidas se realizaron en atmosfera de argón. Para observar la morfología de la muestra de suelo y estudiar los cráteres formados por el impacto del haz láser se empleó la microscopía electrónica de barrido de emisión de campo (FESEM).

Los parámetros físicos del plasma calculados a través de la metodología de calibración "Libre" fueron la temperatura y la densidad electrónica, la primera obtenida a partir de la representación de Saha-Boltzmann de las líneas de Fe y Ti, y la segunda a partir de la anchura de la línea de H que se encuentra a 656,28 nm dando como resultados $T_e$=11294 K y $N_e$=2,46 × $10^{17}$ $cm^{-3}$.

Se determinaron las siguientes especies presentes en la muestra: $Al_2O_3$, $Fe_2O_3$, CaO, $Na_2O$, MgO, $K_2O$, $TiO_2$ y $SiO_2$. Los resultados finales fueron comparados con los proporcionados mediante análisis por ICP óptico obteniendo un error relativo menor al 10% para CaO y $Al_2O_3$ y menor al 50% para el resto de las especies salvo para $Ti_2O_3$.

Estos resultados demuestran que esta metodología puede resultar interesante para hacer un análisis rápido de muestras complejas como son los suelos, para obtener una mayor exactitud se podría realizar un estudio más exhaustivo de los espectros considerando variantes del método que tengan en cuenta la autoabsorción que pueden sufrir algunas señales e intentando calcular los parámetros físicos con mayor precisión [3].

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**MAB-O06**

# SELECTIVE DETERMINATION OF SARTAN DRUGS IN ENVIRONMENTAL WATER SAMPLES BY MIXED-MODE SOLID-PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Sartans constitute an important group of pharmaceuticals, angiotensin receptor antagonists (ARA), widely used in the treatment of cardiovascular diseases, such as arterial hypertension control and to prevent kidney diseases. Seven compounds of this family are currently marketed, and in case of losartan (LOS) and valsartan (VAL), their consumption data according to the AEMPS stay over 10 daily doses, per 1000 inhabitants.
After administration, these compounds are excreted through faeces and urine, reaching the sewage treatment plants (STPs), where they can remain in the water or accumulate in the sludge, depending on their octanol-water partition coefficients. Due to the presence of acidic moieties in their structures (carboxylic acids and/or a tetrazolic ring), these compounds exist as negatively charged species at the pH of environmental water samples [1], which causes a significant increase in their solubility in the aquatic media. Some sartan drugs are stable during oxidative water treatments, so their removal percentages at STPs are variable, reaching surface water reservoirs, including fresh and coastal waters [2,3]. The presence of sartan residues in tap water has been predicted and confirmed in different European countries [4]. In addition to the prescribed drugs, the human metabolite of VAL, valsartan acid (VALA), is particularly concerning as regards its stability and mobility, and recent studies have found this compound in bank filtrated samples and in tap water in a range of concentrations from 57 to 72 ng $L^{-1}$ [5].

This communication describes a new methodology for the simultaneous determination of seven sartan drugs (eprosartan, EPR; olmesartan, OLM; LOS; candesartan, CAN; telmisartan, TEL; irbesartan, IRB; VAL) and VALA, in different water samples, from municipal wastewater to surface and tap water, from a medium size city in the Northwest of Spain. Solid-phase extraction (SPE), based on mixed-mode (reversed-phase and anionic exchange) sorbents and ultra-performance liquid chromatography (UPLC) tandem mass spectrometry (MS/MS) were employed as concentration and determination techniques, respectively. All studied compounds were quantified in wastewater samples in average concentrations over 50 ng $L^{-1}$ and in case of VALA, it was found in all samples, even in tap water in concentrations over 21 ng $L^{-1}$.

## Acknowledgements
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**MAB-O07**

# DETERMINATION OF TRIMETHYLXANTHINES AS ANTHROPOGENIC CONTAMINANTS IN DRINKING AND WASTEWATER BY IN-TUBE SOLID-PHASE MICROEXTRACTION - CapLC

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Methylxanthines (caffeine, paraxanthine -caffeine's main metabolite- and theobromine) are compounds present in coffee, tea, cola drinks, energy drinks, cocoa-containing products and pharmaceutical preparations commonly administered in hospitals. They are considered indicators of anthropogenic activity, because their presence in environmental matrices comes mainly from human use and elimination. Their high stability and consumption makes common their detection in surface water, seawater, groundwater, drinking water and wastewater.

High polar characteristics of methylxanthines (negative values for log $K_{ow}$) increase the difficulty of their isolation from aqueous matrices. In-tube SPME coupled to CapLC with DAD detection to determine theobromine, paraxanthine and caffeine in tap water and wastewater is proposed as a simple and green chemistry sample treatment technique.

The utilization of capillaries coated with C-based sorbent (polystyrene-divinylbenzene) offers a high extraction yields for these compounds, employing only 4 mL of centrifuged and filtered sample, reducing the use of solvents and without additional stages in sample preparation. A good separation for the three analytes was achieved by capillary liquid chromatography with water-methanol gradient in less than fourteen minutes.

The resulting method present a high sensitivity, with limits of detection of 0.1 ng·mL$^{-1}$. Evaluation of method parameters show a good accuracy, with values of relative recovery between 83-103% at three levels of concentration, and adequate repeatability and reproducibility (coefficients of variation less than 6%).

The proposed methodology has been applied to evaluate water samples from Region of Valencia. The results showed the presence of theobromine in samples obtained upstream of a wastewater treatment plant (figure 1), with concentration values in range of 0.99 and 3.50 ng·mL$^{-1}$. Tap water analysis did not detect any of the analytes.

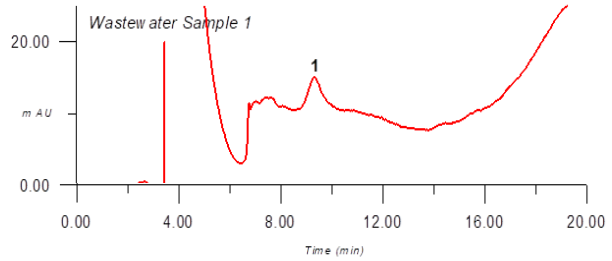


*Figure 1: chromatogram obtained for a wastewater sample (1: theobromine)*

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**MAB-O08**

## $CO_2$ PRINTABLE OPTICAL SENSOR

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Gas sensors monitoring $CO_2$ concentrations have a wide range of applications for several fields from fire detection, indoor air quality, control, biochemical process management, agricultural and food industry to exhalations monitoring. Development of gas sensors on flexible foils is of growing interest, with the aim of achieving low-cost, flexible and wireless devices [1,2].

The work presented here details the fabrication and performance of an optical chemical sensor for gaseous carbon dioxide analysis, which was prepared using inkjet printer deposition. The chemical sensor comprised a phosphorescent inorganic salt (type SKL63) and a pH indicator (α-naphtolphtalein) entrapped in a polymeric matrix of Hydroxypropyl methylcellulose together with a phase transfer agent (Tetramethylammonium hydroxide) and a surfactant (Tween 20). All components were dissolved in water and the composition has been optimized in order to create an ink. This ink was then deposited on flexible foils, testing from Mylar type polymer to different types of papers such as Nitrate cellulose and filter paper.

The sensor has been characterized in terms of sensibility, precision, dynamic response and stability. Figure 1(a) shows a calibration function obtained in the whole range of $CO_2$ and 1(b) shows response and recovery times.

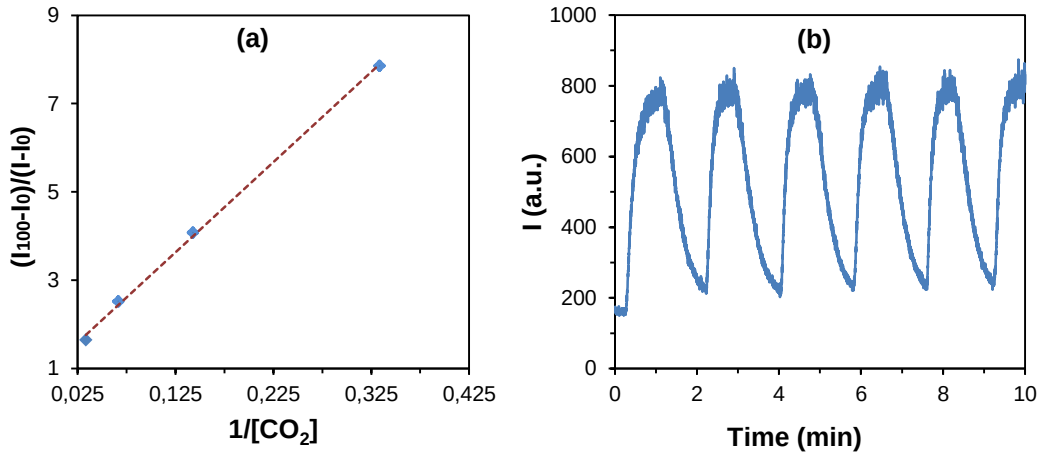


Figure 1: (a) calibration plot; (b) response and recovery times.

The main advantage of this sensor is the possibility of batch-manufacturing using the printing technique, causing considerable cost reduction for its commercialization. In addition, the achieved detection and quantification limits and the use of water as solvent make this sensor suitable for environmental applications.

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**NBO-O01**

# *IMAGING* CUANTITATIVO DE PROTEÍNAS ESPECÍFICAS EN TEJIDOS BIOLÓGICOS MEDIANTE ABLACIÓN LÁSER ICP-MS EMPLEANDO NANOCLÚSTERES METÁLICOS COMO MARCA ELEMENTAL

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La investigación de la distribución elemental y molecular (*imaging*) de analitos a lo largo de estructuras micrométricas en muestras de interés biológico (p.e. secciones de tejidos de donantes *post mortem* y cultivos celulares) es de crucial interés para conseguir una mejor comprensión de diferentes procesos celulares. La combinación de una técnica de muestreo directo de sólidos, como es la ablación láser (LA), con un plasma de acoplamiento inductivo con detección por espectrometría de masas (ICP-MS) ofrece interesantes características para estos estudios, como alta sensibilidad, y posibilidad de realizar análisis multi-paramétricos y análisis cuantitativos con una elevada resolución espacial (del orden de unas pocas micras).

Los últimos avances de la técnica LA-ICP-MS para el análisis de tejidos biológicos están relacionados no sólo con el *imaging* de los heteroátomos naturalmente presentes en las muestras (p.e. cobre, zinc, hierro, etc.), sino también con la distribución de biomoléculas [1]. En este último caso será necesario combinar el análisis por LA-ICP-MS con un protocolo inmunohistoquímico adecuado, empleando anticuerpos marcados con un heteroátomo fácilmente medible por ICP-MS. El uso de nanoclústeres (NCs) metálicos como marca específica para la determinación de proteínas presenta potenciales ventajas frente a otras marcas tradicionalmente empleadas con este fin, como son los quelatos metálicos con DOTA o el polímero comercial MAXPAR$^{TM}$. Los NCs metálicos tienen una mayor relación "número de átomos de metal detectables por tamaño de marca" frente a los quelatos metálicos o a las marcas poliméricas, permitiendo una mayor amplificación con un menor tamaño (cada NC puede tener más de 500 átomos metálicos, con un diámetro entre 2-3 nm) [2].

En el presente trabajo se mostrará la síntesis y caracterización de diferentes NCs metálicos (AuNCs, AgNCs, y PtNCs), así como los pasos llevados a cabo para su correcta bioconjugación con anticuerpos y la caracterización del bioconjugado (anticuerpo:NC). En este contexto, conocer la estequiometría del bioconjugado (es decir, número de NCs que se unen por cada molécula de anticuerpo) es necesario para determinar la concentración absoluta de las proteínas de interés en las imágenes de los tejidos biológicos. Además, con el objetivo de aumentar la paleta de NCs y, por tanto, las propiedades de multiplexado se mostrará la síntesis y aplicación de AgNCs enriquecidos isotópicamente.

La aplicabilidad de la metodología propuesta empleando diferentes NCs metálicos como marcas será mostrada para diferentes tipos de muestras de interés biomédico: (i) Determinación de la concentración de hierro y ferroportina en secciones de cerebro humano de donantes control y con Alzheimer (región del hipocampo), (ii) Estudio de las metalotioneínas 1/2 en secciones oculares humanas (región de la retina), y (iii) Empleo de AgNCs (de abundancia natural y enriquecidos isotópicamente) para estudiar simultáneamente la distribución de MTs1/2 y SOD-1 en la retina humana.

**Agradecimientos**

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**NBO-O02**

## DIAGNOSING METABOLIC SYNDROME BY PLASMA METABOLIC FINGERPRINTING BASED ON FOURIER TRANSFORM INFRARED SPECTROSCOPY

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The metabolic syndrome (MetS) is a common cluster of metabolic disorders that has emerged as a multiplex risk factor for cardiovascular disease (CVD) and other serious health conditions such as diabetes [1]. Three or more abnormal findings out of five cardinal risk factors, which include central obesity, raised triglycerides, reduced HDL-cholesterol, elevated blood pressure and raised fasting plasma glucose, would quality a person for MetS according to National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP III) and International Diabetes Federation criteria [2].
People with human immunodeficiency virus infection and acquired immune deficiency syndrome, in particular, commonly develop metabolic abnormalities (including dyslipidemia, hyperglycemia, insulin resistance and diabetes), as a manifestation of the complex and dynamic interactions between antiretroviral therapy, host factors and HIV infection itself, that resemble metabolic syndrome. Thus, this relatively high prevalence of metabolic syndrome among HIV/AIDS patients makes that a significant proportion of this HIV population could be susceptible to belong to a high CVD risk category [3].
Nowadays, the identification of patients with the metabolic syndrome is solely based on clinical judgement of risk indicators. Nevertheless, taking into account that the metabolic syndrome actually encompasses a set of metabolic changes and disarrangements, it would be possible to approach its diagnosis from a holistic, functional perspective, studying the metabolome comprehensively to identify metabolic patterns or signatures specific and distinctive to MetS, thus facilitating early diagnosis, treatment and follow-up of this condition.
The present study is focused on evaluating how the human plasma metabolomic profile can be altered in patients (both infected and not infected by HIV/AIDS) with MetS and how these metabolic changes, recorded as an infrared signature, can be associated with this pathological condition. Thus, Fourier Transform Infrared (FTIR) spectroscopy of human plasma (a minimally invasive matrix containing a vast amount of high value metabolomics information), combined with a suitable variable selection method and with Linear Discriminant Analysis (LDA) as a classification technique, was used to build an optimal classification for direct diagnosis of patients with MetS. The discrimination strategy proposed is quick, clean and relatively inexpensive since it only relies on IR measurements directly acquired on untreated serological samples. The emphasis in the non-targeted metabolomics approach presented here is not on the selection and identification of individual disorder markers of limited diagnostic value, which would be unable to capture the complexity of MetS-associated metabolic changes, but on extracting specific spectroscopic/metabolic fingerprints able to reflect the variability of the underlying metabolic changes responsible for the development of MetS [4]. Likewise, a main strength of this work resides in the application of an efficient stepwise wavenumber selection method based on LDA to extract a minimum number (maximum parsimony) of significant bands (reduced IR samples signature) from FT-IR plasma spectra which served as the basis for developing improved classifications, providing classification and internal validation rates of nearly 100 per cent. The simplicity and reliability of the classification methodology proposed was reinforced by the satisfactory results obtained in external prediction.
The ultimate aim of the reliable classification approach developed is to use it as simple and sensitive MetS screening and/or diagnostic tool using the reduced IR metabolic fingerprints from new patients as input features.To our knowledge, this is the first time that FT-IR spectroscopy, in combination with chemometric tools, has been used to reveal spectroscopic biomarker signatures that define patients subgroups for the clinical diagnosis and classification of MetS.

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**NBO-O03**

## METABOLÓMICA Y DOSIFICACIÓN DE FÁRMACOS EN PEDIATRÍA

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El ajuste de las dosis de fármacos en población pediátrica se lleva a cabo habitualmente en base a cálculos empíricos optimizados para la población adulta y teniendo en cuenta factores relacionados con la edad, el peso o el volumen corporal [1,2]. Sin embargo, se ha demostrado que en numerosas ocasiones este ajuste no es adecuado, requiriéndose un reajuste de la dosis en función del efecto obtenido. Esto se debe en parte a la variabilidad individual ocasionada por el distinto grado de maduración de los órganos implicados en la eliminación (hígado y riñón principalmente), que da lugar a variaciones en la disponibilidad del fármaco [3,4].

Las dificultades éticas para desarrollar estudios en población pediátrica que puedan aclarar esta problemática hacen que los estudios en modelos animales se conviertan en unas herramientas adecuadas [5,6]. Debido a ello, la determinación de biomarcadores que indicasen el estado de maduración de órganos en modelos animales de diferentes edades, cuyas vías metabólicas sean similares a las humanas, como es el caso de los cerdos, aportarían información útil para incorporar a los modelos semifisiológicos farmacocinéticos, que posteriormente ayudarían al ajuste de una dosis adecuada.

En este trabajo se ha utilizado la metabolómica no dirigida como herramienta en la búsqueda de biomarcadores correlacionables con el grado de maduración de los órganos [7,8]. Para ello, se ha llevado a cabo un estudio de los perfiles metabólicos, obtenidos por la plataforma analítica de espectrometría de masas de alta resolución acoplada a la cromatografía líquida (HPLC-Q-TOF-MS), en muestras de plasma e higado en el modelo animal cerditos mini-pig de diferentes edades.

Los datos obtenidos para cada tipo de muestra se han tratado con el software libre XCMS [9], cuyos parámetros se han seleccionado empleando IPO (Isotopologue Parameters Optimization), un paquete de software diseñado para la optimizaciín del procesado de datos [10]. Posteriormente, el tratamiento quimiométrico a través de MATLAB ha permitido encontrar diferencias significativas entre los diferentes grupos de cerditos mini-pig tanto en plasma como en hígado, asi como, desarrollar un estudio de correlacion que indica que el plasma puede ser un biofluido adecuado para reflejar el estado de maduración del hígado.

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**NBO-O04**

## APTÁMEROS PARA EL RECONOCIMIENTO DEL PATRÓN DE GLICOSILACIÓN EN PROTEÍNAS: APLICACIÓN A LA DETECCIÓN DEL CÁNCER DE PRÓSTATA

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La glicosilación es una modificación postraduccional de las proteínas mediante la cual se enlaza a las mismas un determinado carbohidrato, lo que modula muchas de sus funciones. No es extraño, por tanto, que se hayan identificado una gran variedad de patrones de glicosilación aberrante asociados a etapas clave en el desarrollo y la progresión de diferentes tumores. Muchos de estos cambios se han descrito como biomarcadores tumorales, con gran potencial para mejorar la detección temprana de estas enfermedades. Sin embargo, para su implantación en la práctica clínica es necesario desarrollar métodos de detección selectivos y sensibles, con capacidad para medir en suero sanguíneo las glicoproteínas aberrantes asociadas a la transformación tumoral en una etapa inicial de la carcinogénesis.

El desarrollo de métodos de detección para proteínas con patrones específicos de glicosilación requiere el descubrimiento de receptores específicos que puedan enlazarlas selectivamente [1]. En la Universidad de Oviedo, desde el Grupo de Electroanálisis (GEUO) hemos diseñado un método sencillo y general para dirigir la selección de aptámeros hacia la estructura de glicano de las proteínas, utilizando el antígeno prostático específico (PSA) como glicoproteína modelo. En esta comunicación se describirá esta estrategia, que ha permitido identificar varios aptámeros con capacidad para reconocer diferentes porciones de la glicoproteína PSA. Se ha realizado una caracterización exhaustiva de las propiedades de enlace y selectividad de estos aptámeros, que también se presentará. La integración de los aptámeros identificados con transductores electroquímicos ha resultado en aptasensores que permiten medir PSA en suero a niveles clínicamente significativos [2]. Se describirá la construcción, evaluación y características de respuesta de los mismos. Una evaluación preliminar de estos dispositivos en muestras de suero de pacientes con diferentes patologías, permite prever el enorme potencial de estos ensayos para discriminar cáncer de próstata clínicamente significativo de otras patologías benignas.

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**NBO-O05**

## LIPID IMAGING AND MULTIVARIATE ANALYSIS FOR VISUALIZING RENAL STATUS AND NEPHROPROTECTION STRATEGIES

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Nephrotoxicity is a serious side effect related to several therapies, including antitumoral treatment with cisplatin. Cilastatin, an inhibitor of renal dehydropeptidase-I, was recently proposed as a promising nephroprotector for cisplatin, preventing apoptotic cell death [1]. In this work, cilastatin nephroprotection was further investigated in a rat model. Lipid mapping with 100-µm resolution was performed by using MALDI mass spectrometry imaging (MALDI-MSI) in kidney sections from treated rats. 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacrydine (9-AA) were used as matrices for positive and negative ionization mode lipid analysis. The effect of cilastatin was assessed on 76 altered renal lipids [2,3], induced by cisplatin, mainly phospholipids, sulfatides and cardiolipins, which might be related with topographic, signaling or structural processes in damaged kidney. Immunohistochemical and histological studies were also performed on parallel kidney sections to evaluate renal damage and lipid peroxidation.

Cilastatin was proved to significantly diminish the lipid distribution alterations caused by cisplatin, being lipid levels almost completely recovered to those of control samples. Although our data show that cisplatin-induced toxicity is mainly restricted to cortical proximal tubule, changes in lipid distribution are huge and diverse, affecting the whole renal structure. The extent of recovery of cisplatin-altered lipids by cilastatin turned out to be relevant for discriminating direct or secondary lipid alterations driven by cisplatin. Lipid peroxidation induced by cisplatin was also shown to be reduced when cilastatin was administered.

Importantly, significant groups separation was achieved during multivariate analysis (principal component analysis -PCA- and partial least squares discriminant analysis -PLS-DA) of cortex and outer-medullary lipids, pointing out that damaged kidney can be discerned from the nephroprotected and healthy groups and classified according to lipid distribution. MSI enables hundreds of lipids to be monitored in a single analysis, and, considering the heterogeneous distribution of lipids in kidney, a plethora of possible combinations for visualizing kidney function status is available.

MSI enables simultaneous understanding of complex processes, such as changes in mitochondrial structure and cell membrane phenomena related to apoptosis in acute kidney injury. Moreover, it provides straightforward information on the structural interconnection between the cortex and the medulla during renal damage and its recovery.

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**NBO-O06**

# APLICACIÓN ANALÍTICA DE LA AMPLIFICACIÓN DE LA DISPERSIÓN RAMAN POR OXIDACIÓN ELECTROQUÍMICA DE LA SUPERFICIE

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La amplificación de la dispersión Raman por oxidación electroquímica de la superficie [1] (EC-SOERS) es un nuevo fenómeno descubierto por nuestro grupo de investigación y que puede considerarse paralelo al bien conocido efecto SERS (Amplificación de la dispersión Raman por la superficie) descubierto por Fleischmann en los años 70 [2].

SERS es un fenómeno ampliamente utilizado en el análisis químico, proporcionando una verdadera huella dactilar de los compuestos estudiados, y por lo tanto, dotando a la técnica de una gran selectividad, y a su vez, gracias a la gran amplificación de la señal Raman, la técnica posee una gran sensibilidad. Estas dos ventajas que el efecto SERS confiere a la espectroscopía Raman, también son obtenidas por el efecto EC-SOERS. Por lo tanto, este nuevo fenómeno puede ser utilizado con fines analíticos.

Para conseguir el efecto EC-SOERS se requiere trabajar con espectroelectroquímica Raman resuelta en el tiempo, ya que la amplificación de la señal Raman de la molécula estudiada solo se produce durante la oxidación electroquímica de un electrodo de plata. Además, la señal se extingue completamente cuando se deja de aplicar potencial al electrodo. Este comportamiento inesperado no se puede explicar fácilmente utilizando los modelos clásicos de SERS, y por lo tanto, hace que EC-SOERS sea un fenómeno no solo novedoso sino también intrigante. La Figura 1 muestra los espectros observados para el ácido úrico en un típico experimento de EC-SOERS.

En esta comunicación se mostrarán diferentes ejemplos de moléculas que pueden ser determinadas mediante EC-SOERS y a su vez, dado el intrínseco carácter trilineal de la espectroelectroquímica Raman, se ilustrará el alto potencial de la técnica en análisis cuantitativo.

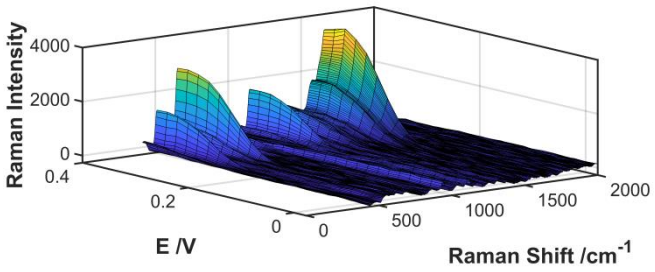


**Figura 1**.Evolución de los espectros Raman con el potencial cuando se realiza un barrido de oxidación desde 0.00 V hasta +0.40 V en una disolución que contiene 1mM de ácido úrico, 0.1 M de $HClO_4$ y 0.005 M de KCl, utilizando un electrodo serigrafíado de plata.

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**NBO-O07**

## ADVANCEMENTS IN THE DETERMINATION OF BIOMARKERS OF OXIDATIVE STRESS: THE IMPORTANCE OF A MULTIPARAMETER APPROACH

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Environmental exposures, smoking, drug intake, unbalanced diet and other stress factors increase the levels of reactive oxygen species (ROS) in organisms, leading to oxidative stress. The extent of oxidative stress in humans is related with health outcomes like diabetes, cancer, respiratory and cardiovascular diseases, among others. [1] The oxidative stress level in humans is usually monitored through the determination of oxidative stress biomarkers (OSBs) in biospecimens. In this work, we highlight the importance of a multiparameter approach to gather complete information about the oxidative stress status in humans. While previous studies focused on the determination of a single OSB, our work highlights simultaneous determination of multiple OSBs generated from the oxidation of different biomolecules: lipids, proteins, DNA and uric acid. The importance of this approach lies in the fact that not all OSBs are equally sensitive in response to a stress factor, therefore the determination of several OSBs from different sources, obtained in the same biospecimen, is meaningful. In this communication we present the advancements in analytical methodology for simultaneous determination of eight OSBs in urine, and results from the application of this approach to the study of oxidative stress in healthy individuals.

The simultaneous determination of the most frequently studied OSBs of the oxidation of lipids: malondialdehyde (MDA) and four $F_2$-Isoprostane isomers (8-$PGF_{2\alpha}$, 11-$PGF_{2\alpha}$, 15-$PGF_{2\alpha}$, 8,15-$PGF_{2\alpha}$); from the degradation of DNA: 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the oxidation of proteins: dityrosine (diY) is accomplished by a novel multiparameter analytical method based on a DNPH derivatization followed by SPE-LC/MS. [2] The levels of allantoin, the product of uric acid oxidation, are determined by a simple and fast D&S (dilute and shoot) LC/MS method. [3] Those analytical methods have been optimized, validated and applied to the determination of OSBs in human urine.

The assessment of the levels and variability of OSBs in healthy individuals is important to establish the suitability of those biomarkers and to establish a baseline for future studies. [4] We show the data obtained from the analysis of 515 urine samples from 19 healthy individuals collected daily for over a month; including the multiparameter OSBs profile, levels, correlations and variability (CV, ICCs). Allantoin (median value 11.9 μg $ml^{-1}$) is the predominant OSB, followed by MDA (11.5 ng $ml^{-1}$), 8-OHdG (3.65 ng $ml^{-1}$), diY (2.13 ng $ml^{-1}$) and $F_2$-Isoprostanes (0.16 to 0.55 ng $ml^{-1}$). These OSBs were detected in all urine samples but for $F_2$-Isoprostanes, with a detection frequency higher than 74%. The oxidative stress profile did not show differences when gender, BMI or age groups were compared. These OSBs were less variable, with ICCs above 0.74, which means that their determination in a spot urine sample is representative of the oxidative stress status of an individual over time.

The simultaneous determination of OSBs from the oxidation of lipids, DNA, proteins and uric acid in urine provides a wide range of oxidative stress indexes and complete information to contribute to the knowledge about molecular routes and mechanisms involved in the development of diseases mediated by oxidative stress. The application of this new multiparameter approach and the results from the study of OSBs levels and variability in healthy individuals will contribute to the better understanding of the role of oxidative stress in the incidence of diseases, as well as the effects of known stress factors in organisms.

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**NBO-O08**

## METABOLOMICS ANALYSIS OF DRY SWEAT BY GAS CHROMATOGRAPHY–TIME OF FLIGHT/MASS SPECTROMETRY AND LIQUID CHROMATOGRAPHY–QUADRUPOLE TIME OF FLIGHT TANDEM MASS SPECTROMETRY IN HIGH RESOLUTION MODE

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The popularity of sweat as clinical sample in metabolomics studies is increasing as this biofluid is non-invasively collected and its composition is influenced by several illnesses. There is a lack of standardized strategies for sampling human sweat. Most studies on this sample have been performed with fresh sweat collected after stimulation. A promising and simple alternative is sampling dry sweat by a solid support impregnated with a suited solvent. This research was aimed at comparing the metabolomics coverage provided by dry sweat collected by two solid supports (gauzes and filter papers) impregnated with three different solvents. The dissolved dry sweat was analyzed by a dual approach: LC–MS/MS and GC–MS. Among the tested sampling strategies, filter paper impregnated with 1:1 (v/v) ethanol–phosphate buffer resulted the combination providing the highest metabolomics coverage (tentative identification of one hundred fifty-six compounds). Dry and fresh sweat were compared by using pools from the same individuals to evaluate compositional differences. Families of metabolites such as carnitines, sphingolipids and N-acyl-amino acids, among others, were exclusively identified in dry sweat. Comparison of both sweat samples allowed concluding that dry sweat is more suited for analysis of low polar metabolites, while fresh sweat is better for polar compounds. As the majority of the identified metabolites are involved in key biochemical pathways, this study opens promising possibilities to the use of dry sweat as a metabolomics source of markers for specific disorders. Sampling of dry sweat could provide a standardized approach for collection of this biofluid, thus overcoming the variability limitations of fresh sweat.

**NBO-O09**

## ESTRATEGIAS ANALÍTICAS PARA EVALUAR LA TERAPIA COMBINADA CON NANOTRANSPORTADORES DE ÓXIDO DE HIERRO Y CISPLATINO EN MODELOS CELULARES.

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Un fármaco ampliamente utilizado para el tratamiento de cáncer de ovario y testículos es el cisplatino (CDDP). La base de su mecanismo radica en el hecho de generar aductos en el ADN para llevar a la célula a la muerte celular a través de un mecanismo de apoptosis. El problema del uso de dicho fármaco reside en sus efectos secundarios, tales como la nefrotoxicidad y la resistencia que adquieren las células.

Las nanopartículas son una buena alternativa para evitar dichos efectos secundarios, permiten una reducción de los efectos nocivos y una mejora en el transporte del fármaco. Recientemente se han desarrollado numerosas estrategias para transportar fármacos en nanoestructuras, tales como nanotubos [1] o nanopartículas [2].

Con el objetivo de minimizar dichos efectos indeseados producidos por el fármaco, también es posible el uso de pro-fármacos [3] tratándose, en la mayor parte de los casos, de compuestos de Pt (IV) que en el interior de la célula se reducen a Pt (II), actuando este último según el mecanismo habitual del cisplatino. Las ventajas del empleo de pro-fármacos es la capacidad de enlazarlos a nano-transportadores lo que permite un transporte dirigido hacia la diana terapéutica correspondiente, así como minimizar las interacciones inespecíficas.

Este trabajo tiene como objetivo el estudio del uso de nanopartículas de hierro para transportar de forma más eficiente un pro-fármaco de Pt (IV) al interior de las células en modelos de cáncer de ovario. El efecto combinado de liberación del cisplatino con la generación de especies reactivas del oxígeno ROS, generadas por el Fe liberado de las nanopartículas podría ser de gran interés terapéutico [4].

Para estudiar dicho efecto, es necesario el empleo simultaneo de técnicas de espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS), microscopía electrónica (TEM) o de dispersión dinámica de luz (DLS) que permitan establecer la eficacia en la síntesis de nanopartículas y en la inmovilización del pro-fármaco sobre su superficie. Así mismo, la incorporación celular del pro-fármaco inmovilizado en la nanopartícula se ha llevado a cabo a través de medidas totales (*bulk*) y haciendo uso de medidas de células individuales (*single cell*-ICP-MS), evaluando también otros parámetros relacionados con la eficiencia del fármaco.

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**NBO-O10**

## ANTITUMORAL EFFECT OF SELENIUM NANOPARTICLES: MECHANISMS, IN VIVO ACTIVITY AND SPECIFIC TARGETING

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Selenium, in its multiple chemical forms, is an essential trace micronutrient that participates in a great variety of biological processes, showing strong antioxidant power and chemotherapeutic properties. On the other hand, nanoparticles have been proposed in different scientific applications due to their unique properties such as large specific surface area, novel optical properties or increased reactivity as compared to bulk materials. Joined together in selenium nanoparticles (SeNPs), nano-scale and selenium properties have arisen a great deal of attention for their potential anticancer properties [1]. In previous studies, we demonstrated the ability of SeNPs to preclude proliferation of cancer cells by inhibiting eiF3, a protein involved in the protein synthesis machinery [2], and Cdk1, a key regulator of cell cycle progression [3]. Based on these results and with the aim of fully understand how SeNPs interact with the cell, specially at the nuclear level, additional assays have been performed.

In the present work, we evaluate the differential gene expression in cells exposed to SeNPs by using whole-transcript microarrays. Validation of the deregulated genes was carried out by qPCR. This approach has allowed us to identify a novel mechanism by which SeNPs inhibit cell proliferation associated to senescence, which provides an additional value to SeNPs as potential antitumoral agents. To study the potential of SeNPs to suppress tumour growth *in vivo*, melanoma tumors were induced in mice by intradermally injection of B16 cells. After the tumors became established (≈50 $mm^3$), SeNPs were injected intra tumor. After 13 days, tumors from control mice and tumors treated with the synthesis media, increased their volume by 8-14-fold, while tumors treated with SeNPs slightly increased by 1.8 fold for the same period of time. Therefore, intra tumour administration of SeNPs substantially suppressed tumour growth.

Based on the good results obtained and for SeNPs to have clinical translation potential, we designed a nanosystem capable to selectively deliver SeNPs to tumor cells. The proposed nanosystem improves the therapeutic profit while preventing harmful side effects on healthy cells. We selected mesoporous silica nanoparticles as a biocompatible vehicle, transferrin as cancer cell targeting ligand, and SeNPs as the active antitumoral drug. Optimization of the synthesis, analytical characterization and functional assays that demonstrate the selectivity of the designed nanosystem towards cancer cells are also presented.

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**NBO-O11**

# SENSITIVE DETERMINATION OF THE HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 (HER2) BY IMMUNO-POLYMERASE CHAIN REACTION WITH INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY DETECTION.

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Sensitive and selective analytical methods are necessary for the determination of clinical biomarkers of breast cancer. The human epidermal growth factor receptor 2 (HER2) is an important breast cancer biomarker since tumors with HER2 protein overexpression (HER2-positive tumors) turn to be more aggressive and likely to recur. Therefore, accurate determination of serum HER2 values is critical to optimize clinical outcomes in patients with breast cancer. To gain sensitivity and selectivity in the determination of HER2, a sandwich immune assay (highly selective) has been implemented using a detection antibody labelled with a DNA marker. Further amplification of the label using the polymerase chain reaction (PCR), followed by phosphorous quantification of the PCR product (amplicon) using inductively coupled plasma mass spectrometry (ICP-MS), completes this novel assay. Considering that the concentration of the amplicon is proportional to the amount of antigen (HER2) that is recognized by the labelled antibody, the concentration of HER2 can be directly obtained by P-analysis. For this aim, a DNA marker of 123 base pairs has been connected to the detection antibody of a sandwich immune assay conducted in pre-coated plates containing the capture antibody of HER2. After the recognition occurred, the PCR amplification was conducted and the PCR product analysed by ICP-MS. Detection limits of 2.5 pg. $mL^{-1}$ of HER2 could be achieved using 35 PCR cycles (7-fold lower than the commercial ELISA method). The developed methodology has been applied to the determination of HER2 in biological samples (human serum and cell culture supernatant of breast cancer cells, MDA-MB-231) obtaining method recoveries of about 80% and 65%, respectively.

**PMU-O01**

# TRATAMIENTO DE LODOS DE DEPURADORA DE AGUAS RESIDUALES URBANAS PARA LA DETERMINACIÓN DE CONTAMINANTES EMERGENTES

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## INTRODUCCIÓN

Los lodos procedentes de la Estaciones Depuradoras de Aguas Residuales (EDAR) urbanas se emplean como abono en lugar de fertilizantes químicos, por lo que existe un gran interés en los contaminantes orgánicos que contienen. Estos "contaminantes emergentes" no se encuentran incluidos en los programas de seguimiento sistemático de la UE (Directiva **2013/39/UE)** y es necesaria una regulación, ya que suponen un importante riesgo (efectos ecotoxicológicos, toxicológicos) así como el control de sus niveles en el medio acuático. Las concentraciones en el medio ambiente se encuentran entre ng/L y µg/L y una exposición continuada puede dar lugar a efectos nocivos para la salud.

## METODOLOGÍA PARA LA DETERMINACIÓN DE CONTAMINANTES EMERGENTES


**Figura 1. Esquema de la determinación de los contaminantes emergentes**

Se llevaron a cabo diversos ensayos y tratamientos para la extracción de estos contaminantes emergentes en lodos de depuradora, resaltándose a continuación los que se han considerado óptimos:

- Centrifugación y liofilización: **10000 rpm x 10'; 72 h**
- Disolvente de extracción: Agua MilliQ / **Agua MilliQ y 5% MeOH**
- Clean up: **Alúmina activa** / Silica gel activo / Hexano/ C18
- Extracción: **MAE** / UAE
- Saturación con **NaCl al 36%** y ajustar el **pH a 3**
- Filtración: **Filtro de jeringa** / filtración a vacío

Las condiciones empleadas en la extracción asistida por ultrasonidos (UAE) corresponden a 2 ciclos de UAE de 30 minutos cada uno. En el caso de la extracción asistida por microondas (MAE) es un ciclo de 40 minutos. Al emplear MAE, el tratamiento mejora los resultados obtenidos con UAE.
Una vez realizado el pretratamiento de la muestra, se lleva a cabo el análisis mediante cromatografía de gases y espectrometría de masas (GC-MS), comunicándose los resultados en este trabajo.

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**PMU-O02**

## IMPRESIÓN 3D EN EL LABORATORIO ANALÍTICO ¿REVOLUCIÓN O UTOPÍA?

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La impresión aditiva o impresión 3D está convirtiéndose en el último elemento integrador de la revolución industrial denominada Industria 4.0, basada en las nuevas tecnologías de la información y comunicación, por la fácil y rápida fabricación de prototipos mediante diseño 3D, alcanzando su máximo apogeo en el campo de la biomedicina y odontología, pero a la vez consolidándose en el ámbito educativo y de la investigación científica [1].
En esta conferencia oral se describirán brevemente las principales tecnologías de fabricación aditiva como son el modelado de deposición fusionada, la estererеolitografía, la sinterización por láser y la tecnología multi-jet fusión [2], así como las posibilidades que ofrecen para la fabricación de nuevas plataformas fluídicas miniaturizadas para el tratamiento de muestra y/o separaciones cromatográficas y electroforéticas [3,4]. Se evaluarán las ventajas y limitaciones de las impresoras comerciales de bajo coste y el uso de materiales poliméricos para la obtención de sistemas fluídicos con especial referencia a su compatibilidad química, rugosidad de las superficies internas, mínimas dimensiones imprimibles y procesos de procesado post-impresión.
Se presentarán características únicas derivadas del uso del propio material fotopolimerizable como material adsorbente para microextracción en fase sólida, de la reactividad química de la misma para posibles funcionalizaciones covalentes, de la impresión de columnas con geometrías en espiral o la propia impresión de membranas de diálisis o difusión gaseosa [5]. Mediante ejemplos ilustrativos de plataformas 3D estereolitográficas diseñadas e impresas en el grupo FI-TRACE de la UIB se demostrará la posibilidad de realizar separaciones miniaturizadas de fosfolípidos o contaminantes emergentes usando columnas monolíticas ancladas covalentemente, nanopartículas magnéticas funcionalizadas con polianilina o sistemas de microextracción en fase líquida usando fibras huecas modificadas con nanofibras de carbono [6,7].
Una de las tendencias de la impresión 3D radica en el diseño tanto de dispositivos de un solo uso como de plataformas integradas (µTAS) de elevada versatilidad y bajo coste, como se demostrará para el caso de las plataformas milifluídicas Lab-on-Valve 3D impresas con un coste inferior a 30 Euros frente a los más de 4000 Euros de las comerciales, y que han dado origen a la nueva generación de análisis por inyección en flujo denominada 3D-microFIA [8], que está fomentado el resurgimiento de los métodos FIA con finalidades separativas o extractivas.

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**PMU-O03**

# TÉCNICAS DE PREPARACIÓN DE MUESTRA EN TOXICOLOGÍA ANALÍTICA FORENSE

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La Toxicología Analítica Forense abarca la identificación, detección y cuantificación de distintas drogas y sus metabolitos en diferentes muestras biológicas. Debido a la naturaleza compleja de dichas muestras bilógicas, como la sangre, los tejidos o el pelo; la preparación de la muestra es un paso muy importante en la toxicología analítica. Esta etapa permite la eliminación de posibles interferencias, como proteínas, sales, ácidos, bases o compuestos orgánicos, consiguiendo reducir el efecto matriz y concentrar los analitos de interés [1].

Existen principalmente dos tipos de extracción: la extracción en fase sólida (SPE) y la extracción en fase líquida (LLE). Con el objetivo de desarrollar procedimientos de preparación de muestra más rápidos y respetuosos con el medio ambiente, han surgido diferentes técnicas de microextracción que han expandido sus aplicaciones en los últimos años al campo forense. Estas técnicas se caracterizan por la miniaturización del dispositivo de extracción que permiten disminuir el consumo de disolventes y el volumen de muestra, reduciendo el tiempo de extracción y favoreciendo la eficacia de la extracción siguiendo los requerimientos de la Química Analítica Verde.

El objetivo de este trabajo es ofrecer una revisión global de las distintas técnicas de microextracción aplicadas recientemente en el aislamiento de diferentes familias de compuestos bioactivos en matrices biológicas. Se presentan aplicaciones de microextraccion con adsorbentes empaquetados (MEPS) y microextracción líquido-líquido dispersiva (DLLME) en análisis toxicológicos forenses y control terapéutico de algunos fármacos [2,3] empleando la cromatografía líquida de ultra resolución para el análisis del extracto final.

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**PMU-O04**

# IN SITU GROWTH OF METAL-ORGANIC FRAMEWORK HKUST-1 IN A POLYMER MONOLITH AS EFFICIENT SORBENT FOR POLYCYCLIC AROMATIC HYDROCARBON DERIVATIVES

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Metal-organic frameworks (MOFs) are a type of highly ordered porous coordination polymers composed of inorganic clusters or chains of metal ions linked together by organic ligands in 2D- or 3D-networks. In the last years, they have received great attention as novel attractive materials for its use as stationary phases in the field of separation sciences due to their fascinating properties such as large surface areas, high porosity, chemical tunability and excellent chemical and thermal stability [1, 2]. Despite its good characteristics, the direct use of MOFs as micro-/nanocrystals is somewhat limited since its packing in solid-phase extraction (SPE) cartridges is troublesome and separation in dispersive mode implies centrifugation or filtration steps. To overcome these limitations, an attractive strategy is the combination of MOF particles with other substrate materials such as organic polymer monoliths for its use in sample preparation. This combination would allow the synthesis of hybrid monoliths supports (composites), which incorporate the best features of both materials. Thus, the porous polymer monoliths have distinct advantages of easy in situ preparation, variable chemical properties, and large chemical stability.

On the other hand, over the last years, attention has been paid to polycyclic aromatic hydrocarbon derivatives, such as nitrated polycyclic aromatic hydrocarbons (N-PAHs) and oxygenated PAHs (O-PAHs), which are emitted primarily by combustion sources and post-emission transformation of PAHs [3]. Some of these derivatives are more toxic, having a greater threat to human health, than some PAHs, due to their direct-acting mutagenicity and carcinogenicity. For this reason, monitoring trace levels of these analytes in complex samples is relevant, being the development of efficient and selective SPE protocols mandatory.

In this work, the *in situ* synthesis of HKUST-1 onto the surface of organic polymer based on methacrylic acid monolith is described. This composite was applied as SPE sorbent for the extraction and preconcentration of N-PAHs and O-PAHs in aquatic samples. Hybrid polymer was morphologically characterized and its potential as SPE phase to isolate PAH derivatives was evaluated. The analytical properties of interest as extraction sorbent were also established (loading capacity, breakthrough volume, preconcentration capacity, etc.). To our knowledge, this is the first time that a hybrid material, built layer-by-layer self-assembly method, is used as sorbent to extract successfully these pollutants from environmental water samples.

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**TAN-O01**

## STRATEGIES FOR IN SITU ANALYSIS

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In the context of current chemistry the necessities of green chemistry have to be considered for a sustainable and environmentally friendly development. In the analytical chemistry field, it leads to the necessity of developing protocols and devices for in situ analysis, use of non-harmful reagents, recycling and minimization of wastes, miniaturization of procedures and instrumentation and reduction of power costs, among others [1]. In our opinion, the development of analytical devices for in situ analysis is basically governed by three factors: the implementation of new technologies and internet, the use of new (nano)materials and the social changes produced in the last years [2,3]. The analytical methods can be classified into three categories: in line, online, and off-line based on the way in which the analytical process is carried out. In line monitoring methods generally involve the use of an analytical device placed directly in contact with the sample. This device automatically provides information about the physicochemical composition of the sample without both, sample treatment/manipulation and analysis in the laboratory. In this sense, optical and electrochemical devices or sensors and also portable instrumentation allow real-time field measurement and thereby, systems for in situ analysis. On line methods involve fixed installations and are generally used for air or water monitoring. These systems are called monitoring stations or bank-side analysers. These analytical instruments are located close to the analysis place. In this case, the sample is drawn into the system for being processed and for analyte(s) determination. These instruments are generally complex and collect a considerable amount of data. As long as the sample is directly processed and its conditions are not modified and/or altered, monitoring stations can be considered as in situ analysis systems. On the other hand, in the case of off line methods, there is a distance from the sampling site to the analytical device or instrument, so the sample is collected in situ and transported to an external testing area.

A balance between performance, equipment cost, complexity, and reliability is required for selecting the best option for a given case and considering the factors involved in the development of the current and future devices for in situ analysis (Fig 1). Examples of different proposed in situ devices by MINTOTA are presented and discussed.

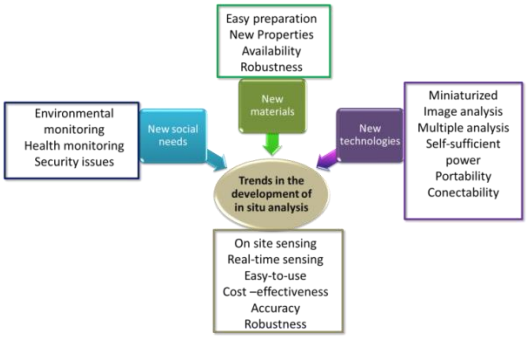


**Figure 1**.

*Schematic representation of the factors involved in the development of the current and future devices for in situ analysis and their remarkable characteristics*

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**TAN-O02**

## IMMUNO AND PEPTIDE-BASED BIOSENSORS FOR THE DETERMINATION OF ABERRANTLY EXPRESSED PROTEINS IN THE TUMOR MILIEU

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Cancer is one of the major causes of death in Europe and across the world, its early detection, evaluation and prognosis being of great importance for patient survival. It is known that there are substances the tumor microenvironment, whose abnormal levels can indicate the presence and, even the tumour stage of this dreaded illness. Two of these molecules of interest are Hypoxia inducible factor 1α (HIF-1α) and trypsin, both of them up-regulated in human cancer cells of lung, prostate, and colon, among others. HIF-1α is a transcriptional factor which allows cells adaptation to reduced oxygen environments while trypsin, a well-known serine protease, is a digestive enzyme secreted by the pancreas that has an important role in protein digestion in the small intestine [1, 2] and has been implicated in tumor growth, invasion, and metastasis.
In this context, this work describes the development of both, immune and peptide-based electrochemical biosensors for the determination of these tumor-related functional biomarkers at cellular level. Thus, the first immunosensing platform is described for the determination of HIF-1α protein, based on the use of immuno-magnetic captors, prepared by covalent immobilization of a specific capture antibody (AbC) onto activated carboxylic-modified magnetic microcarriers (HOOC-MBs), for selective capturing and sandwiching the target protein with a biotinylated detector antibody further conjugated with a streptavidin-HRP complex. Moreover, a novel electrochemical peptide-based biosensor has been developed for trypsin determination, this strategy consisting of the immobilization of a short peptidic sequence, dually labelled with fluorescein isotiocyanate (FITC) and biotin, onto neutravidin-modified MBs, followed by the digestion with trypsin. Once peptide disruption has taken place, the modified MBs are incubated with a specific fluorescein Fab fragment antibody labelled with horseradish peroxidase (HRP-anti-FITC). In order to perform the electroanalytical measurements, in both approaches the modified MBs are magnetically captured at the surface of a screen-printed carbon electrode (SPCE), and amperometric detection is performed using the hydroquinone (HQ)/HRP/$H_2O_2$ system.

Under optimal conditions, the developed bioplatforms display good analytical performance to carry out the required determinations in a very short time (maximum of 3 h and 15 min for trypsin and 1 h and 45 min for HIF-1α). In addition, the good results obtained in the analysis of cell lysates (by using just ≤ 0.5 µg of raw extracts) demonstrate the potential of both affinity biosensors to provide quantitative data after minimal treatments and manipulations. These features make these approaches more compatible than currently available strategies, for their implementation in point-of-care devices so demanded in ambulatory and inpatient routine.

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**TAN-O03**

# NEW MULTINEBULIZATION SYSTEM FOR SPECTROCHEMICAL ANALYSIS: WEAR METALS DETERMINATION IN USED LUBRICATING OILS BY ON-LINE STANDARD DILUTION ANALYSIS (SDA) USING INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

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Engine components undergo continual wear under normal operating conditions, producing metallic particles as well as metallo-organic species. Despite the presence of filters and collectors, most of the wear metals remain in the lubricating oil and they are transported through the whole system. Therefore, an increment in concentration of several key elements in the used lubricating oil may be used as an indicator of problems in some components of the engine, as it exists a relationship between each element and the respective engine component.[1] Nowadays, elemental analysis of used lubricating oils is routinely used for preventive maintenance. This analysis brings economic benefits, reduces potential risks and helps in developing new maintenance procedures.

For this analysis, sensitive techniques are required, since small concentration changes need to be discriminated. However, the first challenge of this analysis lies on the organic matrix itself. Analysis of organic samples by ICP-based techniques requires the use of special calibration techniques in order to minimize matrix effects. Moreover, the high organic solvent load and the formation of carbon deposits on the tulip torch are also remarkable problems raised on organic analysis.

Therefore, the aim of this work is the development of a new analytical methodology overcoming the above mentioned limitations. On one side, a powerful calibration methodology called standard dilution analysis (SDA) has been previously proposed, which simultaneously combines the advantages of the standard addition and internal standard calibration methodologies.[2] On the other side, our research group has introduced a new multinebulizer (MultiNeb®)[3] that has been successfully employed for the elimination of carbon deposits on the tulip torch caused by the high organic solvent load in ICP-OES by the simultaneous introduction of organic samples and aqueous calibration standards.[4] Therefore, the proposed analytical methodology provides ease of operation keeping a minimal sample pretreatment (*i.e.*, dilution) and also avoids the use of organic calibration standards. Hence, the proposed methodology is a simple, fast, relatively economic, ecological, direct and reliable way of wear metal monitoring in used lubricating oil by ICP-OES.

The efficiency of the proposed methodology has been checked by comparing its results with values obtained with conventional standard addition calibration for real lubricating oil samples. Recovery values obtained with the proposed system ranged between 92 and 108 % and a full analytical curve is obtained in 5 min for each sample, achieving a throughput rate of 12 samples per hour. Its greenness has also been demonstrated by the application of the Eco-Scale[5] and the subsequent score comparison with the score obtained with method described in the ASTM-5185 standard.

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**TAN-O04**

## A NEW FRAMEWORK FOR THE IDENTIFICATION OF EMERGING CONTAMINANTS BY NON-TARGET SCREENING LC-QTOF

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Emerging contaminats (ECs) are compounds that are not (yet) included in monitoring programs because they are new or their presence in the environment and/or humans has not been elucidated. However, they may have the potential to exhibit toxicity in humans and wildlife. The impacts of human exposure to mixtures of chemicals are poorly understood, because current biomonitoring campaigns do not include ECs. Therefore, there is an urgent need to establish a set of representative biomarkers to assess the human exposure to mixtures of ECs.

Human urine is a complex matrix and the expected concentration for most of the ECs is at trace levels. In addition, presumably many types of ECs are metabolized through different pathways and (partially) excreted through the urine. Non-target screening analysis of human urine samples by high resolution mass-spectrometry is able to provide an overview of the presence of ECs in the population. However, despite of several attractive features, this novel strategy is facing a lack of harmonization that would permit obtaining comparable and high-quality results.

Quality assurance (QA) is defined as a set of activities or procedures which are adopted in a laboratory to ensure that all quality requirements will be fulfilled. Meanwhile, quality control (QC) refers to operational techniques and activities that are used to fulfill requirements for quality. The achievement an actual and representative fingerprint of ECs in human urine is a challenge that requires the establishment of the proper QA/QC actions for each individual step of the workflow. To facilitate the development of reliable and comparable non-target/suspect screening workflows for the assessment of ECs in human urine by liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS), we have developed a generic QA/QC framework. Some QA/QC actions proposed are the study of selectivity by adding to the urine samples a set of labeled standards in a wide range of chemical properties and the use of Schymanski-scale for compounds identification.

The new framework has been applied to the non-target suspect (NTS) analysis of 35 urine samples belonging to a Flemish-cohort. This study is part of the EU H2020 HBM4EU project to assess the human exposure to new organic chemicals. The urine samples were analysed by using QuEChERS as sample treatment combined with liquid-chromatography time-of-flight-mass spectrometry (LC-QTOFMS). More than 20 ECs were identified at level 4 according to Schymansky´s scale [2]. As a first stage, the identified ECs have been prioritized according to their frequency detection. Highest detection frequency were founded for phosphate flame retardants compounds (100 % for [2-[2-(2-phenylmethoxyethoxy)ethoxy]ethoxy]ethyl dihydrogen phosphate), phthalates (85 % for butyl-cyclohexyl phthalate) and their metabolites (33% for triphenyl phosphate and 75% for 3-carboxy-monopropyl phthalate). Metabolites of alternative plasticizers (35% for MINCH) and pyrethroids (63 % for 3-(4-hydroxyphenoxy)benzoic acid) were also identified.

Although the establishment of a detailed list of QA/QC represents a good starting point to establish a harmonized approach of non-target/suspect screening methodologies in human urine analysis, more effort in this direction is still needed owing to the premature status of the major workflows in this field. The high frequency detection of the identified ECs in this study indicates the high and ubiquitous exposure to these chemicals. Thus, the identification and prioritization of the (new) chemicals is necessary for the development of the appropriate legislation on human and environment safety.

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**TAN-O05**

## ICP-MS-BASED METHODOLOGIES TO STUDY ZINC AND METALLOTHIONEINS IN HUMAN EYE CELLS USING ISOTOPICALLY-ENRICHED TRACERS

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Age-related macular degeneration (AMD), a progressive neurodegenerative eye disease initially affecting the retinal pigment epithelium (RPE), is clinically characterized by the growth of extracellular deposits underlying the RPE. During ageing, oxidative damage to the RPE and inflammatory-mediated processes occurs, contributing to the development and progression of AMD [1]. The inner ocular tissues contain a wide range of antioxidant enzymes, including the zinc-metallothionein (Zn-MT) redox complex, which captures and neutralizes free radicals through cysteine sulphur ligands, releasing zinc in a redox dependent fashion [2]. Clinical studies including a large randomized placebo-controlled age-related eye disease study may support the use of zinc supplementation, in combination with vitamins and antioxidants, to reduce the progression of AMD. However, how zinc supplementation helps to slow down the progression of AMD is not precisely understood and, therefore, the potential roles of zinc in AMD and its protective effects or deleterious interactions are being investigated.

The use of cultured RPE cells as *in vitro* model is a versatile tool to study the protective role of the Zn-MT system against oxidative stress. With this aim, we have previously developed and applied an approach based on HPLC-ICP-MS and the use of isotopically-enriched tracers to quantitate the absolute concentrations of zinc-binding proteins, including MTs, in cultured RPE cells under steady-state conditions and upon exposure to zinc supplementation. However, although the analysis of cultured cells by ICP-MS can provide valuable information on the bulk concentration of a cell population, inter-individual variations are lost and no information about the distribution within a cell is available. Nowadays, laser ablation (LA) coupled to ICP-MS is regarded as a powerful tool for direct trace element and isotopic analysis of solids, permitting single-cell analysis and providing elemental imaging of zinc within cells.

In this communication we will present the combination of several multidisciplinary bioanalytical technologies, including HPLC-/LA-ICP-MS and the use of enriched stable isotopes and fluorescent probes, to study the protective role of the Zn-MT system against oxidative stress and the intracellular distribution of zinc following its supplementation, in an *in vitro* model of RPE cells. We will demonstrate that the Zn-MT system (induced by zinc sulphate) significantly reduces oxidative stress in RPE cultured cells, while oxidative stressors decrease Zn-MT levels. Moreover, the capabilities of LA-ICP-MS combined with isotope pattern Deconvolution (IPD) mathematical tool will be explored for molar fraction imaging in RPE cells treated with different enriched stable isotope tracers of zinc supplements in the form of sulphate and gluconate. Cell analysis by the proposed LA-ICP-MS methodology permits the localization of zinc within the cell and simultaneously allows for differentiating the endogenous contribution of zinc from the exogenous one. Overall, the antioxidant role of Zn-MTs and the nuclear distribution of zinc following its supplementation may be related to their role in protect the cell nuclei from oxidative damage and zinc exchange to transcription factors. Further understanding of the molecular mechanism related to Zn-MTs antioxidant properties, metabolism and zinc homeostasis would contribute the knowledge of the pathophysiology of AMD disease affecting the RPE.

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Acknowledgements: Project ref. CTQ2016-79015-R (AIE/FEDER, UE) and “Cátedra Rafael del Pino”.

**TAN-O06**

## COUPLING D-LFSESI-DMA-MS FOR OLIVE OIL CHARACTERIZATION: A NOVEL APPLICATION IN FOOD ANALYSIS

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Recently, Spanish olive oil industry has been the subject of harsh criticism for false labeling and even adulterations of olive oils. This situation in which both the industry and the population are affected, demands the urgent need to increase the controls that avoid fraudulent activities around this precious product. EEC Regulation 2568/91 and International Olive Council (IOC) have established analytical and organoleptic criteria to define the quality grade of an olive oil[1]. So, it is fundamental that both the chemical analyses and the organoleptic evaluations used to define quality grade are sufficiently objective and reproducible. Until now, the Panel Test is still the only official method accepted for classifying olive oils according to their organoleptic characteristics[2]. On the other hand, there is a lack of official analytical methods to classify olive oil samples according to its organoleptic quality[3]. The objective of this work was to propose a new analytical platform by coupling of electrospray ionization (ESI), differential mobility analysis (DMA) and mass spectrometry (MS) for the analysis of olive oils based on the information obtained from profiling or non-targeted analyses (chemical fingerprint). Two different approaches were proposed for the olive oil analysis: (i) the direct infusion of olive oil after sample dilution with appropriate solvents or (ii) a previous liquid-liquid extraction (LLE) in order to shift the measurement towards a specific part of the composition of the olive oil. To demonstrate the feasibility of our method for analysis of real samples, 30 olive oil samples of three different categories were analyzed (21 samples to elaborate the classification model and 9 blind samples to evaluate it). To classify the olive oil samples after ESI-DMA-MS analysis, principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) were used as multivariate analysis tools. The overall success rate of classification was 89% and 67% for the direct infusion of the diluted samples and for the LLE extracted samples, respectively. However, both methods allowed the differentiation of EVOO from the other categories (VOO and LOO) with a sensitivity of 100%. In general, the results show that ESI-DMA-MS is an interesting alternative tool for the differentiation of olive oils according to their category. In view of the results attained in this study, this novel platform (D-LFSESI/DMA/MS) could be used for fingerprinting and classification of olive oil samples, providing complementary information to the data obtained by others configurations.

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**TSE-O01**

# STUDYING THE CHROMATOGRAPHIC SELECTIVITY BY LINEAR FREE ENERGY RELATIONSHIPS: HILIC, REVERSED- AND NORMAL PHASES

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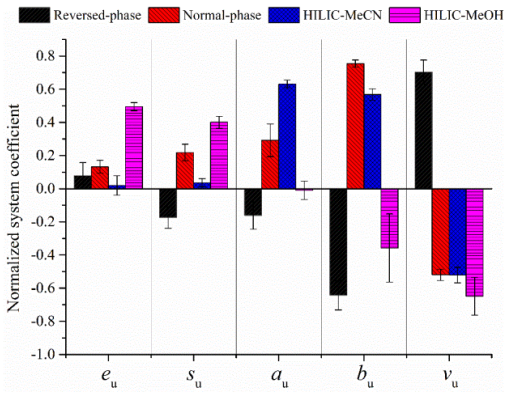
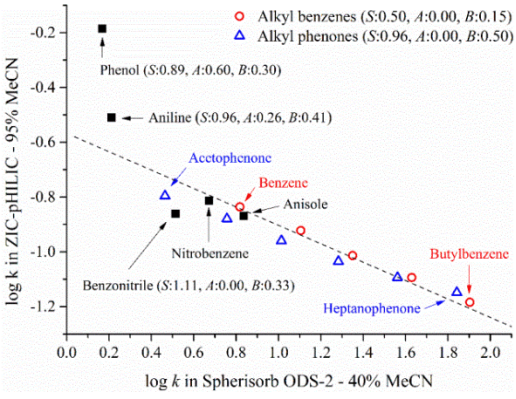
The solute transfer between mobile and stationary phases can be interpreted as the difference in the free energies of solvation of the compound in the two condensed phases. Solute transfer involves first the creation of a cavity in the receptor solvent followed by the newly established solute-solvent interactions. Based on these assumptions, Abraham proposed a LFER model relating these different contributions to the logarithm of the retention factor (log *k*) [1]:

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

The $v \cdot V$ term accounts for the cavity formation, $c$ is a system constant, and the rest of the terms are related to solute-solvent interactions. $e \cdot E$ term models the polarizability contributions from $n$- and $\pi$-electron pairs, $s \cdot S$ the dipole-type interactions, $a \cdot A$ the hydrogen bond donation from the solute to the solvent, and $b \cdot B$ the hydrogen bond donation from solvent to solute. $E$, $S$, $A$, $B$, and $V$ are solute descriptors, and $e$, $s$, $a$, $b$, and $v$ are the system coefficients reflecting the difference in solute interaction between the solvated stationary phase and the mobile phase. The sign (positive or negative) and magnitude of these coefficients lead to the characterization of chromatographic systems, finding the key features responsible for retention and allowing the comparison between different retention modes, columns, and mobile phases [2].

For unionized analytes, the main contribution to retention in reversed-phase and IAM liquid chromatography is the solute molecular size, due to the reduced intermolecular interactions of the stationary phase (C18, lipids…) in relation to the hydroorganic mobile phase. The contrary takes place in HILIC and normal phase, where a highly cohesive water layer adsorbed on the stationary phases is responsible for retention. Generally, polar interactions (polarity, polarizability, hydrogen-bonding…) favor retention in normal phase and HILIC due to the higher polarity of the stationary phase in relation to the eluent, but with the exception of the solute hydrogen-bond basicity in HILIC when methanol is used as mobile phase constituent. The similarity between hydrogen-bond acidity of methanol and water is most probably responsible for this behavior, especially if compared with the aprotic acetonitrile. Regarding this characteristic, HILIC-MeOH and reversed-phase show an unexpected similar behavior. The solute-solvent interactions of HILIC-MeCN, inverse from those of RP-MeCN, explain the complementary retention selectivity of the two stationary phases.

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## TSE-O02

# MODELO PREDICTIVO BASADO EN LA CARACTERIZACIÓN POR EIS (ESPECTROSCOPIA POR IMPEDANCIA) APLICADO A LA EXTRACCIÓN DE PARABENOS EN UN µ-EME-CHIP

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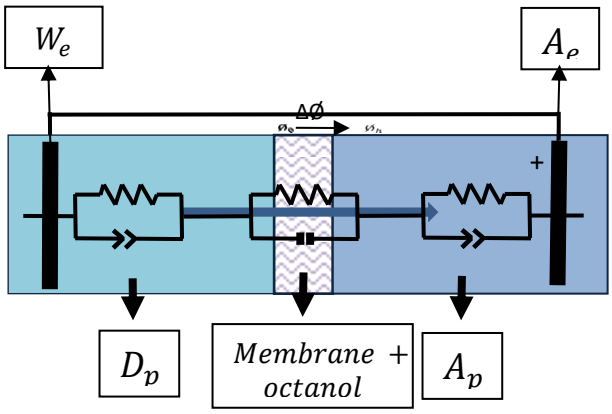
La técnica de microextracción mediante electromembrana (EME) es una técnica muy empleada en el tratamiento de muestra debido a las ventajas que ofrece. EME favorece la migración de los analitos a través de la membrana líquida soportada gracias a un campo eléctrico generado tras aplicar una diferencia de potencial entre dos electrodos (uno inmerso en la fase aceptora y otro en la fase donadora).

En los últimos años, EME se ha implementado en sistemas microfluídicos ya que reduce considerablemente los tiempos de análisis y el volumen de muestra. Con el fin de conocer los parámetros eléctricos que rigen EME, se ha caracterizado el dispositivo microfluidico mediante la técnica de espectroscopia de impedancia (EIS). Los resultados obtenidos nos permiten, por primera vez, predecir y optimizar valores de voltajes por debajo de 5V e intensidades menores a 15mA.

El dispositivo microfluídico empleado en este trabajo, consiste en dos micro-canales (aceptor y donador), en el interior de cada canal se encuentra sumergido un electrodo de platino. Una membrana plana (membrana liquida soportada, SLM) de polipropileno de medidas 17mm de longitud x 3 mm de ancho e impregnada con 4 µL de octanol separa la fase donadora de la aceptora. Tres parabenos fueron seleccionados como modelos de analitos: etil paraben (EtP), isobutil paraben (iBuP) and butil paraben (BuP). Las condiciones óptimas de extracción seleccionadas para este trabajo fueron, pH 11,5 para la fase aceptora, pH 3,5 para la fase donadora, 4V , 3 µL $min^{-1}$ y 1µL min para el flujo donador y aceptor, respectivamente. El proceso de optimización fue satisfactoriamente aplicado a muestra de orina obteniendo unas recuperaciones por encima del 73% para todos los analitos después de 7 minutos de extracción y empleando únicamente 10 µL de muestra.



**TSE-O03**

# POTENTIAL AND COMPLEMENTARITY OF ADVANCED NON-TARGETED METABOLOMIC APPROACHES (LC-ESI/APCI-MS AND GC-APCI-MS) FOR THE CHARACTERIZATION OF *OLEA EUROPAEA* L. DERIVED MATRICES

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Over the last decades, virgin olive oil consumption has been associated to a lower incidence of some chronic diseases such as cancer, diabetes or coronary diseases, largely due to its non-glyceridic minor components. Some of these health-promoting phytochemicals are secondary metabolites of plants that can also be found in other vegetal tissues derived from the olive tree. Thus, the deep characterization of the main parts of the olive fruit and tree might be a key step when trying to find natural sources of bioactive compounds.

In this work, advanced non-targeted MS-based metabolomic approaches (LC-ESI/APCI-MS and GC-APCI-MS) were applied to the study of eight different samples coming from Picudo *cv*. olive trees (lyophilized olive pulp, olive seed, fruit skin, leaves and wood from the branches, as well as virgin olive oil, olive oil obtained from pitted and dehydrated fruits and oil obtained from the seed contained inside the pit). The main goals of this project were to assess the complementarity of the tested methodologies and to unravel the distribution of bioactive compounds on the studied matrices.

Samples were prepared using very unselective liquid-liquid or solid-liquid extraction protocols pursuing the extraction of as many compounds as possible. Afterwards, the extracts were analyzed by LC and GC coupled to a QTOF detector (Bruker) by means of ESI and APCI interfaces in the case of LC and an APCI source in GC. Chromatographic and MS conditions were optimized to facilitate the determination of analytes in a very wide range of polarity/volatility within a single run. In LC, the analytes were eluted in a C18 column (2.1x 100 mm, 1.8 µm), with acidified water and acetonitrile as mobile phases at 40 ºC. The same extracts (after a further silylation step) were analyzed in GC (BR-5 column) with a temperature gradient from 150 to 320 ºC (ramped at 4 ºC/min). Spectra in both positive and negative polarities were acquired and the collected data were processed with MetaboScape® 3.0 (Bruker), which automatically extracted and combined isotopes, adducts and fragments belonging to the same compound into one feature.

Retention time and MS data of more than 40 commercially available pure standards, together with information found in databases and previously published reports were considered in an attempt to assign a feasible identity to most of the detected chromatographic peaks. The identified metabolites belonged to different chemical classes: phenolic compounds (phenolic acids and alcohols, lignans, flavonoids, secoiridoids and some of their glycosides), triterpenic acids and dialcohols, tocopherols, sterols and free fatty acids. Moreover, information obtained from Metaboscape was studied in depth in order to appraise the adequacy of every methodology for the determination of each family of compounds, as well as to describe the distribution of the identified plant metabolites within the selected olive oils and tissues (their presence, absence, relative area in each matrix and relative response in each platform were checked).

The obtained results showed the potential of different non-targeted MS-based approaches to cover the metabolome of eight *Olea europaea* L. derived matrices. The use of LC and GC coupled to a high resolution MS (through different ionization sources) and annotation strategies within MetaboScape 3.0 (based on retention times, accurate masses, isotopic patterns and MS/MS spectra) allowed the identification of around 130 compounds in the profiles, pointing out a great complementarity among the used platforms.


# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER+FLASH*

**ALI-F01**

# TARGETED AND NON-TARGETED UHPLC-HRMS APPROACHES FOR THE CHARACTERIZATION AND CLASSIFICATION OF NUTS BY CHEMOMETRICS

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Due to the complexity of the food chain in a globalized world, food manipulation and adulteration practices have raised in the last years. For instance, replacing authentic substances with masked cheaper inferior quality components has become a common fraud practice, representing not only an economic deception but also a health risk as the adulterant could produce allergy episodes. In this line, a 4% of the food fraudulent cases reported in the European Union in 2016 were related to nuts and seeds [1]. Nuts are food products worldwide consumed and well-known for their health beneficial effects [2]. Even though approximately 70% of their weight is attributed to fat, the amount of saturated fatty acids is very low. Moreover, they have also shown to be important sources of bioactive compounds such as polyphenols, which are a large family of secondary metabolites found in plants [3]. In order to detect and avoid nut adulteration, two main approaches performed by ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) could be proposed: (i) targeted methodologies, where the concentration (or peak signals) of a given group of known selected chemicals is used as food feature, or (ii) non-targeted fingerprinting, which generally consists of peak intensity values recorded as a function of *m/z* and retention time.

In this work, a total of 149 nut samples belonging to different classes (almonds, cashew nuts, hazelnuts, macadamia nuts, peanuts, pinions, pistachios, pumpkin seeds, sunflower seeds and walnuts), some of them processed with different thermal treatment (natural, fried or toasted), were analyzed by UHPLC-HRMS (Q-Exactive Orbitrap). An easy two-split sample treatment consisting of a first extraction with acetone:$H_2O$ 70:30 (*v/v*) and a defatting step with hexane was carried out. The chromatographic separation was obtained by employing a Kinetex C18 (10 cm x 4.6 mm x 2.6 µm particle size) column with an analysis time of 35 min. Regarding the data treatment, both a targeted approach by means of a customized target accurate mass database of more than 100 polyphenolic compounds using TraceFinder™ 3.3 EFS software, and a non-targeted strategy with metabolomics fingerprints obtained at a resolution of 70,000 FWHM (full width at half-maximum) were studied. Both nut polyphenolic profiles and metabolomics fingerprints were evaluated as a source of potential descriptors to be exploited for the classification of nuts according to their type, as well as their thermal treatment, by principal component analysis (PCA) and partial least squares regression discriminant analysis (PLS-DA) using PLS_Toolbox 7.8.2 (Eigenvector Research).

In both strategies, the respective PCA scores plot shows a slight trend between samples according to their nut typology, whereas as expected, PLS-DA improves the observed discrimination. Moreover, PLS-DA models were built in order to study some nuts in pairs, representing the most common nut frauds (e.g. almonds *vs.* hazelnuts or almonds *vs.* peanuts), obtaining a great classification rate. Finally, a good discrimination rate was also observed when studying individual nuts (e.g. almonds, hazelnuts, pumpkin seeds, etc.) regarding their thermal processing treatment. Thus, both targeted and non-targeted UHPLC-HRMS methods have proved to be useful and dependable tools for the classification and authentication of nuts, according to their nut type as well as their thermal treatment, when combined with chemometrics.

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**ALI-F02**

## SOLID-PHASE COLORIMETRIC SENSORS FOR MONITORING MEAT FRESHNESS

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The development of reliable, cost-effective, green and energy-efficient in-situ analysis devices is of increasing interest in the scientific community. In this regard, the research group MINTOTA-UV has developed some solid-phase colorimetric sensors for in-situ monitoring of several atmospheres by passive detection [1]. On the other hand, among the most common volatile markers of meat decomposition, basic nitrogenous compounds such as amines and ammonia are emitted. We proposed here a solid sensor whose chemical recognition is based on the use of the chromophore 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS). It acts as derivatizing reagent that is embedded in a polymeric polydimethylsiloxane (PDMS) matrix modified by the addition of tetraethyl orthosilicate (TEOS). This proposed synthetic strategy includes the addition of the 1-methyl-3-octylimidazolium hexafluorophosphate ionic liquid (IL) as a modifier to introduce extra functionality to the NQS-doped PDMS membrane. The analytical signal has been obtained by measuring the reflectance diffuse at 590 nm expressed as absorbance. It is shown that the NQS-doped PDMS sensor is capable of discriminating between primary and secondary amines atmospheres, which are easily differentiated by eye inspection (Figure 1A). In order to let the sensor be introduced at the meat container, the effect of several factors are addressed. Results indicate that sensor response decreases 50% if it is wrapped individually with parafilm, whereas it is 80% if only one side of the circular sensor is covered. Moreover, it is obtained that the sensitivity of the sensor is different at room temperature or under refrigeration at 4°C, in particular for secondary amines. Additionally, a linear relationship has been achieved between the sensor response and the volume where it is. Particularly, pork samples have been monitored by using the NQS-PDMS sensor jointly with a colorimetric sensor based on the aggregation of silver nanoparticles (Ag NPs) immobilized on a nylon support for the determination of volatile sulfur compounds [2] (Figure 1B). It is shown that a significant response of ammonia, mainly primary amines as well as sulfides is obtained when meat simple is kept for a week, being the meat freshness better preserved under refrigeration conditions. Therefore, the employment of NQS-doped PDMS and Ag NPs colorimetric sensors could be a potential green alternative to in-situ analysis of the meat freshness.

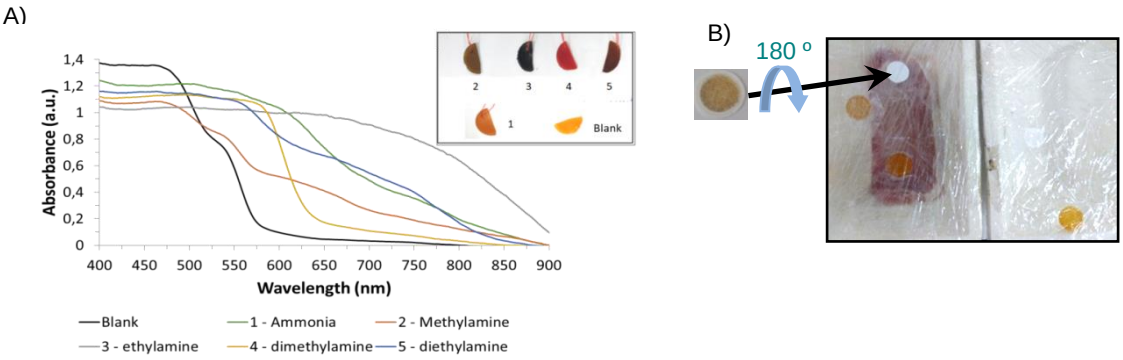


Figure 1. A) Absorption spectra of the NQS-based PDMS sensor for ammonia and amine atmospheres. B) Spatial configuration of sensors in pork sample (left) and blank (right).

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**FyO-F01**

## PREVIOUS CLASSIFICATION OF INKS FOR SUBSEQUENT DATING USING DATUVINK METHOD

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The present study constitutes an extension of a previous ink dating methodology based on a Partial Least-Squares (PLS) regression obtained from UV-vis-NIR reflectance spectra, named DATUVINK [1]. In DATUVINK, the spectroscopic data acquired from a specific brand of commercial ink (Inoxcrom), under accelerated aging, was used to construct a PLS model that was applied to five other pen brands (Milan, Paper Mate, Staedtler, Sierra IB-B and Bic). Although the predictions obtained for two of them were acceptable, inaccurate results were found for the remaining pen inks due to substantial differences in ink formulations. Thus, to broaden the applicability of the method, individual predictive models for each class of ink would be needed.

In this work, ink characterization of ten naturally aged blue writing instruments, such as ballpoint (Pilot, Uniball, Staedtler, Bic, Faber Castell and Paper Mate) and liquid ink (Pilot) pens, was carried out based on vis-spectra by means of microspectrophotometry (in the paper) [2] (wavelength maxima and absorbance) and also on the HPLC-DAD chromatographic profile (retention times and peak area ratio) of the recovered inks (upon extraction from paper). The spectroscopic and chromatographic data were further processed by PCA and HCA for the classification of the inks. According to the type of pen and dyes contained in their formulation, the inks were classified into three groups. The first class consisted of a liquid ink pen (Pilot), the second gathered ballpoint pen inks containing the Victoria Blue dye (Uniball, Pilot, Paper Mate) and the third class gathered inks with Methyl Violet and Crystal Violet dyes (Faber Castell, Bic and Staedtler). Differences were observed with aging in the different inks. This classification will constitute a useful tool for the developing of specific prediction models for each ink group, which can be applied as a previous step for the selection of the appropriate DATUVINK model in a real ink dating scenario.

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**FyO-F02**

# ADVANTAGES OF USING PARAFAC IN THE UNEQUIVOCAL IDENTIFICATION AND QUANTIFICATION OF TERNARY MIXTURES' POLYCYCLIC AROMATIC HYDROCARBONS BY FLUORESCENCE SPECTROSCOPY

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Polycyclic aromatic hydrocarbons (PAHs) are widespread in the environment as products of incomplete combustion or pyrolysis of organic material. Food can be contaminated by PAHs that are present in the air, water or soil, as well as those that are formed during food processing or certain home cooking practices. Several PAHs have been classified by the International Agency for Research on Cancer (IARC) as probably or possibly carcinogenic to humans (Groups 2A or 2B) and benzo[a]pyrene (BaP) directly as carcinogenic to humans (Group 1) [1].
The Scientific Committee on Food (SCF, 2002) decided that BaP may be used as a marker of occurrence and effect of the carcinogenic PAHs in food products [2], but a report of the European Food Safety Authority (EFSA, 2008) shows that BaP is not always detectable in foods containing PAHs and then the Panel on Contaminants in the Food Chain from EFSA concluded that the following PAHs: benzo[a]pyrene (BaP), chrysene (Chry), benzo[a]anthracene (BaA), benzo[b]fluoranthene (PAH4) and additionally other four (PAH8) are currently the possible indicators for the carcinogenic potency of PAHs in food [3]. The maximum level of BaP in smoked meat and smoked fish products is set to 5 μg $Kg^{-1}$ [2] and the additional limit of the sum of PAH4 must be included with a level of 30 μg $Kg^{-1}$ [3].

In the present work, three of the most significative PAHs, i.e. BaP, Chry and BaA, and their ternary mixtures, were analyzed by a spectrofluorimetric method based on the second order calibration of excitation-emission fluorescence matrices (EEMs) and parallel factor analysis (PARAFAC) decomposition. The samples measured were arranged in three-way arrays: trilinearity of the data tensor guarantees the uniqueness of the solution obtained through PARAFAC (the Core Consistency Diagnostic index, CORCONDIA, was higher than 0.99 in all cases), so the factors of the decomposition match up with the analytes. PARAFAC models with two factors were performed for each compound (decomposition model explain variance was 99.73%, 99.26% and 98.27% for BaP, Chry and BaA respectively); while a PARAFAC decomposition with four factors was needed for the ternary mixtures (explained variance equal to 99.89%).
The accuracy was verified with the regression "calculated concentration versus true concentration" ("Accuracy line") that assesses the trueness of the method using the hypothesis tests (for the slope and for the intercept). This was verified for all the analytes: that is, the intercept is 0 and the slope is 1 at the 95% confidence level.

Decision limit (CCα) and capability of detection (CCβ) were 0.107 μg $L^{-1}$ and 0.209 μg $L^{-1}$ for BaP, 0.180 μg $L^{-1}$ and 0.352 μg $L^{-1}$ for Chry, and 0.271 μg $L^{-1}$ and 0.530 μg $L^{-1}$ for BaA, respectively, when the probabilities of false positive (α) and false negative (β) were fixed at 0.05

Additionally, the three analytes were unequivocally identified by the correlation between the pure spectra and the PARAFAC excitation and emission spectral loadings in all the cases.

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**FyO-F03**

## DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF ACRYLAMIDE IN COSMETIC PRODUCTS BASED ON DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

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The possible presence of banned substances in cosmetic products must be supervised since there is the possibility that some of them could be present due to unintentional causes. One of these substances is acrylamide.

Some acrylamide-based polymers can be used in cosmetic formulations with different functions, such as stabilizing agents, antistatic agents, binders, fixatives and conditioners in hair products, etc. [1]. However, the problem that arises in the cosmetic products containing these ingredients in their formulation is that small amounts of unreacted acrylamide monomers can also be present, which are toxic since they can penetrate through the skin and can constitute a risk for users [2].

Currently, there is no official analytical method for the determination of acrylamide in cosmetic products. Therefore, the development of an analytical method for its determination is of great interest.

The proposed method in this work is based on dispersive liquid–liquid microextraction (DLLME), followed by liquid chromatography–ultraviolet (LC-UV) determination. In order to improve the selectivity and sensitivity of the analysis, it was considered appropriate to develop and optimize a previous derivatization step to converts acrylamide into a less polar compound to be extracted by means of DLLME, and to introduce a chromophore group in order to be analyzed by UV spectrometry detection. The main variables involved in the derivatization and DLLME processes were studied to provide the best enrichment factors. The method was successfully validated in terms of linearity, limits of detection and quantification and repeatability, and finally it was applied to the determination of acrylamide in commercial cosmetic samples of different nature. This method is useful to carry out the quality control of cosmetic products containing acrylamide-releasing ingredients.

**Acknowledgements**
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**MAB-F01**

# MICROEXTRACCIÓN DE BISFENOL A Y ANÁLOGOS EN MUESTRAS DE POLVO PROCEDENTES DE DIFERENTES MICROAMBIENTES UTILIZANDO DISOLVENTES SUPRAMOLECULARES

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El bisfenol A (BPA) es un compuesto empleado en una amplia variedad de materiales. Debido a su toxicidad y capacidad de migración al medio ambiente, la industria ha introducido numerosos sustitutos, de los cuales algunos se utilizan ampliamente (ej. bisfenol S y bisfenol F) y su toxicidad y presencia en el ambiente está muy documentada. Otros sustitutos del BPA como 4-hydroxyphenyl 4-isopropoxyphenyl sulfone (D-8), el 4,4′-sulfonylbis(2-allylphenol) (TGSA) o el 4-((4-(allyloxy)phenyl)sulfonyl)phenol (BPS-MAE) han sido escasamente investigados pero su presencia en papel térmico se ha detectado en estudios recientes [1]. La peligrosidad y capacidad de migración al medio de estos compuestos aún no se ha documentado. Dado que el polvo es considerado una importante fuente de exposición a contaminantes orgánicos, se ha seleccionado en esta investigación como matriz de referencia para evaluar la migración de estos nuevos compuestos al ambiente.

El objetivo de este trabajo ha sido la optimización un método para la microextracción de D-8, TGSA y BPS-MAE en muestras de polvo basado en el uso de disolventes supramoleculares (SUPRAS). Los SUPRAS son disolventes nanoestructurados constituidos por ensamblaje de moléculas anfifílicas. Los SUPRAS ofrecen múltiples sitios de unión para la extracción de compuestos de diferente polaridad y exclusión simultánea de macromoléculas, además de favorecer el desarrollo de métodos con bajo consumo de reactivos, simples y rápidos (ya que la etapa de extracción y *clean-up* ocurren simultáneamente). Estas propiedades hacen de los SUPRAS extractantes idóneos para matrices complejas como el polvo doméstico.

Las muestras de polvo analizadas se recogieron en diferentes microambientes en Córdoba, tales como tiendas y otros lugares públicos (donde el uso de papel térmico es frecuente), así como en habitaciones y salones de casas, coches y oficinas. Se analizaron un total de 57 muestras recogidas entre 2017 y 2018. Se tomaron alícuotas de 25 mg (tamizadas con una luz de malla de 0.5 mm) en microcrotubos de 2 mL, donde posteriormente se añadieron el anfifilo y los ingredientes para la formación del SUPRAS (200 μL 1-hexanol, 200 μL THF y 800 μL agua) y una mezcla de estándares internos (BPA-d6 y BPS-d8). A continuación, se agitaron las muestras con vórtex (5 minutos, 3,000 rpm) para favorecer la formación del SUPRAS y la extracción y *clean-up* simultáneos y se centrifugaron (20 minutos, 10,000 rpm) para acelerar la separación de fases. Se analizó la fase superior (SUPRAS con los analitos de interés) mediante LC-MS/MS. Los nuevos sustitutos de BPA (BPS-MAE, D8 y TGSA) se detectaron en un intervalo de concentraciones medias de 20 – 325 $ng \cdot g^{-1}$, 8 – 36 $ng \cdot g^{-1}$ y 7 – 89 $ng \cdot g^{-1}$ y porcentajes de detección comprendidos entre 0 – 55%, 6 – 70% y 69 – 82%, respectivamente, y sin diferencias significativas entre microambientes. Estos resultados reflejan la capacidad que tienen estos compuestos de migrar al medio ambiente. Su presencia en microambientes como habitaciones, salones o coches, demuestra que su procedencia no es exclusivamente del papel térmico.

**Agradecimientos:** Los autores agradecen el apoyo financiero del Ministerio Español de Ciencia, Innovación y Universidades (Proyecto CTQ2017-83823R). A.B.G agradece su beca Ramón y Cajal (RYC-2015-18482).

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**MAB-F02**

# DETERMINATION OF NITRO MUSKS IN ENVIRONMENTAL WATERS BY STIR BAR SORPTIVE DISPERSIVE MICROEXTRACTION FOLLOWED BY THERMAL DESORPTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Musk compounds are obtained as a secretion produced by a gland of the musk deer. They have been widely used as fragrance ingredients in daily consumer products such as cosmetics, detergents, shampoos and perfumes. In order to replace the use of natural musks due to ethical and economic reasons, nowadays the term musk also refers to other compounds with totally different chemical structure but possessing musk-like odour properties. Nitro musks are a group of these synthetic musks whose structures consist of dinitro and trinitro substituted benzene derivatives. The nitro musk group is mainly constituted by musk ambrette (MA), musk ketone (MK), musk moskene (MM), musk tibetene (MT) and musk xylene (MX). However, despite their pleasant aroma, they have shown health risks related to dermatitis, carcinogenic effects and endocrine disruption [1]. For this reason, MA, MT and MM are banned in European cosmetic products according to the European Regulation on Cosmetic Products [2], whereas the use of MX and MK is restricted.

It should be said that nitro musks are indirectly released into the environment via wastewater treatments plants, or directly from, for example, swimming activities [3]. Moreover, these compounds are persistent in the environment and degrade slowly. Therefore, it is important to develop new analytical methods for the determination of these compounds, and thus to evaluate its potential for bioaccumulation on the environment.

Among the vast array of microextraction techniques currently developed, magnetic-based approaches have gained popularity due to the magnetic properties of the extraction phases that allow their easy and fast retrieval by means of an external magnetic field. Moreover, the coating of the magnetic material can be modified to extract specific analytes on demand. However, to the best of our knowledge, the high potential of magnetic materials has never been used before for the extraction and subsequent determination of the complete family of nitro musks compounds.

In this sense, the aim of this work is to develop, for the first time, a high sensitive magnetic-based analytical method for the determination of the nitro musks in environmental water samples. This method is based on stir bar sorptive-dispersive microextraction (SBSDME) [4], developed by our research group, as extraction technique followed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) analysis. A polydopamine-coated magnetic nanoparticles composite (i.e., $CoFe_2O_4$@pDA) is proposed here as sorbent material.

**Acknowledgements**
Authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-70301R).

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**PMU-F01**

## STIR BAR SORPTIVE-DISPERSIVE MICROEXTRACTION MEDIATED BY A MAGNETIC NANOPARTICLES-METAL ORGANIC FRAMEWORK COMPOSITE FOR THE DETERMINATION OF N-NITROSAMINES IN COSMETIC PRODUCTS

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The current European Regulation on cosmetic products [1] includes a list of compounds whose presence in cosmetics is prohibited in order to assure the safety of the users. An example of this are the so-called N-nitrosamines, prohibited in the EU since 1992 due to their mutagenic, carcinogenic, and teratogenic effects. However, their presence in cosmetics at trace levels is a persistent problem, since they are easily formed by the reaction of a secondary or tertiary amine and a nitrosating agent, such as nitrite, oxides of nitrogen, and cosmetic ingredients containing nitro groups. In this sense, with the purpose of ensuring the safety of the users, the European Scientific Committee on Consumer Safety (SCCS) established a maximum limit of 50 µg/kg for all N-nitrosamines contained in raw materials as well as all N-nitrosamines potentially formed in the finished cosmetic product [2]. For this reason, the analytical control of N-nitrosamines in both cosmetics and raw materials is a matter of interest.

The stir bar sorptive-dispersive microextraction (SBSDME) [3], a solid phase-based microextraction approach recently developed by our research group combines the principles of stir bar sorptive extraction (SBSE) and dispersive solid-phase extraction (DSPE), and it consists in the use of a neodymium stir bar coated with a magnetic material as extraction device, in such a way at low stirring rate the magnetic material remains onto the surface of the stir bar like in SBSE, whereas at high stirring rate the material is dispersed into the donor phase like in DSPE. Once the stirring is over, the magnetic material containing the extracted analytes is retrieved by the stir bar without requiring any additional magnetic field. Finally, the analytes can be desorbed in an appropriate solvent prior to its injection into a chromatographic system, or they can be directly introduced into a GC system by thermal desorption.

A new analytical method based on the recently proposed SBSDME technique has been developed for the determination of eight hazardous N-nitrosamines in cosmetic products. The method makes use of a magnetic nanoparticles-metal organic framework composite, $CoFe_2O_4$@MIL-101(Fe), as sorbent to entrap the target analytes, which are later chemically desorbed and measured by LC-MS/MS. The variables involved in the SBSDME process were studied to obtain the highest analytical signal. Under the selected conditions, the method was successfully validated showing good linearity; enrichment factors up to 62 depending on the target compound; limit of detection values from 3 to 13 ng $g^{-1}$; and good repeatability (RSD <17.0 %). Finally, the proposed method was applied to different cosmetic samples, composed either of lipophilic or hydrophilic matrices. Quantitative relative recovery values (96 – 109 %) were obtained by using standard addition calibration. This work expands the applicability of the SBSDME technique, as the analytical features of the proposed method, besides of its simplicity and affordability, make it useful to perform the quality control of cosmetic products.

**Acknowledgements**
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**TAN-F01**

# DETERMINATION OF PHOSPHATE CONCETRATION IN ARTIFICIAL EYEDROPS BY DIGITAL IMAGE ANALYSIS

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In the recent years, several corneal calcification cases have arisen in relation to the use of eyedrops with high phosphate concentration in their chemical composition. Therefore, the quantification of phosphate concentration in eye drops and artificial tears is essential in order to avoid complications in high-risk patients [1]. The main objective of this work is to develop a method for the determination of phosphate in artificial eye drops by digital image analysis.

The molybdenum blue reaction has been used for the colorimetric determination of phosphate. Samples were placed into a microtitration plate as it can be seen in Figure 1(a) and digital images were subsequently taken by a desktop scanner and a smartphone. Best conditions for image analysis were optimized and data processing was carried out by ImageJ and MatLab.

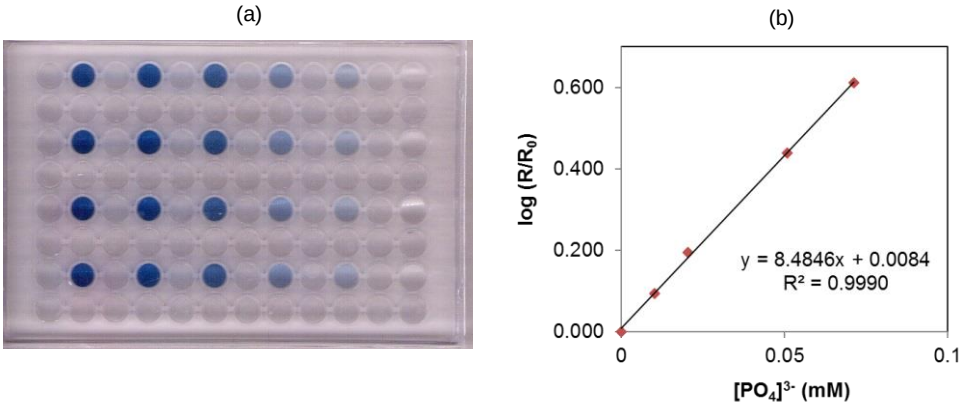


Figure 1. (a) Image obtained by desktop scanner for blue molybdenum complex in the presence of varying amounts of phosphate. (b) Calibration line for phosphate using information on the R channel in image (a).

A good calibration was obtained with the proposed method, Figure 1(b), and it was applied to various eye drops used for the treatment of glaucoma. Ultraviolet-Visible spectroscopy and ionic chromatography have been used as reference and not significant differences were observed.
Summarizing, an accurate, precise and robust method has been developed which can be used to determine phosphate concentration in eye drops in a simple and rapid way.

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**TAN-F02**

# ELECTROCHEMISTRY OF GALLIUM AND ELECTROCHEMICAL FORMATION OF Cu-Ga INTERMETALLIC COMPOUNDS IN CHOLINE CHLORIDE-ETHYLENE GLYCOL (1:2)

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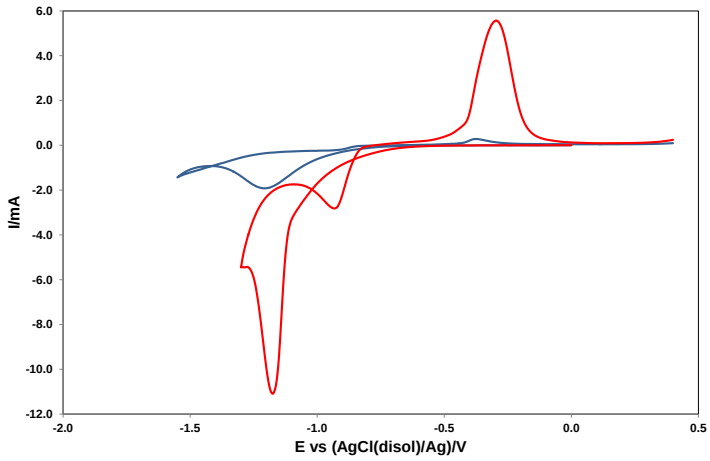
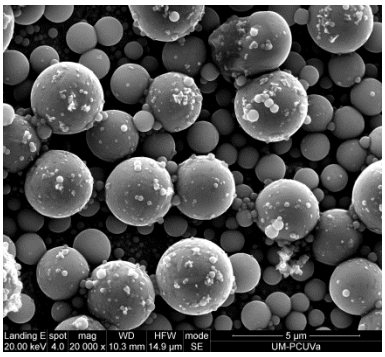
Gallium is a suitable material for producing semiconductor compounds (e.g. GaAs, GaInAs, $CuGaSe_2$ and $Cu(In,Ga)Se_2$) used in electronic and optoelectronic technology. As a part of a project to look into the ability of deep eutectic solvents (DES), as reaction media, to deposit high-quality semidonducting films, the present work is concerned with the electrochemical behaviour of gallium, in the eutectic mixture Choline Chloride – 2 Ethylene Glycol (ethaline). The study has been carried out using different substrates as working electrodes: i) Pt, Mo, W for the electrochemical deposition of Ga(liq) and ii) Cu for the electrochemical formation of Ga-Cu intermetallic compounds .



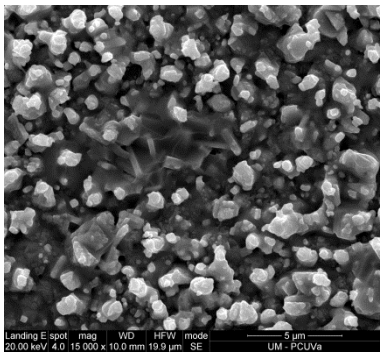

Cyclic voltammograms of a $GaCl_4^-$ solution on a Mo electrode at 80 and $100^0$C

Ga electrodeposition is difficult from aqueous solution due to its low standard potential and the interfering hydrogen evolution reaction. The use of Ethaline, with a better thermal stability and larger potential window, eliminates the interference of solvent breakdown reactions during Ga deposition on Pt, W and Mo electrodes.

The electro-reduction of $GaCl_4^-$ solutions was also investigated at a copper substrate. Ga-Cu alloy films were obtained by continuous potentiostatic electrolysis and intensiostatic pulse electrolysis. The obtained samples, characterized by XRD and SEM, revealed the formation of $CuGa_2$.



SEM image of a deposit of Ga on a W electrode



SEM image of $CuGa_2$

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *ALIMENTACIÓN*

**ALI-P01**

## HPLC-UV and HPLC-FL fingerprints to authenticate the origin of coffee samples by Partial Least Squares regression – Discriminant Analysis

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Coffee is today one of the most popular beverages in the world. More than one billion cups are consumed every day, with an annual consumption per capita over 5 kg, on average, in Europe. Intake of coffee is associated with a reduced risk of several diseases, such as type 2 diabetes in healthy individuals, probably due to its antioxidant activity. The proven health benefits of coffee brew plenty justifies the consideration of this infusion as a functional food [1]. Polyphenols are among the most interesting bioactive compounds found in coffee. In fact, coffee is the major source of chlorogenic acids (polyphenolic family of esters of caffeic, ferulic and *p*-coumaric acids with quinic acid) in the human diet and there is plenty of evidence of their important antioxidant activity. Polyphenolic coffee content seem to be related to features such as the coffee variety (i.e. Arabica, Robusta), production region and climate conditions, among other parameters. As a consequence, bioactive compound fingerprinting (mainly polyphenols) can be used as a source of analytical data to achieve sample characterization and classification, and to authenticate the origin, the variety and the roasted grade of coffee.

In the present work, C18 reversed-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescence (FL) detection has been applied to the characterization of Arabica and Robusta coffee samples from different production regions by using Partial Least Squares Regression – Discriminant Analysis (PLS-DA) chemometric method.

For that purpose, a set of 120 commercially available coffee samples belonging to different groups depending on the variety (Arabica and Robusta) and the growing region (Ethiopia, Colombia, Nicaragua, Indonesia and India) were analysed after brewing the coffee (espresso machine) and filtrating. HPLC separation in a Kinetex C18 reversed-phase (100×4.6mm i.d., 2.6µm) column under gradient elution using 0.1% aqueous formic acid and methanol was applied. Data corresponding to HPLC-UV and HPLC-FL fingerprints were considered as a source of potential descriptors to be exploited for the characterization and classification of the coffees. The plot of scores obtained after PLS-DA using both UV and FL detection fingerprints revealed patterns that were perfectly correlated to the production regions, both coffee varieties and the different roasted grade of samples. Moreover, sample classification and discrimination tend to be related to polyphenolic content. Potential discriminant polyphenolic compounds of each coffee variety and production region were tentatively identified. In addition, PLS-DA models were validated by pairs of class groups obtaining a great classification rate.

The results obtained in this work suggested that HPLC-UV and HPLC-FL fingerprints can be useful for the characterization and classification of coffee samples to authenticate their variety and production region, thus helping to prevent consumer frauds.

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**ALI-P02**

## IMPACT OF BOILING IN PHYTOCHEMICAL PROFILE OF VIOLET CAULIFLOWER (*Brassica oleracea* L. var. *botrytis*)

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In the last years, diets rich in food of plant origin have gained popularity due to their content of bioactive compounds, such as polyphenols [1]. The presence of these characteristic compounds in vegetable products directly relates their consumption to some health benefits. In this line, a particular attention has been focused in violet cauliflower (*Brassica oleracea* L. var. *botrytis*), a less common Brassicaceae species which biodiversity is not only visible in its outer appearance but also in term of phytochemical profiles and concentrations [2]. However, cauliflower phytochemical composition have been proved to be strongly affected by cooking treatment due to its hydrophilic and heat-sensitive nature [3], being crucial the ratio between cooking time and temperature in phytochemicals integrity and liberation from the plant matrix. Ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) targeted approaches, where peak signals of defined compound families are proposed as food features, have become one of the most common tools to study variations in food products due to any kind of thermal process.
In the present study, an evaluation of variations in the phytochemical content of violet cauliflower caused by boiling treatment was carried out. Thus, raw and boiled (10 and 25 min of cooking time) violet cauliflower samples were analyzed by a UHPLC-ESI-HRMS (Q-Exactive Orbitrap) method. A simple solid-liquid extraction with acetone:water:formic acid (70:29.9:0.1, *v/v/v*) as extracting solvent was employed and an analysis time below 23 min was achieved by using an Ascentis Express C18 (15 cm x 2.1 mm x 2.7 µm particle size) column. The obtained HRMS data was then processed by means of customized target accurate mass databases of flavonoids, glucosinolates and anthocyanins using TraceFinderTM 3.3 EFS software. Moreover, phytochemical profiles were exploited by principal component analysis (PCA) and partial least squares regression discriminant analysis (PLS-DA) to address the classification between raw and boiled samples, as well as according to boiling time applied, by using PLS_Toolbox 7.8.2 (Eigenvector Research). The obtained PCA scores shows a trend between raw and boiled samples, and as expected, PLS-DA improves the observed discrimination. Besides, a difference in phytochemical composition was observed among those samples with different boiling time.

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**ALI-P03**

## QUANTIFICATION OF OXYTETRACYCLINE IN BEEKEEPING PRODUCTS WITH VOLTAMMETRIC METHODS

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The oxytetracycline (OTC) is a tetracycline broad-spectrum antibiotic. The study of this type of antibiotics is important due to their use in food, especially in beekeeping products. As consequence of their side effects, the residual quantity in honey and beekeeping products is severely controlled. For instance, the use of antibiotics in beekeeping in the European Union is prohibited. According to the Technical Regulations of the Customs Union TR CU 021/2011 "On food safety", the allowed maximum concentration limit of OTC in honey is 0.01 mg/kg [1].

To determinate this molecule in beekeeping products, high pressure liquid chromatography coupled to a mass-spectrometric of fluorescence detectors, conventional microbiological methods, and immunoenzyme method (ELISA) can be used [2]. Usually these techniques are used as confirmatory techniques. Voltammetric methods can also be used as rapid, early detection, and accurate determination of the OTC in beekeeping products, compared to more complex instrumental techniques.

The principal aim of this study was to develop sensitive voltammetric methods for the determination of OTC in commercial honey and beewax samples. The main problem with this kind of samples has been the high concentration of sugars which can interfere in these determinations. Sugars affect significately and can be reduced at the same potential of the antibiotic, producing electrochemical interferences, for which adecuate and rapid sample pretreatments were studied.

Different voltammetric techniques have been studied to achieve good analytic properties (sensitivity, detection limit, selectivity, reproducibility, and detection limits). Modified electrodes with gold nanoparticles and multi-wall carbon nanotubes have been studied for increasing the surface of the electrode and improve the charge transfer rate between the antibiotic and the electrode. Although the sensitivity is good, 17 nA/mg $L^{-1}$, the detection and the quantification limits are not (about 10 mg $L^{-1}$ of OTC), so modified electrodes with mercury were studied with the aim to improve sensitivity.

Mercury film electrodes (MFEs) have been the best option to analyse this molecule by differential pulse voltammetry. Adsorptive Stripping Voltammetry with MFEs, as a preconcentration method, has resulted the method with lowest detection and quantification limits, 10 ng $mL^{-1}$ and 100 ng $mL^{-1}$, respectively, and higher sensitivity, about 94 nA/ng $L^{-1}$. Good recovery values have been reached by the AdSV with mercury film electrodes, about 90-95% in honey and beewax samples, at the sub ng $mL^{-1}$ level.

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**ALI-P04**

## VALORIZACIÓN DE BORRAS DE CAFÉ CON DISOLVENTES SUPRAMOLECULARES

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El café es la segunda bebida más consumida a nivel mundial. Se cultiva en alrededor de 80 países, de los cuales el 90% son países en vías de desarrollo. Para producir una taza de café se genera un 95% de biomasa residual. En estos residuos se encuentran sustancias bioactivas que pueden ser de interés para nuevos procesos y/o productos, permitiendo de esta manera disminuir los impactos negativos del desecho de residuos y generando un valor añadido a la agrocadena del café. La extracción con fluidos supercríticos o extracción con disolventes orgánicos asistida por ultrasonidos o microondas o utilizando elevadas presiones o temperaturas han sido las técnicas más investigadas para su valorización. En este estudio se emplean disolventes supramoleculares (SUPRAS) [1] como un alternativa sostenible, económica y eficiente para la valorización de los residuos de café. Los SUPRAS son líquidos nanoestructurados que ofrecen múltiples interacciones y sitios de unión para la extracción de compuestos en una amplia polaridad y que se caracterizan por baja volatilidad, inflamabilidad y toxicidad.

Entre los compuestos con interés funcional presentes en residuos de café, la cafeína y los ácidos clorogénicos son los de mayor interés. La cafeína tiene efecto estimulante del sistema nervioso central y los ácidos clorogénicos (CGAs) son compuestos con elevada capacidad antioxidante. Estos tipos de compuestos bioactivos se seleccionaron como representativos para la optimización del proceso de extracción de borras de café húmedas.

En este estudio se investigaron SUPRAS formados espontáneamente a través de autoensamblaje y coacervación de agregados de 1-hexanol o ácido decanoico (8-24% v/v) en una fase hidro-orgánica de etanol o THF (20-40 % v/v) y agua (36-72 %) para la extracción de compuestos bioactivos de borras de café húmedas procedentes de la variedad *Castillo* cosechada en Colombia. Los SUPRAS se sintetizaron in situ en presencia del residuo de café y se investigó la capacidad de extracción de los mismos para cafeína y ácido clorogénico. El SUPRAS que produjo la máxima eficiencia de extracción fue el sintetizado a partir de 24% v/v 1-hexanol, 30% etanol v/v y 46% agua v/v. El proceso consistió en agitación de la mezcla durante 1 min a 3,000 rpm y centrifugación para la separación del residuo del extracto de SUPRAS. Estos extractos mostraron altos contenidos en cafeína (3.3 $mg.g^{-1}$) y 5-CGA (4.3 $mg.g^{-1}$), elevado valor en polifenoles totales (60 mg/g), así como alta actividad antioxidante (evaluada con diferente métodos: DPPH, FRAP, ABTS) y cierta actividad antimicrobiana, especialmente ante bacterias gram-negativas (*S. enterica* and *P. putida.)*.

En base a estos resultados podemos concluir que los SUPRAS presentan una alternativa verde y viable para la valorización de borras de café.

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**ALI-P05**

# DETERMINACIÓN DE BISFENOL A, SUS DERIVADOS CLORADOS Y ANÁLOGOS ESTRUCTURALES EN VEGETALES DE CONSUMO HUMANO

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Los disruptores endocrinos son un grupo de sustancias naturales o sintéticas capaces de interferir con la función normal del sistema endocrino en animales y humanos. Los bisfenoles pertenecen a este grupo de compuestos [1, 2]. El máximo representante de esta familia es el bisfenol A (BPA), con una producción anual de 2-3 millones de toneladas. Es la base para la síntesis de plásticos policarbonato y de las resinas epoxi utilizadas en la producción de una gran variedad de productos manufacturados. En los últimos años, debido al incremento en la preocupación por la demostrada actividad biológica del BPA y su presencia en productos de consumo, el compuesto se está reemplazando gradualmente por análogos estructurales, cuyo impacto ambiental y riesgos asociados no se conocen aún [3].

En este trabajo, se presenta un método analítico para la determinación de 11 bisfenoles con demostrada actividad estrogénica (BPA, sus derivados clorados (Cl-BPA, $Cl_2$-BPA, $Cl_3$-BPA y $Cl_4$-BPA) y los análogos estructurales (bisfenol B (BPB ), bisfenol E (BPE), bisfenol F (BPF), bisfenol P (BPP), bisfenol S (BPS) y bisfenol AF (BPAF)) en muestras de vegetales. El procedimiento implica la extracción de los analitos de la muestra usando una extracción con ultrasonidos focalizado con acetona seguido de una etapa de limpieza del extracto mediante extracción en fase sólida dispersiva empleando PSA. La determinación analítica se realiza mediante cromatografía gaseosa acoplada a espectrometría de masas de triple cuadrupolo. Se optimizaron los principales parámetros de la extracción utilizando técnicas estadísticas de diseño experimental. Los analitos se separaron en 11 min. Los límites de cuantificación estuvieron entre 0.05 y 1 ng $g^{-1}$. Se obtuvieron valores de exactitud, expresada en términos de recuperaciones relativas, en el rango entre 74-105%. La precisión del método, expresada en términos de desviación estándar relativa, fue inferior al 12%. El método se aplicó a la determinación de estos compuestos en muestras de zanahoria de un comercio local. Se detectaron BPA y Cl-BPA en la mayoría de las muestras analizadas a concentraciones de hasta 8.91 y 6.13 ng $g^{-1}$, respectivamente. Esta es la primera vez que estos compuestos se analizan en vegetales.

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**ALI-P06**

# OPTIMIZATION OF THE TBA-MLD REACTION IN NON-AQUEOUS MEDIUM. DIRECT ANALYSIS OF LIPIDIC SAMPLES BY FLUORIMETRIC-HPLC

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Malondialdehyde (MLD) is the principal compound formed during the lipid peroxidation, and this process is the responsible of rancidity in stored foods and related to heart disease, cancer, and aging in animals. The most useful method for the determination of MLD, both in food samples and in biological samples, is its reaction with thiobarbituric acid (TBA) in acidic aqueous medium [1]. In these conditions takes place the formation of a 1:2 (MLD:TBA) pink adduct that absorbs at 532 nm. Also, the namely thiobarbituric acid reactive substances (TBARS), including saturated and unsaturated alkanes, among other compounds, react with TBA and thus contribute to overestimate the extent of lipid peroxidation [2]. Usually, before TBA reaction, MLD is extracted from lipid samples but this process exhibit a low yield.

Now, and with the object to analyze directly MLD in lipidic samples, such as oil samples, incompatibles with aqueous medium, and get a most realistic vision of the lipid peroxidation, the reaction has been carried out in non-aqueous medium, and a HPLC method with fluorimetric detection, has been optimized. Propanol was selected as more adequate solvent, and all reagent solutions, TBA, trichloroacetic acid and MLD were prepared in this solvent. The concentration of TBA, thricholoracetic, percentage of $H_2O$ and time and heating temperature were tested and optimized. The elution was performed with methanol and phosphoric acid 1% using a gradient mode. Three peaks at 0.95, 3 and 4.3 min have been observed. The nature of the compounds formed in non-aqueous medium were identified by HPLC-UV and HPLC-MS using negative ionization The m/z of the first peak is coincident with the described by Jardine et al, that corresponds to a yellow product that absorbs at 450 nm [3]. The absorption spectra of peaks 2 and 3 are identical with an absorption maxima at 532nm but its m/z are very different. The third peak has been assigned to the MLD:TBA adduct and a good correlation between 0.05-1 μg $mL^{-1}$, $R^2$ = 0.9977, was obtained.

Olive oil and skin oil samples were analysed using the addition standard method. For each sample 0.5 g of oil were diluted with propanol to a volume of 10 mL Aliquots of 0.5 mL were transferred to a 10 mL volumetric flasks and increasing volumes of MLD standard solution, 6 mL of TBA and 1 mL thricholoracetic acid 5 M in propanol, were added. Samples were heated at 75 ºC during 30 min and cooled. Aliquots of 2 mL were centrifuged at 20000 rpm during 2 min. Supernatants were filtered through a 0.22 μm nylon filter, and aliquots of 5 μL were injected in the chromatographic system.

At the same time, the samples were spectrophotometrically analysed at 532 nm with the aim to compare the obtained results.

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**ALI-P07**

## A 3-YEAR SURVEY OF PESTICIDE RESIDUES IN FRUIT-BASED SOFT DRINKS

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Pesticides are highly used to control the plagues, which affects the majority of the crops around the world. However, a misuse can also have negative effects on the human health. In order to protect to the consumer, a plethora of regulation is available for pesticide control in fruits, vegetables or drinking water. However, there is not still a legislation about derivate products such as fruit-based soft drinks. Our research group has revealed the presence of pesticides in these type of product in several occurrence studies in the last 10 years. Thus, a follow-up study on the occurrence of multiclass pesticides has been proposed.

To undertake this study, a sensitive and selective liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method has been developed for the determination of over 100 pesticides in fruit-based soft drinks. The separation was performed by using a reversed phase $C_{18}$ column Zorbax Eclipse Plus RRHD (2.1 mm × 100 mm, 1.8 µm). Mobile phase was water and acetonitrile both them with 0.1% of formic acid. The mass spectrometer was a TSQ Quantiva triple quadrupole analyzer (QqQ) (Thermo Scientific, USA) operating in selected reaction monitoring mode. A heated electrospray ionization probe working in positive mode was employed.
Sample treatment was based on solid-phase extraction (SPE) using polymer-based SPE cartridges. LOQs ranged 1-24 ng/kg was obtained, being less than maximum residue level (MRL) established by the European Union in the case of citrus fruits or water intended for human consumption. To obtain a wide perspective of the concentration of pesticides in this type of products, over 200 samples have been collected worldwide over the period 2017-2019. Some preliminary results show that some of the pesticides sought were detected, while the overall content of pesticides exceeded, in some cases, 500 ng/L, the pesticide threshold set for water intended for human consumption for the sum of pesticides, for several samples studied. Therefore, the exposure to these chemicals through these beverages still represents a challenge, which should be adequately addressed.

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**ALI-P08**

# AN ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF DIFFERENT TYPES OF ENDOCRINE DISRUPTING CHEMICALS IN DAIRY PRODUCTS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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There is growing interest in the potential health threat posed by endocrine disrupting chemicals (EDCs), which are increasingly present in our environment, foods and consumer products. EDCs and can interfere with hormone biosynthesis, metabolism, homeostatic control and reproduction. In addition, these compounds can cause diabetes and hormone-sensitive tumors in female organs, prostate, thyroid, and the neurodevelopmental and neuroendocrine systems [1]. About 1000 compounds have so far been listed as EDCs that fall into various chemical families. Identifying EDCs is often made difficult by their sharing no similarity other than a usually small molecular mass (< 1000 Da). It is therefore difficult to confirm whether a given organic molecule may exhibit endocrine disrupting activity. Physiologically, EDCs can be of two types, namely: natural or synthetic. Some natural chemicals including phytoestrogens (e.g., coumestrol and genistein present in human or animal food) act as EDCs, and so do many synthetic chemicals including polychlorinated and polybrominated biphenyls, dioxins, plasticizers such as phthalates, plastics such as bisphenol A, pharmaceuticals such as diethylstilbestrol, and pesticides such as methoxychlor, dichlorodiphenyltrichloroethane, chlorpyrifos and some fungicides (e.g., vinclozolin) [2].

EDCs can usually be sensitively detected and accurately quantified with instrumental techniques such as gas chromatography coupled with mass spectrometry (GC–MS) or high performance liquid chromatography in combination with mass spectrometry or fluorescence detection. These tools provide signals that are usually sensitive enough for the identification of EDCs. However, these compounds generally contain functional groups such as hydroxyl and carboxyl that require derivatization in order to ensure the obtainment of symmetric chromatographic peaks, and a high sensitivity and/or precision in their GC–MS determination [3].

In this work, we developed an analytical method for the determination of five EDC classes (viz., alkylphenols, phenylphenols, bisphenol A, parabens, triclosan, and organophosphorus pesticides) in dairy products. The method involves ultrasound-assisted extraction in combination with automated solid phase extraction to remove the sample matrix and preconcentrate the analytes. The proposed method is highly sensitive; thus, it allows the analytes to be determined at the ng/kg level with good precision (relative standard deviation < 7.5%) and accuracy (near-quantitative recovery). Its applicability was checked by using it to determine 24 EDCs in various dairy products (milk, cheese, yogurt and butter). The samples were found to contain some of the analytes, albeit at concentrations invariably below the current maximum residue levels (MRLs) set by the European Commission.

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**ALI-P09**

# DETERMINACIÓN DE AMINOÁCIDOS LIBRES EN JAMÓN IBÉRICO MEDIANTE CROMATOGRAFÍA DE GASES DE ALTA RESOLUCIÓN. APLICACIÓN A LA DIFERENCIACIÓN DE ESTADOS DE CURACIÓN

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Los aminoácidos son componentes de las proteínas y, además, son intermedios en la biosíntesis de nucleótidos, vitaminas y metabolitos secundarios. La actividad proteolítica ejercida por las enzimas endógenas y/o microbianas produce alguno de los cambios bioquímicos más importantes en la carne y sus derivados [1]. La degradación de las proteínas de la carne da aminoácidos libres, péptidos, aldehídos [2], ácidos orgánicos y aminas [3]. Los aminoácidos contribuyen a las características organolépticas de la carne [4]. Debido a ello, su determinación es de suma importancia.

El objetivo de este trabajo ha sido desarrollar un método altamente eficiente para la extracción, derivación y posterior análisis, mediante cromatografía de gases de alta resolución (HRGC), de aminoácidos libres en jamón ibérico. Los aminoácidos se utilizan como descriptores químicos para diferenciar las etapas del proceso de curación.

El primer paso del procedimiento es la eliminación de la grasa utilizando una mezcla de n-hexano-dietil-éter (4:1 v/v). Posteriormente, se lleva a cabo una extracción con metanol-acetonitrilo (1:1 v/v), obteniéndose recuperaciones entre 68 y 164%. Los aminoácidos se derivatizaron con BSTFA, formando trimetilsilil derivados. Se utilizó metimazol como patrón interno.

La fracción de aminoácidos libres se analizó mediante HRGC, utilizando un cromatógrafo de gases equipado con una columna DB-17HT (30 m x 0,25 mm i.d., 0,15 µm de espesor de fase), usando hidrógeno como gas portador, con inyección split y detección con ionización de llama. El programa de temperatura tiene una temperatura inicial de 85 ºC mantenida durante 2 min., una rampa de 1 ºC $min^{-1}$, hasta 100 $^{o}$C, una segunda rampa de 6 ºC $min^{-1}$ hasta 258 ºC, manteniendo esta temperatura durante 5 min. La identificación de los aminoácido libres se llevó a cabo utilizando un detector de espectrometría de masas, con un rango de 25–650 uma y una fuente de ionización de impacto electrónico a 70 eV. El método desarrollado permite una separación y cuantificación rápida, sensible y altamente reproducible de los diferentes aminoácidos libres, en muestras de jamón ibérico.

Se detectaron dieciocho aminoácidos en muestras estudiadas, considerando tres niveles de curación. En todos los casos se comprobó un aumento significativo de las concentraciones de aminoácidos, excepto en el caso de la L-alanina, obteniéndose la siguiente relación entre la concentración y la duración del proceso de curación: [aminoácido] = 5.7767x(Días) + 4714.6.

Mediante la aplicación de análisis discriminante lineal (LDA) se consiguió diferenciar completamente los tres períodos de curación utilizando como descriptores, L-alanina, L-tirosina, L-glutamina, L-prolina, L-2-aminobutírico, L-cisteina y L-valina. Los valores de los aminoácidos libres totales en postsalado, secado y bodega, respectivamente, se pueden utilizar para predecir el tiempo de curado.

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**ALI-P10**

# DISCRIMINATION OF WHITE WINES' HARVEST YEAR AND VARIETIES BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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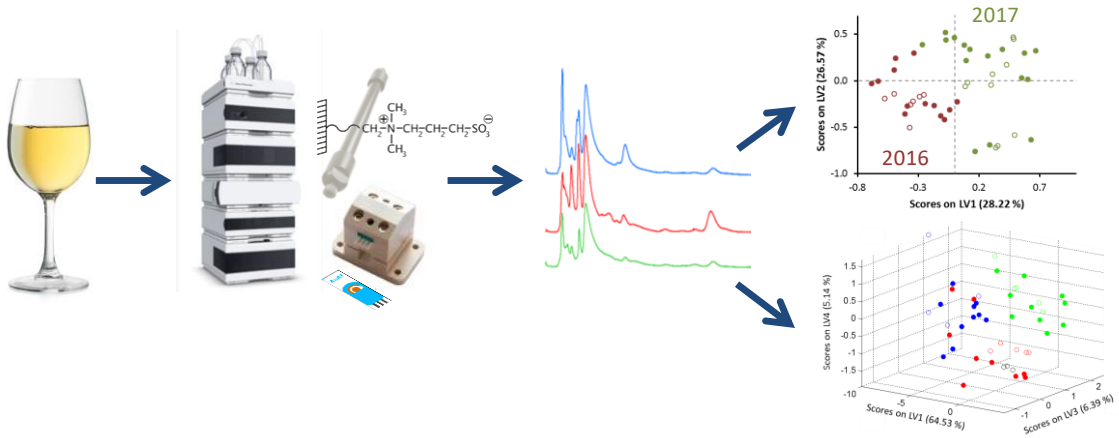
In recent years food quality and food authentication have become important aims for food industry and society since consumers give more value to the quality and origin of food products. In this sense, one of the products in which these quality labels have been historically most valorised is wine. Regarding wine quality, both quality producers and final consumers usually pay special attention to sensory aspects that are usually related to wine variety, aging processes, style and aroma. In this sense, small soluble aminothiols such as glutathione, cysteine and related compounds have been reported as important biomarkers involved in the protection of white wines from both browning and losses of flavour and aroma [1].

Considering the coexistence of different aminothiols in wine, the use of separation techniques like chromatography or capillary electrophoresis is required to determine the occurrence of aminothiols in wine samples. Particularly liquid chromatography (LC) coupled with UV, fluorescence or mass spectrometry detection has been among the most exploited methods. However, UV and fluorescence detection methods require derivatisation treatments into UV absorbing or fluorescent species since these compounds present low UV-vis absorbance whereas the detection by means of mass spectrometry is quite expensive. A more affordable alternative detection method that can avoid tedious derivatisation procedures is electrochemical detection (EC), which takes advantage of the electroactive character of aminothiols, being the oxidation of thiol groups to disulfide the main electrochemical reaction involved.

In EC the selection of the amperometric sensor plays a key role. On the one hand, it is well-known that noble metals, like gold and silver, are very convenient for the determination of aminothiols due to their affinity for thiol groups. On the other hand, the use of screen-printed electrodes (SPE) has been postulated as a very convenient alternative to conventional solid electrodes due to their intrinsic characteristics, such as the disposable and low-cost character and three-electrode configuration printed on the same strip.

Given the importance of these aminothiols in white wines [1], a simple HPLC-EC method using gold screen-printed electrode as amperometric sensor has been developed for the determination of aminothiols in white wines. This method introduces, for the first time, hydrophilic interaction liquid chromatography (HILIC) with electrochemical detection, allowing wine analysis without any sample treatment. The analysed aminothiols have been used as biomarkers for wine aging processes, combining the developed HPLC-EC method with partial least squares discriminant analysis (PLS-DA) to achieve wine classification in terms of harvest year. Moreover, the developed HPLC-EC method also generated characteristic fingerprints that were combined with PLS-DA to classify wines according to three wine varieties [2].

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**ALI-P11**

# PHENOLIC COMPOSITION AND BIOACTIVITY OF *ELAEAGNUS UMBELLATA* AND *SAMBUCUS LANCEOLATA*: EFFECT OF *IN VITRO* GASTROINTESTINAL DIGESTION

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Plants are rich in phenolic compounds, which are known to present many beneficial health effects. Many edible fruits, such as berries, and infusions or decoctions from leaves of berry-producing plants are commonly used in folk medicine. However, in many cases, the exact composition and bioactivity of these plants are still unknown. Here, we present a study on two plant species: *Elaeagnus umbellata* and *Sambucus lanceolata*.

The phenolic profiles of *E. umbellata* and *S. lanceolata* leaves and berries have been determined by high performance liquid chromatography with electrospray ionization mass spectrometry detection (HPLC-ESI-$MS^n$). The most important phenolics were quantified using HPLC with diode-array detection. The antioxidant activity was evaluated by several *in vitro* assays, such as ABTS, DPPH, nitric oxide, and superoxide. Then, the enzyme-inhibitory properties (α-glucosidase, α-amylase, lipase, aldose reductase) and anti-glycation activity were also evaluated. Finally, we carried out a simulated *in vitro* gastrointestinal digestion, observing that although the phenolic contents decreased, both leaves and berries extracts still retained considerable bioactivity.

**ALI-P12**

# OPTIMIZACIÓN DE LAS CONDICIONES INSTRUMENTALES EN EL ANÁLISIS MEDIANTE LIBS DE PRODUCTOS PELETIZADOS QUE COMPARTEN UNA MISMA MATRIZ

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En un planeta cuyo estigma es el consumo de recursos a gran escala para satisfacer la necesidad de su población, resulta de vital importancia el desarrollo y optimización de técnicas analíticas que nos permitan determinar los niveles de ciertos contaminantes para cada tipo de alimento. La aplicación del láser como técnica espectroscópica (Laser-Induced Breakdown Spectroscopy o LIBS) ha tenido un crecimiento progresivo con el paso de los años, algo que se puede evidenciar desde la página oficial de WoS (Web of Science), llegando a publicarse más de 200 artículos relacionados durante el 2018 [1].

Ecuador es un país con fuerte actividad pesquera a nivel industrial, el aprovechamiento de subproductos del procesamiento de pescado se basa principalmente en la transformación de estos en harina de pescado, la misma que a su vez se utiliza para la elaboración de alimento balanceado por su alto contenido proteico [2]; en el presente estudio se analizaron ocho productos peletizados que les fue adicionada harina de pescado durante su elaboración, para la optimización se seleccionó uno de los productos, el mismo fue molido en un mortero de ágata, se tomaron 0,6 gramos y se compactaron en una prensa hidráulica Perkin Elmer a 75000 newtons durante 80 segundos, la instrumentación LIBS fue un láser Nd:YAG (Quantel, modelo Ultra CFR nm, ancho de pulso 7,7 ns y una energía de pulso máxima de 50 mJ a 1064 nm) unido a un espectrógrafo Echelle (Andor, modelo Mechelle ME5000,) sincronizados mediante un trigger externo para la obtención de espectros; se utilizó una metodología para la obtención de superficie de respuesta [3], donde se estudiaron 3 variables a dos niveles, dando un total de 19 experimentos (50 espectros por experimento distribuidos en 5 medias de 10 acumulaciones cada uno), y para cada experimento se calculó la deseabilidad individual y media de 20 líneas espectrales. El experimento no permitió obtener una superficie de respuesta, pero se obtuvo la mejor respuesta señal-fondo en el experimento 17 (tiempo de retraso: 1,8 µs, tiempo de lectura: 2,34 ms, energía del láser: 37,5 mJ), bajo estas condiciones se obtuvieron 125 espectros de 10 acumulaciones cada uno para el posterior análisis de las muestras mediante calibración libre.

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**ALI-P13**

# DETERMINATION OF NATURAL AND SYNTHETIC HORMONES IN EDIBLE ANIMAL TISSUE BY CONTINUOUS SOLID-PHASE EXTRACTION IN COMBINATION WITH GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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In recent years, safe food production of animal origin has become an important concern because of the presence of steroid hormones in edible animal tissue. Several studies have shown that these contaminants can potentially harm the human body through endocrine disrupting or carcinogenic effects leading to breast, ovary or prostate tumors, for example [1]. The illegal use of steroid hormones as growth promoters in animal husbandry is one specific source of their residues in animal products. As a consequence, compounds such as diethylstilbestrol, 17β-estradiol, testosterone and progesterone were banned by the European Union in 1986 (EC Directive 86/469) [2]. Safety in animal production is assured by international bodies such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the United States Federal Drug Administration (US-FDA) [3]. The other major source of hormone residues in meat and fish is contamination by environmental pollution via effluents from wastewater treatment plants and agricultural runoffs. As a result, a number of hormones have been included as priority or emerging pollutants in the European Water Framework Directive [4].

Effectively protecting consumers' health requires developing analytical methods for determining hormones at trace levels in complex matrices such as meat and fish in order to detect the illegal use of growth promoters in meat production and to help establish new regulatory policies for acceptable limits. In this work, we developed a method for isolating steroid hormones from animal products and removing their high lipid and protein contents, which can interfere with detection of the target compounds. Once protein and lipids are removed by precipitation and centrifugation, samples are enriched and cleaned up by continuous solid-phase extraction on an Oasis-HLB column. The resulting extracts are used to determine the analytes by gas chromatography–mass spectrometry with good selectivity, resolution and sensitivity, and very short chromatographic run times.

The proposed method was validated by using it to determine 13 estrogenic, progestogenic and androgenic hormones in meat and fish samples. Precision was good (relative standard deviations < 7%), and so were selectivity and sensitivity (low limits of detection, and near-quantitative analyte recoveries).

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**ALI-P14**

## EXPLORACIÓN DE LAS POSIBILIDADES DE LA FLUORESCENCIA FRONT-FACE PARA LA OBTENCIÓN DE LA HUELLA DACTILAR DE MIELES.

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En esta comunicación se presenta una serie de estudios enfocados a explorar las posibilidades de la fluorescencia molecular en modo front-face, en combinación con herramientas quimiométricas, para obtener una huella dactilar de la miel, que ayude a su caracterización. La modalidad clásica de la fluorescencia es útil al tratar con muestras diluidas y transparentes, con absorbancia menor de 0.05 [1], condiciones en las que la intensidad de la fluorescencia emitida es proporcional a la concentración de fluoróforos. Tradicionalmente, se ha recurrido a diluir las muestras con objeto de cumplir con este requisito, sin embargo al diluir las muestras, se altera el ambiente en el que los fluoróforos se encuentran de manera original en la matriz. Como solución a este problema, surge la fluorescencia en modo *front-face*, la cual permite examinar muestras intactas, concentradas, turbias o incluso sólidas [2]. La principal diferencia con la metodología clásica consiste en un cambio en el ángulo formado entre el haz de excitación y la perpendicular a la cara iluminada de la muestra. El valor de este ángulo es 0º en la técnica clásica, mientras que ronda los 30º en la modalidad *front-face*.

Se han obtenido las matrices de excitación-emisión de mieles sin tratar en los rangos 215 – 450 nm y 275 - 600 nm, para excitación y emisión, respectivamente. Una vez obtenidas dichas matrices se realizó un análisis exploratorio de las mismas mediante PARAFAC (Paralell Factor Analysis), lo que ha permitido obtener los perfiles de excitación y emisión de los principales fluoróforos presentes de manera natural en mieles. Además, el análisis detallado de los valores de score obtenidos en la descomposición por PARAFAC, ha permitido agrupar las muestras según su origen floral.

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**ALI-P15**

# DESARROLLO DE UN MÉTODO DE CROMATOGRAFÍA LÍQUIDA ALTA RESOLUCIÓN PARA LA DETERMINACIÓN DE ACRILAMIDA EN ACEITUNAS NEGRAS OXIDADAS

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La acrilamida es una sustancia química que se genera al cocinar a elevadas temperaturas alimentos ricos en carbohidratos (Pérez Nevado et al., 2018). Diversos estudios señalan que su consumo puede aumentar el riesgo de cáncer. Las aceitunas negras oxidadas al estilo Californiano son una fuente potencial de acrilamida dado que requieren altas temperaturas de esterilización para su correcta conservación (Casado et al., 2014).

Como apenas se han desarrollado métodos de análisis de acrilamida en aceitunas negras oxidadas dado que las aceitunas constituyen una matriz de difícil tratamiento, surge la necesidad de establecer un método cromatográfico para determinar la acrilamida en el menor tiempo posible, selectivo, repetitivo y sensible. Por ello, se procedió a la puesta a punto de un método cromatográfico de análisis de acrilamida acoplado a masas seleccionando las condiciones de extracción óptimas y su validación en términos de sensibilidad, límites de detección y cuantificación, repetitividad y recuperación. Para establecer la recta de calibrado por patrones externos se procedió a preparar por triplicado ocho niveles de concentración de patrones de acrilamida dentro del rango 1-40 μg/kg obteniendo un coeficiente de determinación $R^2$ de 0,9995. Para evaluar la exactitud, se prepararon patrones de concentración conocida y se obtuvo su porcentaje de recuperación de cada uno siendo el rango del 94,2-102,3%. El límite de detección fue de 0,28 μg/kg y el de cuantificación 0,92 μg/kg. Para evaluar la repetitividad se prepararon dos patrones de 5 y 20 ppm, y se midieron diez veces cada uno, siendo su coeficiente de variación de 1,40 y 1,53%. Para valorar la reproducibilidad se han analizado muestras de patrones preparados de forman independiente y en días distintos para dos niveles de concentración 5 y 20 ppm. El coeficiente de variación para 5 ppm fue 2,77% y para 20 ppm fue 1,51%. También se valoró la recuperación añadiendo diferentes cantidades de acrilamida a la misma cantidad de aceitunas por triplicado obteniendo valores de recuperación entre 80,2 y 109,8%. Por tanto, con los resultados obtenidos se dispone de un método rápido y fiable para la determinación de acrilamida en aceitunas.

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**ALI-P16**

## PREDICTION OF TOTAL POLAR COMPOUNDS IN USED SUNFLOWER OIL BY SMARTPHONE

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This study presents a mathematical model to predict total polar compounds (TPC) in sunflower oil from the color of the sample measured with a calibrated smartphone. The image treatment is a fast, cheap and green analytical procedure to determine whether fried oil is still fit for human consumption without harmful effects for health, taking into account the maximum TPC rate allowed by law in Spain. The colorimetric profile of the camera was obtained by measurements of RGB responses to a set of chips from the Munsell Atlas, covering the color region of the oil samples, and used to estimate the oil samples CIELAB color descriptors. Generalized linear models were derived to predict TPC values from lightness (L*), the red-green and blue-yellow responses (a* and b*) and chroma (C*). The models predict the concentration in samples with errors of the order of 10 % of TPC as estimated by a Testo 270 instrument, which permits the direct quantification of TPC without sample treatment.

For direct analysis, sunflower oil sample was heated alone and heated with ultrafrozen potatoes in two different fryers. One of the fryers was exclusively used for heating the oil and the other exclusively for frying. 140 g of potatoes were weighted in a balance and put inside the fryer basket, frying them during 4 min at 170°C. After this time, 5 mL of samples was taken and put inside a circular container high-density polyethylene (HDPE). Photographs were taken with a Samsung Galaxy S7 Edge (Seul, South Korea) and two Iphone 7 Plus (China). Three photographs were taken with each of the smartphones.

TPC concentration values increases with the number of cycles, reaching higher values in fried oil, although the slopes of bot curves are similar, except during the first four cycles, where the increment rate for fried oil is the largest. Four fried treatments is enough to increase the TPC from 9.5 % to 15 %. The blank oil, after 40 heating cycles, reached a TPC value of 18 %, well below the allowed limit of 25 contemplated in the Spanish legislation to be considered suitable for human consumption.

Models built for the prediction of the TPC (%) shows than for IPhone 1 was obtained a root mean square error of 1.8661, the model for Iphone 2 was obtained a root mean square error of 2.1921 and the model for Samsung S7 was obtained a root mean square error of 1.5892. Agreement between model predictions and experimental TPC values has been analyzed by Bland-Altman plot [1]. The models tend to overestimate low TPC values and underestimate high TPC values.

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**ALI-P17**

# FRESHNESS SENSOR FOR APPLICATIONS IN PACKAGED MEAT

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Here we present, a freshness colorimetric sensor that has been integrated within pork meat packages. The sensor responds to $CO_2$ concentration in the package that has been correlated with meat spoilage, as $CO_2$ levels increase with bacterial growth [1].
The chemistry is based on the acidity of the $CO_2$ molecule, a water based colorimetric sensor has been used containing the pH indicator m-cresol purple along with glycerol, hydrogencarbonate and hydroxyethyl cellulose which composition has been optimised and characterized [2]. All components are non toxic in order to obtain a food grade sensor. The membrane is prepared using just 2 µL of a water based cocktail deposited onto different materials such as nylon, nytran spc and protran BA. The sensors have been incorporated inside meat packages prior sealing. The colour of the sensor has been correlated with bacteria content present, and therefore with the state of meat. A custom made app has been developed as detection system, in this way, just taking a photograph of the sensor with a smartphone, the quality of meat can be checked (Figure 1 shows the scope of the proposed freshness sensor). All measurements have been validated using as reference method the Checkpoint Analyser and the results suggest it can provide the basis for a quick test of the quality of the packaged pork.

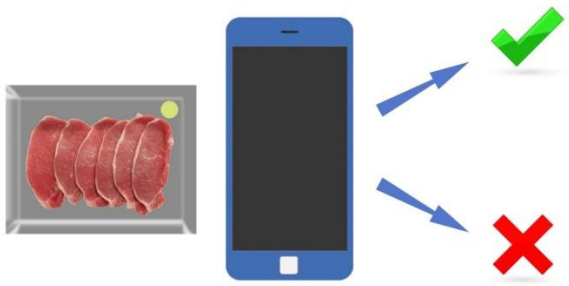

Figure 1. Designed system for freshness detection

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**ALI-P18**

# DESARROLLO DE UN MODELO CINÉTICO MULTIVARIABLE PARA LA DETERMINACIÓN DEL TIEMPO DE VIDA ÚTIL DE ACEITES VEGETALES COMESTIBLES

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Las características físico-químicas y organolépticas de los aceites vegetales comestibles no son estables, sino que están sujetas a cambios provocados por diferentes procesos de degradación, que se pueden agrupar en tres mecanismos generales: (i) degradación oxidativa; (ii) degradación térmica; y (iii) degradación hidrolítica. Todos estos procesos entran en juego desde el mismo momento de la extracción del aceite. Una vez que éste está envasado, o filtrado y almacenado en bodega, y siempre que se conserve en acondiciones aceptables (no expuesto a fuentes de calor o luz intensa), la principal alteración se debe a procesos oxidativos, principalmente de autooxidación, denominados genéricamente de enranciamiento, y que dan lugar en una primera etapa a peróxidos o hidro-peróxidos de los ácidos grasos insaturados, y posteriormente a productos de oxidación entre los que destacan los compuestos volátiles característicos de la rancidez.

Por ello, es común encontrar en el mercado aceites de oliva etiquetados bajo una categoría comercial superior a la que realmente pertenecen. Estos aceites estarían incurriendo en un fraude alimentario. Los consumidores de productos alimentarios valoran cada día más la calidad alimentaria y en consecuencia, es de interés avanzar en la información analítica suministrada a éstos, con un buen soporte científico, para la caracterización, en término de su calidad, de aceites y otros productos alimentarios de alto contenido graso.

Existen numerosas publicaciones y avances en este sentido, sin embargo, el número de parámetros que pueden ser considerados para evaluar la estabilidad o el grado de deterioro de los aceites es excesivamente amplio para que examinando la evolución de cada uno sea posible en la práctica, adquirir una idea clara de su comportamiento. Por este motivo, se presenta la posibilidad de establecer un índice global de estabilidad desarrollando un modelo cinético multivariable que permita tener una estimación del periodo de vida útil del aceite considerado.

En la comunicación se presentarán los parámetros fisicoquímicos considerados tanto para aceites vegetales como para aceites de oliva virgen extra; las descripciones del banco demuestras utilizado y las condiciones experimentales y metodología llevadas a cabo.

El estudio se enmarca dentro del proyecto de investigación 'Avances analíticos para la mejora de la información sobre calidad y seguridad de aceites vegetales comestibles y otros productos alimentarios vegetales de alto contenido graso' (RTC-2017-6170-2).

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**ALI-P19**

# ESTUDIOS ANALÍTICOS PARA LA CERTIFICACIÓN DE MATERIALES DE REFERENCIA PARA ANÁLISIS SENSORIAL DE ACEITES DE OLIVA VIRGEN EN EL MARCO DEL PROYECTO "GRUPO OPERATIVO INTERPANEL"

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Hasta la fecha, el aceite de oliva virgen es el único alimento que además de cumplir una serie de parámetros físico-químicos, requiere ser sometido a análisis sensorial para verificar su correcta clasificación en las diferentes categorías comerciales. Dicho análisis sensorial se lleva a cabo por un conjunto de catadores entrenados que conforman un panel de cata, y su metodología de aplicación es similar a la de cualquier instrumento de medida.

Sin embargo, existe una insuficiente coincidencia entre los resultados emitidos por diferentes paneles de cata respecto a la clasificación de una misma muestra de aceite de oliva. Este hecho afecta de forma negativa al sector del aceite de oliva ya que puede suponer determinadas pérdidas económicas o de prestigio comercial, y provocar cierta inseguridad jurídica de los operadores del mercado.

La existencia de discrepancias entre diferentes paneles puede tener un origen muy diverso, por ejemplo, una inadecuada aplicación del método de análisis sensorial denominado “panel test”; un entrenamiento insuficiente de los integrantes del panel; o a una falta de armonización en los criterios aplicados durante la valoración sensorial. El proyecto denominado "SISTEMA DE REFERENCIA Y ARMONIZACIÓN DE PANELES DE CATA DE ACEITE DE OLIVA VIRGEN DE ANDALUCÍA – Grupo Operativo INTERPANEL" trata de disminuir dichas discrepancias, aplicando criterios de armonización y haciendo uso de materiales que sirvan como referencias de la intensidad organoléptica de determinados atributos sensoriales.

Precisamente estos materiales deben reunir una serie de condiciones específicas que los hacen candidatos válidos para su reconocimiento como materiales de referencia certificados (MRC). Para ello, han de ser sometidos a diferentes estudios (homogeneidad, estabilidad y caracterización) y cuyos resultados le dan validez a dicho certificado.

El objetivo último de esta comunicación es dar a conocer la metodología aplicada durante los diferentes estudios analíticos utilizados en cada uno de los procesos necesarios para lograr la certificación de materiales de referencia, que serán utilizados dentro del marco del proyecto Grupo Operativo INTERPANEL para alcanzar los objetivos propuestos dentro del mismo.

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**ALI-P20**

## ANALYTICAL ASSESSMENT OF PHENOLIC COMPOUNDS FROM VIRGIN OLIVE OIL: CAN WE EXPECT EQUIVALENT RESULTS AMONG SPECIFIC AND GLOBAL METHODS?

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The link between olive oil consumption, phenolic compounds and health is so solid that the European Union approved in 2012 a specific health claim on virgin olive oils (VOOs) containing at least 5 mg of hydroxytyrosol (HTY) and derivatives (e.g. oleuropein complex and tirosol (TY)) per 20 g of oil [1]. The existing analytical methods to determine VOO phenolic compounds can be classified in two categories: specific methods, which allow the quantification of individual phenols, and non-specific or global methods, which generate a "total phenolic content" result. Comparison among the results from global methods and an artificially created total amount obtained when applying profiling approaches is questionable from a mathematical/chemical point of view. Moreover, the disparity of criteria regarding results expression and the difficulties to reliably quantify compounds lacking commercially available pure standards lead to incomplete phenolic fraction characterizations and contradictory results. This causes great confusion among VOO producers, consumers and even the legislative bodies.

Despite the general recognition of the problems with the quantitative estimation of VOO phenolic compounds, no consensus method has yet been published. The scientific community is making efforts to point at the most convenient analytical approach, but different views and methods of choice coexist so far. This project aimed to evidence the difficulties for carrying out a reliable determination of this important kind of VOO minor compounds achieving comparable results when diverse approaches are applied.

To do it so, a specific methodology (LC-MS using various reference pure standards for individual quantification [2]) and three global strategies (the Folin-Ciocalteau (FC) assay [3], the International Olive Council (IOC) HPLC method [4] and an approach based on the hydrolysis of complex phenols and subsequent detection of resulting HTY and TY by HPLC [5]) were assessed. The four methodologies were applied to the analysis of 50 VOO samples (covering all the possible ranges in terms of phenols concentration) and their outcomes were very thoroughly compared. Their equivalence (or not) were carefully evaluated, establishing possible correspondence factors and discussing the advantages and disadvantages of each considered option. The main points of reflection were identified, with special emphasis on the need of reformulating the health claim associated to hydroxytyrosol and its derivatives.

The LC-MS establishment of individual absolute concentration values (using pure standards of all the phenolic substances under study) was the optimal and most reliable situation. Total phenolic content obtained by summing up individual phenolic compounds levels was higher than the values given by the three non-specific methods; in any case, FC assay, IOC method and the hydrolysis approach (using validated and efficient protocols) could be considered as feasible strategies when a global value is pursued. Good correlations between their results were found ($R^2 > 0.89$), with the following equivalence factors: $FC_{(mg\ caffeic\ acid/kg)} \approx 0.60\ IOC_{(mg\ TY/kg)}$; $FC_{(mg\ HTY/kg)} \approx 1.04$ Sum hydrolysis $products_{(mg\ TY+HTY/kg)}$; $IOC_{(mg\ TY/kg)} \approx 1.27$ Sum hydrolysis $products_{(mg\ TY+HTY/kg)}$. The strategy based on the hydrolysis of bound forms could be considered as a reasonable compromise solution concerning the health claim on "olive oil polyphenols".

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**ALI-P21**

# OLIVE FRUIT PROCESSING METHOD ENTAILING STONE REMOVAL AND DEHYDRATION TO OBTAIN OLIVE OILS AND FLOURS WITH ENHANCED CONTENT OF BIOACTIVE SUBSTANCES: QUANTITATIVE CHARACTERIZATION OF TWO PROMISING PRODUCTS

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Virgin olive oil (VOO) is still produced using, essentially, the same principle implemented by Romans, which involves huge simultaneous waste generation (mainly olive pomace and mill wastewater). Over the last decades, the interest in looking for a cost-efficient, technically feasible and environmentally sound solution for the residues generated from olive oil industry has considerably increased. One of the innovative proposed strategies consists on performing a stone removal treatment from clean olives, followed by a dehydration process and a cold press. This procedure drives to two products: 1) an olive oil with numerous potential uses which could certainly meet the increasing demand for high-quality oils (with a very high content of bioactive compounds); and 2) a pulp pellet that can be converted into 'olive flour' by grinding and which is expected to contain high levels of fiber and bioactive compounds, fulfilling the criteria to act as a potential ingredient in functional food. Carrying out the chemical characterization of both products is essential to estimate its industrial viability and to check the advantages that the novel processing method could bring to the VOO sector.

Thus, the main objective of the present work was to accomplish the comprehensive qualitative and quantitative characterization of the metabolic profiles of the olive oils and flours obtained by means of the described processing method and to evaluate the effect of the dehydration temperature on the composition of the resulting products.

Fresh fruits from Lechin de Granada *cv.* were harvested, conditioned (washing and size-sorting) and subsequently, they were stoned and dehydrated at four different temperatures (35, 55, 75 and 100 ºC). Afterwards, dry pulp was pressed with a screw press to obtain olive oil and defatted pulp separately. Finally, the obtained oils were filtered through a paper filter to remove solid particles and the stoned, dehydrated and defatted pulp was grinded to obtain 'olive flour'. Additionally, 'conventional' VOO was obtained through an Abencor® laboratory oil mill (two-phase system), in order to facilitate the comparison with the new ones.

The prepared samples were analyzed by means a powerful LC-MS multi-class method capable of determining around 60 metabolites belonging to different chemical classes (phenolic compounds, pentacyclic trirterpenes and tocopherols) within the same run [1]. Both the flours and the new oils presented considerable amounts of olive fruit metabolites that are usually absent from VOOs. In addition, qualitative and quantitative differences were found among VOOs and oils produced using different dehydration temperatures probably due to the inhibition of some enzymes (e.g. β-glucosidase and polyphenoloxidase) caused by the temperature increase or the absence of water during the processing.

In general terms, all the evaluated chemical families were found at higher concentration levels in samples produced from fruits dehydrated at 100ºC. The oils obtained in these conditions were also richer than the conventional VOO in terms of most of the determined metabolites except for phenolic acids and aldehydes, three minor secoiridoids and the aglycone flavonoids.

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**ALI-P22**

## UHPLC-APCI-HRMS SIMULTANEOUS CAROTENOID AND CAPSAICINOID PROFILE FOR THE CHARACTERIZATION AND CLASSIFICATION OF PAPRIKA

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Paprika is a red dried and ground spice obtained from different varieties of red pepper, whose production in Europe is limited to Spain as well as certain countries of the Eastern Europe. It is characterized for its distinctive organoleptic properties that are mainly attributed to their content of bioactive substances. For instance, carotenoid natural pigments and capsaicinoids are strongly related to their color and flavor, respectively [1]. Thus, this profile could be interesting in terms of nutritional information but also it may be employed as a source of potential chemical descriptors to achieve the classification of paprika samples according to their region of production.

In this work, a UHPLC-APCI-HRMS (Q-Orbitrap) method was developed for the simultaneous quantification of carotenoids and capsaicinoids in order to characterize paprika samples. A simple and fast solid-liquid extraction with methanol:acetone 50:50 (*v/v*) was performed to extract the target compounds from the samples. The method was achieved by using an Accucore C18 column (100 mm × 2.1 mm id., 2.6 µm particle size), a quaternary mobile phase (water:methanol:acetonitrile:acetone) and working in positive full scan acquisition mode (range 50-700 *m/z*). Moreover, the obtained profile results were statistically evaluated as a food feature by chemometric techniques based in Principal Component Analysis (PCA) and Partial Least Squares regression Discriminant Analysis (PLS-DA) using the PLS_Toolbox 7.8.2 software (Eigenvector Research).

The optimized chromatographic separation of both carotenoid and capsaicinoid families was achieved in less than 13 min under gradient elution with the quaternary mobile phase. All the studied compounds could be ionized with APCI in positive ion mode generating the protonated molecule ion $[M+H]^+$ as base peak of the mass spectra, although in the case of lutein the in-source collision-induces dissociation (CID) fragment ion $[M+H-H_2O]^+$ dominated the mass spectrum. Moreover, tandem mass spectrometry was used to obtain structural information for further confirmation of these analytes in paprika samples. The performance of the proposed method showed satisfactory quality parameters providing low limits of detection (0.01 – 0.2 mg $L^{-1}$), good precision (RSD% <10%), high extraction efficiencies (>85%), low matrix effects (<15%) and accurate quantification (relative error % <10%).

More than 100 paprika samples belonging to different geographical origins (Spain, Czech Republic and Hungary) and varieties (sweet, bittersweet and hot) were analyzed by the developed UHPLC-APCI-HRMS method and quantified. A slight trend between samples was observed in the PCA scores plot according to their production region. This discrimination was improved when using PLS-DA. Besides, hot paprika varieties were clearly classified due to their capsaicinoid content as remarked in the loadings plot obtained in PLS-DA.

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**ALI-P23**

# CROMATOGRAFÍA IÓNICA: HERRAMIENTA ANALÍTICA PARA LA CARACTERIZACIÓN DE EFLORESCENCIAS EN PRODUCTOS CÁRNICOS

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En la actualidad, la calidad de los alimentos ha cobrado especial relevancia en la sociedad. Los consumidores son cada vez más exigentes a la hora de adquirir los alimentos. A pesar de los grandes avances en los procesos de elaboración y en los sistemas de control de calidad existentes en la industria alimentaria, en ocasiones los productos finales no presentan las características sensoriales que se espera de ellos. En este sentido, en los embutidos de cerdo (chorizos), se observa en la superficie del producto un velo blanco (eflorescencias). Este hecho provoca grandes pérdidas económicas en la industria cárnica, debido a que dicha modificación de la capa externa del chorizo hace que los consumidores rechacen el producto y en ocasiones sean devueltos por los comercios[1].

Las eflorescencias se presentan en forma de cristales blanquecinos y según su composición se pueden clasificar en tres tipos: hidrogeno fosfato disódico heptahidratado o dodecahidratado (reversible), lactato de magnésico y/o cálcico (irreversible), y creatina monohidrato (irreversible). La formación de las distintas eflorescencias dependerá de la temperatura de almacenaje, la velocidad de secado, el pH del producto e incluso de los ingredientes y posibles aditivos empleados[2 3].

El objetivo de este trabajo ha sido el desarrollo de un método analítico mediante cromatografía iónica con detección conductimética, para la caracterización de las eflorescencias existentes en la superficie de los chorizos[4]. El método cromatográfico desarrollado y validado ha permitido la caracterización de las eflorescencias en base a lactato y fosfato. Así mismo, se ha estudiado la difusión de dichos analitos desde el interior a la superficie del producto alimenticio. Los resultados obtenidos confirman que la difusión en el proceso de secado de los chorizos es el fenómeno implicado en la formación de este tipo de eflorescencias[5].

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**ALI-P24**

## ADVANTAGES OF USING PARAFAC WITH HPLC-DAD DATA FOR THE IDENTIFICATION AND QUANTIFICATION OF MELAMINE MIGRATED FROM MELAWARE

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Melamine (2,4,6-triamine-1,3,5-triazine) is one of the components of the melamine-formaldehyde resin, which is used in different applications like in the manufacture of kitchenware. When melaware comes into contact with food, migration of this substance to the food is highly probable. European legislation establishes maximum migration level, known as specific migration levels (SML), specifically for melamine migration from plastic materials in contact with food is fixed at 2.5 mg $kg^{-1}$ [1].

In this work, migration of melamine from melaware into simulant B (3% acetic acid (w/v) in aqueous solution) has been analysed according to regulation in force [2,3]. For this task, three bowls were exposed to three consecutive migration tests and were analysed in triplicate. The quantification and identification were carried out using PARAFAC/PARAFAC2 decomposition with the signals obtained from liquid chromatography coupled to a diode array detector (HPLC-DAD). The chromatographic conditions were the same that in ref. [4].

This three-way decomposition allows the unequivocal identification and quantification of melamine even though some interferences co-elute in the same retention time as those of melamine. To do this, data from HPLC-DAD (ultraviolet-visible spectra recorded at different elution times) were arranged in tensors with dimensions (I×J×K). The first dimension of these tensors refers to the chromatographic mode (number of scans), the second one to the spectral mode (number of wavelengths) and the third one to the sample mode (number of samples). PARAFAC2 was needed when shifts in the retention time of melamine peak appear in the chromatographic mode.

For the bowl tensor (76x151x41) was needed to build a PARAFAC2 model with two factors. The model has a corcondia consistency index equal to 100%, so trilinearity was assured, and explains 97.46% of variance. The amount of melamine migrated from the bowl was 0.34 mg $L^{-1}$ (mean of nine data for the third exposure). Pearson correlation coefficient between the pure melamine spectra and those obtained in the PARAFAC2 decomposition was 0.999. The accuracy line 'PARAFAC2 predicted concentration' versus 'true concentration' indicates that the procedure is unbiased (slope equal to 1 and intercept equal to 0 at a signification level of 5%). Decision limit (CCα) and detection capability (CCβ) were also evaluated with probabilities of false positive and false negative of 0.05, being 0.11 and 0.21 mg $L^{-1}$ respectively.

Also a migration kinetic curve was built. In this case, a different tensor (35x151x30) was used to build the PARAFAC model with two factors. The model has a corcondia consistency index equal to 100% and the explained variance was 84.52%. The equation of the kinetic curve for the bowl for sixteen cycles of 30 min was $Y = 0.299 + 0.046x$ ($R^2$=99.58%).

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**ALI-P25**

# ESTUDIO ANALÍTICO DE BIOMARCADORES DE LA TRAZABILIDAD DE LAS DOP DE ACEITES DE OLIVA VIRGEN EXTRA

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El gran valor del aceite de oliva se debe a que, aunque su producción es menor que la de otros aceites comestibles, tiene un efecto beneficioso sobre la salud que le proporciona un alto valor añadido en el caso de los vírgenes, principalmente debido a sus propiedades saludables y sus excelentes propiedades organolépticas, que son muy valoradas por los consumidores. El aceite de oliva virgen extra es también, en algunas circunstancias, un alimento de calidad diferenciada y, por tanto, protegido por la normativa de la UE que garantiza el cumplimiento de unos requisitos superiores a los exigidos para el resto de productos alimentarios. Las Denominaciones de Origen Protegidas (DOP) e Indicaciones Geográficas Protegidas (IGP) constituyen un sistema utilizado en Europa para el reconocimiento de una calidad diferenciada, consecuencia de características propias y diferenciales, debidas al medio geográfico en el que se producen las materias primas, el lugar donde se elaboran los productos, y la influencia del factor humano. El marco legal de la denominación de origen protegida (DOP) en Europa se ajusta a las expectativas de los consumidores y mejora la protección de los aceites de oliva vírgenes con características botánicas, químicas y sensoriales particulares. Sin embargo, a veces el registro de nuevas DOP asociadas a nuevas demarcaciones de áreas geográficas se basa en aspectos administrativos más que en datos químicos objetivos, siendo necesario disponer de herramientas que permitan proteger estos productos [1,2].

El objetivo de este trabajo es evaluar, desde un punto de vista analítico, la capacidad de diferentes compuestos químicos (esteroles, dialcoholes terpénicos, alcoholes lineales, alcoholes triterpénicos, 4,4 metil esteroles, hidrocarburos y compuestos volátiles), presentes en los aceites de oliva vírgenes con DOP, para ser utilizados como biomarcadores de trazabilidad, con el fin de desarrollar metodologías analíticas que permitan protegerlos. Para ello se hace necesario poner a punto metodologías que consideren diferentes aspectos que afectan a los aceites y que se ven reflejados en su composición química, así como crear una base de datos con la información necesaria.

Se han utilizado muestras de diferentes DOP españolas, así como de variedades que forman parte de ellas, aplicándose métodos de análisis espectroscópicos y cromatográficas para la determinación de los diferentes analitos y parámetros considerados, y se han aplicado procedimientos estadísticos uni- y multivariantes para la evaluación de la capacidad de los diferentes biomarcadores en la diferenciación de los aceites.

En el estudio se ha evaluado el papel de los compuestos en base a la procedencia geográfica, la variedad, el procesado, la autenticidad y la alteración de los aceites, para establecer el papel de cada uno de los biomarcadores en la caracterización y autenticidad de las DOP. Como resultado, los esteroles, alcoholes terpénicos e hidrocarburos han demostrado su utilidad para la caracterización del origen geográfico de los aceites. Los perfiles de compuestos volátiles, junto a hidrocarburos y ácidos grasos ofrecieron muy buenos resultados en la discriminación de los aceites por variedades. Por otro lado, durante el procesado que sufre el aceite de oliva virgen puede modificarse el contenido de componentes volátiles, dando lugar a compuestos responsables de determinadas notas sensoriales positivas o negativas que varían la calidad del producto final. Se ha puesto de manifiesto que la presencia o ausencia de estos compuestos volátiles es importante para la trazabilidad del aceite ya que ofrece huellas de las buenas/malas prácticas seguidas durante el procesamiento. Algunos compuestos volátiles como el 2-octenal, ácido acético, butanol, pentanal, algunos ésteres, etc. se comportan como biomarcadores del procesado y su presencia es indicativa de determinadas prácticas en el mismo. Por otro lado, los hidroperóxidos y principalmente los compuestos volátiles responsables de la rancidez (aldehidos y ácidos) han permitido establecer si la muestras habían sufrido algún tipo de proceso oxidativo que implicase una disminución de su calidad.

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**ALI-P26**

## POTENCIAL DE LAS TÉCNICAS ESPECTROSCOPICAS (UV-VIS, NIR) EN LA DIFERENCIACIÓN DE VINAGRES Y LA PREDICCIÓN DE SU BIOFUNCIONALIDAD

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Los vinagres andaluces poseen unas características organolépticas propias y singulares, debidas fundamentalmente a las materias primas utilizadas en su elaboración. Ser un producto obtenido por acetificación de un vino, le confiere unas características que lo hacen único y específico, y de gran tradición histórica en regiones vitivinícolas de Andalucía como Jerez, Huelva o Córdoba. Estos dos últimos tienen también reconocidas sus denominaciones de origen, ampliando la gama de vinagres europeos amparados: Denominación de Origen de Jerez, Módena, Condado de Huelva y Montilla-Moriles.

Con vistas a garantizar la autenticidad de estos productos, en el presente trabajo se han analizado un total de 71 muestras representativas de las tres D.O. para evaluar el potencial de las espectroscopías UV-Vis y NIR, como técnicas rápidas y sencillas para el control de calidad de los vinagres. El uso combinado de los datos espectrales con métodos quimiométricos multivariantes permite diferenciar los vinagres según su origen geográfico, así como utilizar esta información para predecir parámetros de calidad tales como su capacidad antioxidante o su contenido en compuestos fenólicos totales mediante el empleo de técnicas de regresión.

La aplicación del análisis discriminante lineal (LDA) a la información proporcionada por los espectros UV-vis ha permitido diferenciar los vinagres estudiados según su denominación de origen, siendo las longitudes de onda más discriminantes las incluidas en el intervalo entre 280 y 400 nm. Sin embargo, cuando se utilizan como descriptores los datos espectrales NIR la discriminación de los vinagres según su denominación de origen, no fue satisfactoria. Asimismo, se han construido modelos de regresión utilizando mínimos cuadrados parciales para predecir el contenido de fenoles totales y la actividad antioxidante, a partir de datos UV-vis obteniéndose en todas los casos buenos modelos con valores de coeficientes de determinación múltiple, $R^2$, superiores a 0.6. El mejor ajuste se obtiene para la actividad antioxidante. En el caso de la espectroscopía NIR se han construido modelos de regresión utilizando distintas técnicas multivariantes para predecir la acidez, el pH, el contenido de fenoles totales y la actividad antioxidante. En el caso de la regresión lineal multivariante, se obtuvieron buenos modelos con valores de coeficientes de determinación múltiple, $R^2$, superiores a 0.8, excepto en el caso de los fenoles totales.

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**ALI-P27**

# TRAZABILIDAD GEOGRÁFICA DEL GARBANZO EN BASE A SUS CARACTERÍSTICAS FÍSICO-QUÍMICAS Y PERFIL MINERAL.

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Las legumbres son conocidas como una excelente fuente de los principales nutrientes (almidón, proteínas, minerales), así como componentes que presentan efectos beneficiosos para la salud multidireccionales (por ejemplo, tocoferoles, compuestos fenólicos). En España el consumo de legumbres está en retroceso desde hace años y se sitúa alrededor de 3 kilos por persona año, y a escala mundial el consumo es bajo, debido a que tienen poco prestigio social, en gran parte porque se asocian a tiempos de escasez. Las leguminosas contienen gran aporte de proteínas promedio de 20 a 22% cuyo aminoácido limitante es la metionina, lo que hace que con los cereales cuyo limitante es la lisina exista complementariedad aminoacídica entre vegetales y mejore la calidad proteica de la dieta. En algunas regiones del mundo, las semillas de leguminosas son el único suministro de proteínas en la dieta [1]. El garbanzo es una leguminosa de grano cultivada principalmente en áreas con clima templado y semiárido. Se caracteriza por un alto contenido de proteínas, grasas, vitaminas, fibra y un contenido de carbohidratos más bajo que la harina de trigo [2], y su consumo y producción se extiende a todo el mundo, debido a que resulta un alimento barato y saludable [1].
El perfil multielemental de los productos agroalimentarios resulta muy útil en estudios de autenticación y trazabilidad geográfica de alimentos, dado que la composición mineral depende de la demanda biológica de la planta, así como de la biodisponibilidad y movilidad de los compuestos minerales del suelo [3, 4].
En este trabajo se han determinado las características físico-químicas (dureza, capacidad de absorción de agua, ratio piel/albumen, proteínas y humedad) y el perfil de elementos minerales de 40 muestras de garbanzos procedentes de diferentes localizaciones de Andalucía y Castilla. Este estudio completa los trabajos previos realizados en el grupo de investigación sobre los garbanzos de la IGP "Garbanzo de Escacena" ampliando el número y la procedencia de las muestras así como introduciendo nuevas variables relacionadas con la calidad de esta legumbre y que pueden ayudar al establecimiento de la trazabilidad geográfica.

El perfil multielemental se determinó mediante ICP-MS previa digestión ácida en sauvillex. Los datos generados por el ICP-MS junto con los parámetros físico-químicos fueron tratados mediante técnicas estadísticas multivariantes para encontrar diferencias entre las muestras según su origen geográfico. El análisis de la varianza y los métodos de análisis multivariante (ACP, LDA y PLS-DA) muestran diferencias significativas entre los grupos estudiados. Por lo tanto, cabe destacar que los compuestos minerales pueden considerarse como buenos descriptores para construir modelos de clasificación (LDA, PLS-DA) con el objetivo de diferenciar los garbanzos de Escacena del resto y encontrar marcadores de trazabilidad adecuados.

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**ALI-P28**

# ESTUDIO PARA LA DETERMINACIÓN DE ASPARAGINA Y ACRILAMIDA EN MUESTRAS DE HARINA Y PAN

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La acrilamida es un compuesto que se forma en algunos alimentos cuando son sometidos a procesos de horneado, asado o fritura, reacción de Maillard (1). Este compuesto está siendo ampliamente estudiado desde que en 1994 fue incluido por la Agencia internacional de Investigación del Cáncer (IARC) como probable cancerígeno en humanos debido a sus propiedades genotóxicas. Uno de los componentes principales a partir del cual se puede generar la acrilamida es la asparagina, aminoácido que puede encontrarse en múltiples alimentos como: harinas, patatas, frutos secos…etc (2-3). En el trabajo, se presenta un estudio para la determinación de acrilamida y asparagina en muestras de harina de trigo y pan de molde, mediante cromatografía de líquidos HPLC-UV/Vis y espectroscopia NIR. El procedimiento seguido fue el siguiente:

Las muestras se marcan con adiciones sucesivas de acrilamida y asparagina, a efectos del estudio del rendimiento en los procesos de extracción y su posterior determinación.

La extracción de los compuestos se lleva a cabo mediante: a) Baño maría, con y sin agitación a diferentes temperaturas, con dos tipos de disolventes, agua y metanol, y b) extracción soxhlet con metanol. Los mejores resultados se obtuvieron realizando la extracción con agua, procedimiento a).

Para la determinación de acrilamida y asparagina por cromatografía de líquidos (HPLC-UV/Vis) en pan y harina, se realiza un estudio previo con patrones de ambos compuestos en agua. Las condiciones de la fase móvil fueron: 60%agua/ 30%acetonitrilo/ 10%metanol. Se obtiene para la acrilamida un pico alrededor de 1,5 minutos con LOD del orden de la 0,1 ppm y otro para la asparagina a 1,2 minutos y LOD del mismo orden. El procedimiento se aplica a los extractos acuosos de las muestras.

Se realiza una pre-calibración para la determinación de acrilamida en muestras de pan mediante la tecnología NIRS, con el objetivo de estudiar la viabilidad de esta técnica que permita una rápida y sencilla determinación de este compuesto en los procesos relacionados con la industria alimentaria.

## Conclusiones

La acrilamida y la asparagina son compuestos de gran interés debido a su posible presencia en alimentos básicos. Con la técnica cromatográfica se observa una buena separación entre los picos lo que permite su identificación y cuantificación. Los resultados se relacionan con los obtenidos por espectroscopía NIR con el fin de proponer un método sencillo por esta técnica que facilite un análisis rápido y en continuo de este compuesto en muestras comercializadas.

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**ALI-P29**

# GREEN INFRARED ANALYSIS OF SYZYGIUM AROMATICUM L. IN ADULTERATED COMERCIAL SAMPLES

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*Syzygium aromaticum* L. (*Eugenia aromatica* or *Eugenia cariophylata*), commonly named as clove, is widely cultivated in tropical and sub-tropical countries. This high production lies in its essential oil, mainly characterized by the high presence of eugenol. Clove oil has been listed as "generally recognized as safe (GRAS)" by US Food and Drug Administration (FAD) and used throughout the world as flavouring agent in food, being recognized for its biological activity as antibacterial, antifungal, insecticide and antioxidant properties. Furthermore, have also several therapeutic effects, including antiphlogistic, analgestic, antivomiting, antiseptic and antispasmodic. However, nowadays its quality control and the detection of adulterants require the development of analytical methodologies that allow a quick action.

Two fast and green procedures, alternative to chromatography procedure, have been developed for the determination of eugenol in clove oil commercial samples. Methods are based on the use of transmission NIR or ATR-FTIR measurements followed by chemometric treatment. A set of 17 samples, including dried clove buds and commercial clove essential oil samples, obtained from Spanish and Argelian markets were employed. The essential oil from dried clove buds was extracted by employing hydrodistillation (HD), steam-distillation (SD) and microwave assisted extraction (MAE). For external calibrations, the effect of different clove batches and essential oil extraction proceses, and diferent comercial oils employed as diluent (sunflower, corn and olive) were studied to evaluate the influence of these variables on the PLS models. Several models were built though the evaluation of the wavenumber range, data pre-processing and selection of appropriate number of latent variables, using as external calibration mixtures of eugenol diluted with the aforementioned edible oils. From NIR spectra, a PLS model, using the first derivative (FD) followed by Standard Normal Variate (SNV) normalization and mean centering pretreatment of 8001.4 - 4462.6 $cm^{-1}$ spectral region with 3 latent variables, was selected. Model was characterized by a RMSEC and RMSECV values of 0.120 % v/v and 0.282 % v/v, respectively. The same data pretreatment was employed to develop the model from ATR-MIR spectra. In this case, model included the 1558-1481 $cm^{-1}$ spectral region using 3 latent variable, obtaining values of 0.118 % v/v and 0.191 % v/v for RMSEC and RMSECV, respectively. For both, NIR and MIR PLS models, RPD values were adequate for analytical pourposes with an excellent accuracy.

For the evaluation of the performance of PLS-NIR and PLS-ATR-FTIR methods, the accuracy studies were carried out employing four commercial clove oil samples spiked with pure clove essential oil at different levels of concentration between 2 and 3.5 % v/v. The average recovery values of clove pure essential oil spanned from 97.1% to 103.3% for PLS-NIR, and from 92.4% to 107.0% for PLS-ATR-FTIR.

Eugenol in commercial clove oils adulterated with vegetable oil was determined using the two proposed methodologies, and results obtained were comparable with values obtained by a reference GC-MS methodology. The developed methodologies have allowed the identification of eight adulterations from seventeen samples and the identification of the presence of other components in a qualitative analysis.

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**ALI-P30**

# DETERMINACIÓN DE TIRAMINA EN UNA MUESTRA REAL DE QUESO PARA LA VALIDACIÓN DE UN MÉTODO ÓPTICO ENZIMÁTICO

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La tiramina es una amina biógena que aparece en alimentos (especialmente, en diferentes tipos de queso), por la acción de ciertos microorganismos. Altos niveles de este compuesto pueden desencadenar, según la persona que los sufra, diversos problemas de salud que van desde intolerancias hasta intoxicación. Por ello, se requiere disponer de métodos rápidos y sencillos de análisis que se puedan aplicar para el control de calidad de este tipo de alimentos, incluso en sus puntos de distribución.

En este trabajo se ha desarrollado un método óptico enzimático para la determinación selectiva de tiramina, basado en el esquema de reacciones mostrado en la Figura 1 (donde TAO y HRP representan a la enzimas Tiramina Oxidasa y Peroxidasa respectivamente, y TMB al cromógeno Tetrametilbencidina). En las condiciones de reacción óptimas encontradas, el método ofrece un rango lineal que va desde $3{,}4\cdot10^{-7}$ M hasta $1{,}0\cdot10^{-5}$ M. Estudios de interferencias de otras aminas biógenas que también suelen encontrarse en este tipo de muestras (histamina, cadaverina y putrescina) demostraron que la TAO es muy selectiva hacia la tiramina, y que la presencia de ellas, en las concentraciones relativas en las que se suelen encontrar en el queso, no interfieren en la determinación.

El método se validó mediante su aplicación a una muestra real de queso, previamente analizada por el Laboratorio de Salud Pública de la DGA (acreditado por ENAC para la determinación de tiramina en muestras alimenticias). Posteriormente se empezó a estudiar la inmovilización de esta metodología en diferentes soportes sólidos, para poder llevar a cabo una determinación más rápida y visual. En una primera fase, se utilizaron tiras reactivas comercializadas para la determinación de $H_2O_2$ sobre la que se adicionó TAO observándose una respuesta cuantitativa (Figura 2). Actualmente se está estudiando la inmovilización de las enzimas y el colorante sobre diferentes tipos de plásticos con el objeto de desarrollar envases inteligentes, que sean capaces de auto-indicar la presencia de contenidos elevados de esta amina.

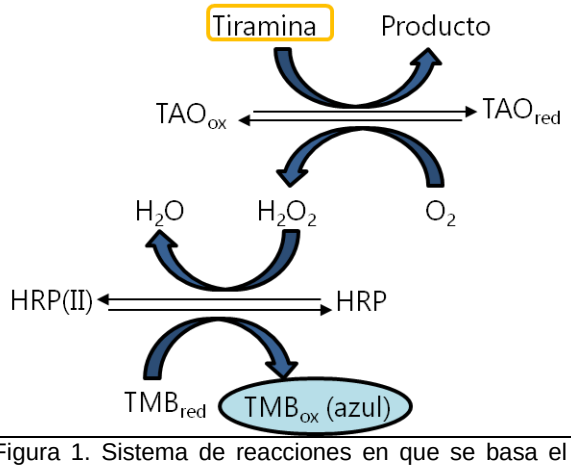


Figura 1. Sistema de reacciones en que se basa el método desarrollado.

| Tira | [Tiramina], mg/kg |
|---|---|
| 1 | 0 |
| 2 | 55 |
| 3 | 220 |
| 4 | 550 |
| 5 | 1100 |
| 6 | 2700 |

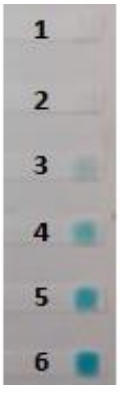


Figura 2. Rango de respuesta de la calibración en tiras reactivas y resultado visual en las mismas.

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**ALI-P31**

# NUEVAS POSIBILIDADES ANALÍTICAS DEL ABTS EN BASE A LAS PROPIEDADES DEL $ABTS^{2+\cdot}$ APLICACIÓN EN DETERMINACIONES ENZIMÁTICAS.

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El ABTS es un cromóforo ampliamente utilizado en determinaciones colorimétricas en las que está involucrada la reacción enzimática HRP/$H_2O_2$. En ellas, el ABTS (incoloro) es oxidado a ($ABTS_{ox}$), presentando una coloración azul característica y una absorción a 730 nm que permite la determinación cuantitativa de $H_2O_2$, y otros sustratos que, mediante reacciones previas lo generen. Sin embargo, esta metodología presenta dos limitaciones importantes: a) En determinadas condiciones, la especie $ABTS_{ox}$ puede sufrir una segunda oxidación $ABTS_{ox2}$ [1] que no es estable en disolución y puede interferir en la medida del $ABTS_{ox}$; b) Los aminoácidos de las proteínas son capaces de reaccionar con el $ABTS_{ox}$ reduciéndolo a su forma inicial [2], dando lugar a que la intensidad observada será menor a la esperada, lo que imposibilita la aplicación de este método colorimétrico en estas condiciones.

Sin embargo, en diferentes estudios realizados hemos observado que la presencia de una alta concentración de proteína en el medio, permite la estabilización de $ABTSox_2$, dando lugar a un compuesto de color violeta que presenta un máximo de absorción en torno a 560 nm; la evolución espectral de las diferentes especies del ABTS, es la que se muestra en la figura 1. Tomando esto como base se ha desarrollado una metodología para la determinación de $H_2O_2$ por formación de $ABTS_{ox2}$ en presencia de albúmina (la concentración óptima encontrada de proteína es de 1,5 mg/mL). Los resultados indican que no hay una gran especificidad en cuanto a la proteína, por lo que este hecho puede servir para determinaciones de $H_2O_2$ en muestras ricas en este tipo de compuestos.

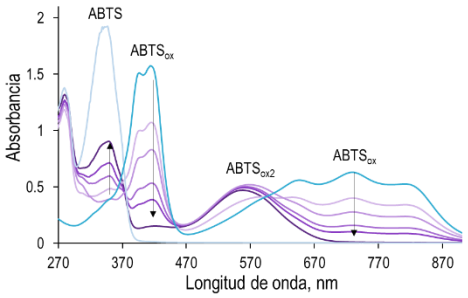


Figura 1: Evolución espectral de las especies del ABTS. [HRP] = 0,62 U/mL, [Alb] = 1,5 mg/mL, [ABTS] = $5{,}01\cdot10^{-5}$ M, [$H_2O_2$] = $5{,}98\cdot10^{-5}$ M

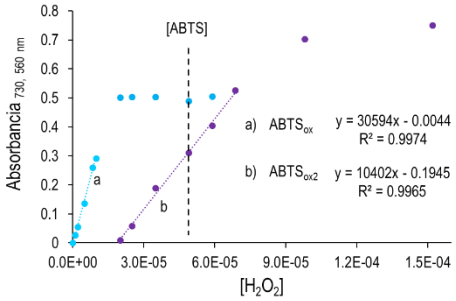


Figura 2: Rangos de respuesta lineal $Abs_{max}$ frente a la [$H_2O_2$] del $ABTS_{ox}$ (a) y del $ABTS_{ox2}$ (b).

Tras la optimización de las condiciones experimentales, se han estudiado los diferentes rangos de respuesta de las dos especies coloreadas ($ABTS_{ox}$ y $ABTS_{ox2}$), tal y como se muestran en la figura 2. Como puede observarse, una vez que el $ABTS_{ox}$ se ha formado en su totalidad, éste puede actuar ahora como reductor de la HRP, formando así el $ABTS_{ox2}$, haciendo que el rango lineal del método para la determinación de $H_2O_2$ se amplie.

Con este estudio se han podido establecer nuevas bases para el uso del ABTS como cromóforo detector de reacciones enzimáticas donde se produzca $H_2O_2$. Actualmente esta metodología se está aplicando al desarrollo de métodos para la determinación de aminas biógenas en alimentos.

Agradecimientos: Este trabajo ha sido realizado con cargo al Proyecto CTQ2016-76846R (MINECO) y las ayudas a grupos de investigación DGA-FEDER (E25_17R). Jesús Navarro agradece a la DGA por la concesión del contrato predoctoral en formación (Construyendo Europa desde Aragón).

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *DOCENCIA*

**DOC-P01**

# DESARROLLO DE UN EVALUADOR DE UN EJERCICIO INTERLABORATORIO EN DOCTUS, UNA APLICACIÓN DE AUTOAPRENDIZAJE

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En previos trabajos se ha demostrado la utilidad de Goodle, una herramienta de evaluación basada en la web, para el desarrollo de evaluadores automáticos en el ámbito de la docencia en Química Analítica [1-3]. DOCTUS es una versión completamente renovada de este sistema que expande sus funcionalidades y presenta un espacio de trabajo amigable y moderno, basado en potentes herramientas de programación. Una ventaja importante de DOCTUS, en comparación con Goodle, es que los autoevaluadores pueden ser escritos no solo en MatLab, sino también en Excel, lo que hace que la elaboración de los mismos sea mucho más sencilla, al minimizar la tarea de programación, haciendo la herramienta más accesible a un público más amplio.

En esta comunicación presentamos el desarrollo de un autoevaluador en Excel, y su implementación en DOCTUS, consistente en la emulación de un ejercicio inter-laboratorios, utilizando la determinación analítica de fosforo en un detergente. La determinación analítica de este analito se realiza en el laboratorio por grupos de estudiantes, simulando cada grupo a un "laboratorio" independiente. Se utiliza el parámetro z-score para llevar a cabo la evaluación externa de la calidad de los resultados de cada uno de los laboratorios emulados. Dicho parámetro está internacionalmente aceptado como una medida de la desviación de los resultados obtenidos por cada laboratorio con respecto al valor nominal. Asimismo, se aplican los tests de Cochran y Grubbs simple- y doble- para la detección de resultados anómalos. DOCTUS permite a los estudiantes enviar sus resultados a un servidor y recibir su calificación, así como una indicación de los posibles errores cometidos. Así, la evaluación automática permite a los estudiantes conocer inmediatamente su calificación y les fuerza a entender los procedimientos que les pueden haber llevado a una calificación negativa. El código de evaluación puede ser fácilmente modificado por el profesor en función de los objetivos en cada momento.

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**DOC-P02**

# RESULTADOS DE LA INTEGRACIÓN DE LA HERRAMIENTA DE GAMIFICACIÓN *KAHOOT!* COMO INSTRUMENTO DE EVALUACIÓN EN UNA ASIGNATURA TEÓRICA DE QUÍMICA ANALÍTICA

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La interactividad durante las clases es un complemento importante que fomenta el compromiso e implicación de los estudiantes, lo que permite mejorar la experiencia de aprendizaje. En los últimos años, la inclusión de dispositivos móviles, donde se pueden utilizar diversas herramientas de gamificación, en la metodología docente universitaria ha permitido mejorar esta interactividad. En particular, la inclusión de dispositivos móviles en el aula tiene utilidad tanto para realizar un seguimiento del método de enseñanza-aprendizaje como para aumentar la motivación y participación de los estudiantes. Una de las herramientas de gamificación que ha ganado popularidad entre los docentes por su sencillo uso y su capacidad de establecer dinámicas de trabajo activas en el aula es *Kahoot!*.

En el presente estudio se presentan los resultados obtenidos después de integrar la herramienta de gamificación *Kahoot!* como instrumento de evaluación en dos grupos diferentes de una asignatura teórica de tercer año en el Grado en Química (Química Analítica II) en el curso 2018-2019. Este estudio recoge parte de los resultados de dos proyectos de innovación docente financiados por la Universidad de Valladolid (PID 12-curso 2017-2018; PID 14-CURSO 2018-2019). Se compararon los resultados académicos de estos dos grupos de estudiantes entre sí, y estos a su vez con los obtenidos en cursos anteriores, en uno de los cuales la herramienta *Kahoot!* no fue empleada (curso 2016-2017) o fue utilizada una metodología empírica-analítica diferente en ambos grupos de estudiantes y además la participación fue voluntaria ya que no tenía peso en la calificación final (curso 2017-2018). Los resultados mostraron, en todos los casos, que el uso de *Kahoot!* había llevado a un aumento significativo en las calificaciones generales y en el número de estudiantes que aprobaron la asignatura. También se observaron diferencias en el rendimiento académico de los estudiantes según el grupo cuando se utilizó *Kahoot!*, que no fueron tan acusadas al comparar los resultados obtenidos entre los cursos donde se utilizó *Kahoot!* bien de forma voluntaria (2017-2018) o como instrumento de evaluación (2018-2019). Se puede concluir que el uso de una herramienta de gamificación (*Kahoot!*) en una asignatura teórica de Química Analítica en general mejoró el aprendizaje y las calificaciones de los estudiantes. Además, el empleo de *Kahoot!* como instrumento de evaluación, mejoró la participación y la asistencia a las clases presenciales de manera general. Por último, cabe reseñar que los beneficiarios del empleo de *Kahoot!* han sido tanto los estudiantes, ya que han mejorado su rendimiento académico en todos los aspectos (formativo y calificativo), como los profesores, debido a que la motivación, esfuerzo y participación de los estudiantes se ha visto incrementada, lo que ha repercutido positivamente en su rendimiento académico.

**Agradecimientos**

Los autores de este trabajo agradecen al Área de Formación e Innovación Docente de la Universidad de Valladolid la financiación recibida (Proyecto Nº 14).

**DOC-P03**

# APRENDIZAJE BASADO EN PROYECTOS COMO ESTRATEGIA METODOLÓGICA EN LABORATORIOS DE QUÍMICA ANALÍTICA

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El Espacio Europeo de Educación Superior (EEES), fundamentado en la Declaración de Bolonia, busca potenciar tanto la calidad como la competitividad internacional de la educación superior en Europa. Se realiza un cambio importante en el paradigma de la educación situando al estudiante en el centro del proceso de aprendizaje-enseñanza y desplazando al profesor a una posición de apoyo, *coach* o experto. De esta manera, el EEES ha provocado cambios significativos en las metodologías docentes con el fin de que el estudiante adquiera tanto las competencias específicas de su titulación como las transversales. En el caso de la titulación de Química, muchas universidades nacionales han optado por un aprendizaje experiencial, donde se busca que el estudiante aprenda a través de su propia experiencia, siempre dentro de un marco conceptual y operativo concreto y bien desarrollado. Esto es lo que se pretende con asignaturas de laboratorio, donde se establece como marco conceptual los conocimientos adquiridos previamente en una asignatura teórica. De esta manera, se busca que el estudiante adquiera competencias como la resolución de problemas y toma de decisiones, la capacidad de organización y planificación o la capacidad comunicativa y de trabajo en equipo. No obstante, en muchos casos, las asignaturas experimentales se basan en un cúmulo de experimentos, que se realizan de forma individual donde, aunque los estudiantes adquieren ciertas habilidades y se enfrentan a problemáticas analíticas, éstas muchas veces no se terminan de relacionar con un problema socio-económico real. Esta metodología carece de un nexo de unión entre las diferentes prácticas dando lugar a una pérdida del sentido global de la asignatura. Además, ello provoca que los estudiantes no terminen de realizar una evaluación más amplia de los resultados obtenidos, perdiendo así una oportunidad de favorecer su razonamiento crítico.

Entre las estrategias metodológicas existentes, el aprendizaje basado en proyectos (ABP) proporciona un contexto a las prácticas que permite a los estudiantes buscar soluciones a problemas no triviales, hecho que implica que tengan que formular preguntas, debatir ideas, realizar predicciones, diseñar y planificar experimentos, recolectar y analizar datos, extraer conclusiones y comunicar sus resultados [1]. Además, el ABP también fomenta el trabajo colaborativo entre estudiantes, lo cual puede influir muy positivamente en su éxito académico. Es por ello que esta estrategia metodológica puede tener un gran potencial, especialmente en el campo de las ciencias experimentales, ya que permite la aplicación directa de los conocimientos y las competencias trabajadas durante los estudios de grado a un caso real que puede tener trascendencia social.

Así, en este trabajo se propone el ABP como estrategia metodológica en una asignatura de laboratorio de Química Analítica. Inicialmente, se establecieron diversas temáticas a partir de las cuáles los diversos grupos de trabajo seleccionaron la problemática que querían resolver, plantearon una hipótesis y seleccionaron los experimentos y las muestras a analizar. Los resultados de estos análisis fueron compartidos mediante el uso de hojas de cálculo participativas donde los estudiantes intercambiaban la información obtenida. Finalmente, los diversos grupos de trabajo trataron los datos obtenidos, extrayendo y presentando las conclusiones obtenidas en base a los proyectos planteados. Esta estrategia metodológica nos permite afrontar tres grandes retos con los que se pretende mejorar el proceso de aprendizaje de los estudiantes: (i) otorgar un sentido más global a la asignatura, (ii) aumentar la capacidad de evaluación crítica de los estudiantes y (iii) favorecer la colaboración entre estudiantes.

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**DOC-P04**

# APRENDIZAJE BASADO EN PROYECTOS Y USO DE CUADERNOS VIRTUALES EN LA ASIGNATURA LABORATORIO AVANZADO DE QUÍMICA ANALÍTICA

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La incorporación en nuestra actividad docente de metodologías de aprendizaje activo, como el aprendizaje basado en proyectos (*project-based learning, PBL*), favorece el desarrollo de competencias transversales, generales y específicas. Asimismo, se consigue motivar al estudiante al abordar problemas reales de la vida profesional [1].

En un enfoque de aprendizaje activo, en lugar de observar o escuchar al profesor o seguir procedimientos de laboratorio similares a recetas de cocina, los estudiantes trabajan de forma colaborativa en grupos pequeños (3-5 estudiantes), en actividades de laboratorio o aula preparadas cuidadosamente. Los laboratorios basados en proyectos han demostrado ser más efectivos en la mejora del rendimiento del aprendizaje y para alcanzar resultados de aprendizaje como las habilidades para la resolución de problemas, el pensamiento crítico, la capacidad de diseñar e implementar experimentos, y de aplicar el conocimiento en situaciones reales; que no se consiguen con los experimentos de laboratorio tradicionales [1]. Además, con esta metodología los estudiantes adquieren confianza en sí mismos e independencia.

Durante los cursos 17-18 y 18-19 se ha usado la metodología PBL en la asignatura Laboratorio Avanzado de Química Analítica, optativa de cuarto curso del grado en Química (4.5 ECTS) de 45 horas presenciales, de las cuales 30 horas corresponden a prácticas de laboratorio y 15 horas a clases en aula.

Al comenzar las clases se propone a los estudiantes un reto analítico real, por ejemplo, en el curso 17-18 fue la determinación de parabenos en geles de baño y lociones corporales y en este curso 18-19 ha sido la de triclosán en jabones y pasta de dientes. De esta forma el estudiante se ha enfrentado a una situación profesional real como es el desarrollo y la validación de un método nuevo en su laboratorio.

En las sesiones de aula se aprende a buscar bibliografía sobre métodos de análisis, elegir patrones y reactivos consultando páginas web de casas comerciales, se repasan conceptos de validación (propiedades analíticas) y de desarrollo de métodos (incluyendo el diseño de experimentos usando R) y se muestran ejemplos de métodos oficiales, informes de validación y de análisis como los que ellos serán responsables de elaborar al final del curso.

Durante las sesiones de laboratorio los estudiantes trabajan en grupo, de manera autónoma y trabajando competencias generales (como trabajo en equipo, aprendizaje autónomo, organización y planificación, razonamiento crítico, toma de decisiones, análisis y síntesis, entre otras) a la vez que las específicas correspondientes al análisis químico. El papel de la profesora es de supervisora y consultora experta, dejando toda la iniciativa y el desarrollo del proyecto en manos del grupo. Los estudiantes registran el trabajo de cada sesión y la toma de decisiones en un cuaderno virtual en la nube que todos pueden consultar. Al finalizar el semestre los estudiantes entregan un protocolo normalizado de trabajo para el problema analítico que se les ha planteado con el correspondiente informe de validación y el informe del análisis de las muestras.

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**DOC-P05**

# EVALUACIÓN FEEDFORWARD DE PRÁCTICAS DE LABORATORIO PARA LA INTERIORIZACIÓN DE CONCEPTOS CON LA AYUDA DE CUESTIONARIOS EN MOODLE

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La evaluación no debería ser simplemente una herramienta para medir el grado de adquisición de conocimientos de los estudiantes. La evaluación puede (y debe) ser un medio y no un fin dentro del proceso de enseñanza-aprendizaje (E-A). Por eso, la evaluación debe estar dirigida a la adquisición de conocimientos (learning-oriented assessment). Para ello es necesario que se cumplan unas condiciones concretas según Padilla y Gil[1]:

1. Plantear tareas de evaluación que se consideren como tareas de aprendizaje
2. Proporcionar *feedforward* (retroalimentación orientada a la ejecución futura) a los alumnos para que utilicen esa información para progresar en su aprendizaje (frente al tradicional *feedback* donde solo se valora la tarea realizada)
3. Implicar a los alumnos en su propia evaluación.

Durante las prácticas de laboratorio en el grado de química, uno de los grandes problemas es la obtención de la resolución de las diferentes prácticas de laboratorio de una forma rápida y sencilla, para saber si la práctica se ha realizado correctamente. La rapidez en el *feedforward* es indispensable según Gibbs y Simpson[2] para que esa valoración sea útil. En la mayoría de los casos, los alumnos desconocen si han realizado correctamente hasta que el profesor haya corregido los informes o la memoria de la práctica. Eso dificulta la comprensión y el conocimiento de la práctica, dado que cuando han recibido el *feedback*, los alumnos se encuentran realizando otro nuevo ensayo y prestan poca (o nada) atención a lo realizado inicialmente.

Tener el *feedforward* de la práctica realizada al momento, mejora la implicación de los alumnos en la realización de las practicas ya que, si no han obtenido una valoración positiva, los alumnos tienen la oportunidad de reflexionar sobre sus errores y aprender de los mismos. Es decir, que la propia evaluación estimule a los alumnos a mejorar su tarea involucrándose en una mayor medida. Ello conlleva a una mayor reflexión, creatividad y comprensión más allá de la memorización de los contenidos[3].

Es complicado que un profesor puede estar pendiente de todos sus alumnos a la vez. Para esa labor existen herramientas muy útiles como la plataforma Moodle (eGela en la UPV/EHU), que facilita ese *feedforward* mediante la aplicación de los cuestionarios. Estos son muy útiles para valorar la adquisición de los contenidos teóricos y para la valoración de los resultados obtenidos en el laboratorio.

Estos cuestionarios se plantean como informes breves de laboratorio. Los diferentes tipos de preguntas que ofrece se ajustan a diferentes ejemplos de informe, así pues, pueden ser preguntas tipo "*multiple choice*", verdadero-falso, respuestas numéricas, etc. En el caso de estas últimas se establecen diferentes puntuaciones dependiendo del error cometido en el resultado. De esta manera, la puntuación se verá afectada en una mayor medida cuando el resultado de los alumnos se aleje del valor verdadero o no entre el en el intervalo de confianza propuesto.

Dado que parte de la nota dependerá directamente de los resultados obtenidos en el laboratorio. Los alumnos prestarán más atención durante la preparación de la práctica y eso se verá reflejado en que adquirirán los conocimientos con mayor profundidad.

Estos cuestionarios ya se han puesto en práctica durante las prácticas de tercero del grado de química en la asignatura "Experimentación en Química Analítica" y han sido valorados de manera muy positiva por los alumnos. En estas prácticas, se les da una muestra real para que determinen el contenido de un analito mediante diferentes técnicas espectrofotométricas, cromatográficas y potenciométricas. En algunos casos, la información de la concentración en la muestra es conocida mientras que en otros casos se utiliza como resultado, una media de valores obtenidos por los propios alumnos de otros grupos y/o de cursos anteriores.

La ventaja del uso de estos cuestionarios en prácticas de laboratorio es que los alumnos saben al momento si la práctica la han realizado bien antes de empezar con otra nueva y eso les da la oportunidad de poder repetirla (o parte de ella, como puede ser la revisión de los cálculos). En el caso de que repitiesen o rehicieran los cálculos, se proporciona una nueva oportunidad de hacer el cuestionario y la nota final obtenida sería la media de los cuestionarios repetidos.

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**DOC-P06**

# APRENDIZAJE EN ASIGNATURAS DEL GRADO EN CIENCIAS AMBIENTALES A TRAVÉS DE LA IMPLEMENTACIÓN DE ESTUDIOS DE CASO

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El empleo de estudios de casos para el aprendizaje de las Ciencias ha sido propuesto en varios niveles de educación. Una de las principales ventajas, es que los casos resultan adecuados para el formato de aprendizaje colaborativo/cooperativo en grupo pequeños así como en clases de discusión en grupo grande. El aprendizaje adquirido empleando estudios de caso permite una comprensión de la realidad, el desarrollo de la capacidad de análisis y síntesis, integrar conocimientos y vivencias y la toma de decisiones.

Dentro del marco de Evaluación de la contaminación en suelos y aguas (asignatura obligatoria tercer curso del Grado en Ciencias Ambientales), se cubren aspectos fundamentales y aplicados estrechamente relacionados con los diversos flujos y etapas del ciclo de agua. El ciclo integral del agua comprende desde el abastecimiento a través de las estaciones de tratamiento de aguas para su potabilización, el uso (industrial o doméstico) que hacemos de la misma, el saneamiento y depuración de las aguas residuales y su posterior vertido y la devolución a cauces de ríos o el compartimento que corresponda. La calidad del agua debe ser controlada en estos distintos pasos para preservar la misma ya que es un bien relativamente escaso. Pese a la importancia de este campo para egresados del Grado en Ciencias Ambientales por la gran diversidad de potenciales empleos que genera la industria del tratamiento, saneamiento y potabilización de agua, el conocimiento de partida en esta disciplina en el inicio de la asignatura es muy escaso.

Esta iniciativa de innovación docente implementada durante el curso 2015-2016 planteaba como objetivo incorporar dentro de la dinámica de la asignatura el estudio de caso del saneamiento del agua residual de la ciudad de Jaén. Para tal fin, se diseñaron e implementaron, dentro de las actividades académicamente dirigidas, distintas actividades (visitas, salida de campo y recolección de muestras, seminarios y prácticas de laboratorio), que haga que los alumnos participen en la distintas etapas de un estudio real desde la búsqueda de documentación y legislación, la planificación de actividades a desarrollar para abordar el problema, la ejecución de salidas de campo, el desarrollo de experimentos destinados a obtener los resultados del estudio, su interpretación y la elaboración de un informe completo sobre el estudio de caso. Esta propuesta puede estimular el aprendizaje no sólo de las competencias específicas descritas anteriormente, sino también de competencias transversales como la capacidad de trabajar en equipo y comunicarse de forma oral o escrita con precisión.

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**DOC-P07**

# MODELOS DE ORGANIZACIÓN DE LOS CONTENIDOS DE CARÁCTER EXPERIMENTAL DEL ÁREA DE QUÍMICA ANALÍTICA

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Ya han transcurrido 5 años desde la completa implantación del Grado en Química en la Universidad de Jaén (curso 2012-2013) y se dispone de experiencia sobre los resultados de las diferentes asignaturas de carácter obligatorio del área de Química Analítica. Es llamativo observar las diferentes tasas de aprobado en asignaturas del módulo obligatorio de Química Analítica. Pese a que los contenidos en principio son comunes, se observan grandes discordancias entre los resultados de las diferentes asignaturas

Dentro de los diferentes planes de estudios de los Grados en Química existentes en España, pese a que las competencias y los contenidos de carácter obligatorio son prácticamente idénticos, éstos se organizan de forma completamente diferente, bien sea a través de asignaturas principalmente teóricas con una fracción de prácticas incluidas en ellas o asignaturas específicas de laboratorio. Teniendo en cuenta los esquemas tan heterogéneos hay que plantear estrategias para ajustar la evaluación de competencias de forma más exhaustiva, como el empleo de exámenes de naturaleza completamente práctica o el uso de rúbricas que permitan una valoración más objetiva y eficaz del trabajo diario del alumno en sesiones de carácter práctico/experimental.

En este trabajo, se presenta un estudio de la organización distribución de los contenidos prácticos en diversas universidades. Se pretende examinar si realmente las asignaturas de carácter experimental intensivo, que tienen un coste asociado por crédito relativamente elevado, ofrecen o no, ventajas para el aprendizaje de los conceptos, resultando más eficaces que las asignaturas de carácter eminentemente teórico, con una pequeña fracción de prácticas o directamente sin ellas. Esto puede llevar a la posibilidad de implementación de nuevos modelos más propios de las metodologías actuales donde se potencie la faceta práctica/experimental de las titulaciones y se fomente el protagonismo del estudiante como centro del proceso enseñanza-aprendizaje..

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**DOC-P08**

# SOCRATIVE COMO HERRAMIENTA DE APRENDIZAJE Y MOTIVACIÓN: EXPERIENCIAS DE SU APLICACIÓN EN ASIGNATURAS TEÓRICAS Y PRÁCTICAS DEL ÁREA DE QUÍMICA ANALÍTICA

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Actualmente, la enseñanza de la Química Analítica y de otras disciplinas del ámbito científico se encuentran desde hace algunos años en un estado de transición, desde la implantación del Espacio Europeo de Educación Superior, en el que se han ido incorporando paulatinamente poco a poco nuevas estrategias o propuestas, a veces efectivas, destinadas a promover firmemente el aprendizaje autónomo y activo del estudiante. Además, dichas estrategias deben ir encaminadas a fomentar la falta de motivación y de interés por parte de los estudiantes causado, entre otros motivos, por el enfoque demasiado magistral y descriptivo de los contenidos teóricos de las asignaturas de esta área. Otra problemática también frecuente en los estudiantes de nuestra disciplina es la falta de preparación de la sesión de prácticas antes de acudir al laboratorio, traduciéndose en una pérdida efectiva del tiempo disponible para la experimentación, y una merma en la adquisición de conocimientos y habilidades, entre otros aspectos.

Por otro lado, en los últimos años, la incorporación de nuevas metodologías basadas en tecnologías móviles ha permitido diseñar escenarios de aprendizaje fluidos donde profesores y estudiantes puedan interactuar sin limitaciones espacio-temporales [1]. Este tipo de metodologías ha permitido la participación en tiempo real del alumnado respondiendo a las preguntas planteadas a través de algún dispositivo electrónico. Un ejemplo de ello es la aplicación *Socrative* (https://socrative.com/). Dicha plataforma posee un entorno amigable y permite medir en tiempo real y en línea el progreso de los estudiantes que hacen uso de dispositivos móviles [2].

En la presente comunicación se describe una experiencia de innovación docente realizada en asignaturas (de índole teórica y práctica) impartidas en diferentes cursos por estudiantes pertenecientes a diversas titulaciones de Grado y de Máster usando la herramienta *Socrative*. En concreto, se han diseñado pruebas (basadas mayoritariamente en cuestionarios tipo test) para ser usadas durante las sesiones teóricas, así como tests o sistema de evaluación previo de entrada al laboratorio y tras finalizar el mismo. Los resultados de dicho estudio mostraron mejoras en la adquisición de conocimientos y en la participación de los alumnos en el desarrollo de las clases teóricas, así como una mejora significativa en la preparación de los estudiantes previa al laboratorio. Asimismo, los estudiantes demostraron un elevado grado de satisfacción por este tipo de estrategias docentes, mostrándose propicios a responder las preguntas a través de sus móviles o tabletas. Así pues, los datos obtenidos apoyan la utilidad de emplear *Socrative* en la actividad docente universitaria como herramienta para la fomentar el aprendizaje activo y la motivación en asignaturas del área de Química Analítica.

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**DOC-P09**

# DESIGN OF EXPERIMENTS APPLIED IN JOB-LIKE SITUATIONS TO FULFIL EXTERNAL REQUIREMENTS IN A SUSTAINABLE WAY

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Doing experiments is probably the activity that best define what all areas of Chemistry has in common, also shared with any other experimental sciences. Consequently, knowing the methodology of experimental design [1] is at the core of the academic background every chemist, especially analytical chemist, must have.

In a problem-based learning (PBL) pedagogical strategy, students of a master´s degree in Chemistry should solve an open problem as part of an assignment in the subject 'Advanced Methods in Experimental Design and Natural Calculation' of the M*áster en Química Avanzada* that is taught at the University of Burgos. The activity carries the intention of strengthening cognitive skills in students, generating a new way of thinking, reasoning and acting. Additionally, this activity is related to the reflection about social and ethical responsibilities, particularly the concern for the environment, as well as the ability of applying knowledge into practice, which are part of the competences that the master explicitly intends to provide.

The assignment is meant to place the student mimicking a 'professional laboratory situation' with a microwave assisted Soxhlet extraction procedure that must be improved with limited resources. In particular, the efficiency of the extraction (response) depends on four factors, those detailed in Table 1 along with their variation.

Table 1. Experimental factors and experimental domain

| | Experimental factor | Allowable range | |
|---|---|---|---|
| | | Minimum | Maximum |
| 1 | Extractant | dichloromethane | *n*-hexane |
| 2 | Volume (mL) | 30 | 50 |
| 3 | Irradiation power (W) | 100 | 200 |
| 4 | Irradiation time (s) | 20 | 60 |

All the factors in Table 1 influence the efficiency of the extraction, the irradiation time possibly in a nonlinear way. Furthermore, irradiation time and irradiation power are likely to interact, while the remaining interactions do not make sense. The goal is to improve the efficiency of the extraction, by only conducting 12 experiments and reducing the quantity of chemicals used (less cost and more sustainable environment). The work will discuss the results obtained with the different approaches proposed by three students and the consequences of the choice of design.

**Proposal 1**. Dichloromethane is cheaper than *n*-hexane but it is likely to be carcinogenic in humans, so the student decides to use *n*-hexane as extractant (less toxic) but with the minimum volume (reducing both cost and quantity of extractant). Then, student 1 explores the effect of irradiation time and irradiation power by using a central composite design to account for the possible nonlinear effect, and adding three replicates at some point of the experimental domain.

**Proposal 2**. Student 2 prioritizes exploring all the factors, but only takes into account the extreme values in every factor. From the sixteen experiments of the $2^4$ full factorial, a fractional $2^{4-1}$ experimental design was done, and the remaining four experiments are replicates at some point.

**Proposal 3**. From the 24 experiments of the needed full factorial design for four factors (three at two levels and one at three levels), student 3 selects a D-optimal design with 10 experiments, then adding two replicates at some point.

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**DOC-P10**

# VÍDEOS PARA LA PRÁCTICA DE TÉCNICAS ANALÍTICAS EN UN ESCENARIO *FLIPPED TEACHING*

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Abordar, con una calidad y eficacia adecuada, el proceso de enseñanza/aprendizaje de las técnicas analíticas, particularmente las instrumentales y aquellas técnicas o procedimientos que implican tratamiento de muestra de cierta complejidad, desde un punto de vista teórico-práctico, supone un auténtico reto tanto para el estudiante como para el docente. Por un lado, la concepción descriptiva y poco práctica que adquieren los estudiantes de éstas en materias o asignaturas teóricas vinculadas a titulaciones del área de Ciencias que las contemplan en sus guías docentes, resulta poco alentadora y motivadora, limitándose los estudiantes a memorizar los contenidos aprendidos, pero no a relacionarlos con sus aspectos prácticos. Por otro lado, el alto número de alumnos y el bajo ratio instrumento/estudiante hace también más difícil asumir esta tarea. Además, de los aspectos anteriores, hay que sumarle los desajustes existentes entre teoría y experimentación en muchos de los planes de estudios actuales que conlleva a que los alumnos lleguen al laboratorio con unos mínimos conocimientos sobre los instrumentos o técnicas que van a utilizar, resultándoles difícil incluso imaginar cómo son y cómo funcionan los mismos. Así pues, todo ello obliga al docente a adoptar nuevas estrategias metodológicas y a diseñar recursos propios que sean realmente útiles para solucionar estos problemas y alcanzar así un aprendizaje efectivo y práctico.

El enfoque metodológico "clase invertida" o también denominado *Flipped-Teaching* o *Flipped classroom* consiste en invertir el modelo tradicional de docencia, donde el alumnado visualiza vídeos en casa con las explicaciones del docente, y en clase se resuelven dudas y se participa en actividades creativas/colaborativas, recibiendo el correspondiente *feedback*. Un elemento clave de esta metodología consiste en proporcionar una oportunidad para que el alumnado conozca el contenido de aprendizaje antes de que éste se trabaje en el aula o en el laboratorio [1]. En este modelo, el vídeo se ha venido utilizando como un material típico de enseñanza pre-clase [2].

En esta comunicación, se describe la elaboración de diversos vídeos educativos que cubran distintas técnicas instrumentales avanzadas (como cromatografía de gases con detección de espectrometría de masas) y de preparación de muestra (extracción en fase sólida, Kjeldahl, QuEChERS entre otras) correspondientes a diversas prácticas de laboratorios pertenecientes a asignaturas del título de Grado de Química y Máster en Técnicas Experimentales en Química de la Universidad de Valencia para aplicar la metodología de *Flipped teaching* en dichas asignaturas experimentales. Además, los materiales audiovisuales generados estarán disponibles con acceso libre en la página web de la Universitat de València, lo cual permitirá una distribución global tanto a nivel nacional como internacional, lo que aumentará considerablemente su difusión y uso. Aunque se trata de un estudio preliminar, puede afirmarse que el diseño de vídeos y su uso bajo este tipo de metodología pueden repercutir positivamente en la práctica instrumental dentro y fuera del aula.

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**DOC-P11**

# KAHHOT COMO ESTRATEGÍA METODOLÓGICA APLICADA A LA ENSEÑANZA DE QUIMICA ANALÍTICA EN FARMACIA

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Las Tecnologías de la Información y la Comunicación constituyen instrumentos de gran valor para su uso en la innovación de la metodología docente universitaria [1]. Por otra parte, se ha demostrado que el aprendizaje basado en juegos, denominado "gamificación" es un recurso con múltiple ventajas para el discente, como son la ayuda al razonamiento, aumento de motivación, mejora del aprendizaje activo, fomento de la imaginación, etc [2]. Considerando lo anteriormente expuesto, en este curso académico 2018/19 se utilizó la herramienta "Kahoot" como plataforma de aprendizaje basado en el juego y en el conocimiento, con el fin de aumentar la participación del estudiante en el aula y mejorar el proceso de enseñanza-aprendizaje. Esta plataforma, permite la realización de cuestiones tipo test a los estudiantes asistentes a una clase en tiempo real. Cuando el juego acaba se genera un archivo Excel con todos los resultados. Con el uso de esta herramienta se involucra al alumno en su aprendizaje en un ambiente relajado y agradable, y, además le permite al profesor una forma de evaluación continua.

La herramienta descrita se utilizó en la asignatura obligatoria "Química Analítica II" impartida en segundo curso del Grado en Farmacia de la Universidad Complutense de Madrid. Con el uso de esta herramienta se pretendían alcanzar 3 objetivos: Objetivo 1) Favorecer el aprendizaje activo del estudiante, Objetivo 2) realizar la evaluación continua del alumnado y Objetivo 3) mejorar el rendimiento académico del alumnado. Para comprobar el alcance de estos objetivos, se establecieron unos indicadores de calidad dentro de los objetivos descritos: Para el objetivo 1, se calculó la diferencia entre el número de aprobados entre los estudiantes que realizan los kahoots y aquellos que no participan en la actividad así como la relación entre la nota media obtenida en los kahoots y la nota obtenida al final de curso, para el objetivo 2 se calculó la nota media obtenida al final de curso en los Kahoots realizados y para el objetivo 3 se midió la relación entre la nota final de la asignatura y la realización o no de Kahoots.

El uso de la herramienta kahoot favoreció el aprendizaje activo del estudiante ya que comprobamos que el 90% de los alumnos que participaron en la actividad aprobaron, mientras que sólo aprobó el 57,9% de los que no lo hicieron. Además, hubo una relación positiva y significativa entre la nota media obtenida en los kahoots y la alcanzada a fin de curso ($B=0,396\pm0,092$; $p<0,001$). Por último, parece que es de utilidad para mejorar el rendimiento académico del alumnado, ya que se obtuvo que los alumnos que realizaron Kahoots (al menos 2 de los 3 llevados a cabo) obtuvieron una mayor nota media en la asignatura que aquellos que no participaron activamente en la actividad ($6,8\pm1,3$ vs. $5,7\pm1,9$; $p<0.05$).

Ante los resultados obtenidos parece que la plataforma Kahoot es una herramienta de utilidad para favorecer el aprendizaje activo del estudiante, realizar la evaluación continua del alumnado y mejorar el rendimiento académico del mismo.

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***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *FARMACIA y OTRAS*

**FyO-P01**

## DETERMINATION OF HYDROXYLATED INGREDIENTS WITH PRESERVATIVE ACTIVITY IN COSMETIC PRODUCTS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Some hydroxylated compounds commonly used in cosmetic formulations show antimicrobial activity, although they are not considered as preservatives according to the current European Regulation on Cosmetic Products [1]. These 'alternative preservatives' are in fact cosmetic ingredients such as emollients, solvents, etc. used for other purposes. However, their biologic activity makes also necessary their control to ensure quality and safety of the final product [2]. Some methods have been described in the literature for the analysis of some of these compounds, but not for the simultaneous determination of all of them in cosmetic samples.

In this sense, the aim of this work is to develop and validate an analytical method for the simultaneous determination of seven of these ingredients in cosmetic samples: propylene glycol, pentylene glycol, hexylene glycol, caprylyl glycol, methylpropanediol, phenylpropanol and methylbenzyl alcohol.

The research was carried out focused on finding an efficient and reliable method that could be easily applied to the quality control of cosmetic products. In this sense, a method based on gas chromatography with mass spectrometry detection (GC-MS) after ultrasound-assisted lixiviation of the analytes from the cosmetic matrix is proposed.

The optimum working conditions for the instrumental measure and the quantification of the analytes were selected and the quality parameters of the method were evaluated. The limits of detection and quantification were between 0.001 and 0.013 % (w/w) and between 0.004 and 0.041 % (w/w), respectively. Good recoveries (on the order of 80-120 %) and repeatability (1-15 % RSD) were obtained. Finally, the method was successfully applied to the analysis of eight commercial cosmetic samples and two laboratory-made samples, used to evaluate the accuracy of the method (relative errors where between 0.4 and 15.7 %).

The analytical features of the proposed method and the obtained results make the method to be in accordance with the “Green Analytical Chemistry” principles and make it to be a useful tool for controlling these alternative preservatives in the cosmetic industry in order to guarantee consumers safety.

**Acknowledgements**
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**FyO-P02**

## A GREEN ANALYTICAL METHOD FOR THE DETERMINATION OF HYDROXYETHOXYPHENYL BUTANONE IN COSMETIC PRODUCTS

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According to the current European Regulation on cosmetic products [1], 'cosmetic product' means '*any substance or mixture intended to be placed in contact with the external parts of the human body […] with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours*'. Cosmetic formulations generally include a variety of different types of ingredients, such as excipients and active principles, besides of several additives such preservatives, which are added to avoid microbial growth. The list of the currently permitted preservatives in cosmetic products, and their restrictions to be used (including their maximum concentration), are listed in the Annex V of the aforementioned European Regulation.

Since the ban of certain parabens in 2014, the cosmetic industries have been continuously looking for new compounds that can perform the preservative function effectively and safely. In fact, some cosmetic ingredients can play more than one role in a cosmetic formulation, leading to cosmetic products that are free of preservatives or self-preservatives. In this sense, hydroxyethoxyphenyl butanone (HEPB), which is normally used as skin conditioning, is being used as preservative in cosmetics in concentrations up to 2%, according to a recent opinion published by the Scientific Committee on Consumer Safety (SCCS) [2]. Nevertheless, the SCCS concluded that only a maximum concentration of 0.7% of HEPB in cosmetic products can be considered safe, as potential toxicity due to repeated exposure has been shown. Consequently, the inclusion of HEPB in the Annex V of the European Regulation, in order to restrict its maximum concentration, is expected to occur.

However, there are no methods to determine it. In this sense, a green and rapid analytical method for the determination of HEPB in cosmetic products has been developed and validated for the first time [3]. The method is based on a simple ultrasound-assisted lixiviation of the analyte from the cosmetic matrix followed by LC-UV. Under selected conditions, the method limit of detection and limit of quantification values were 30 and 90 μg $g^{-1}$, respectively. The method was validated with good recovery (86-103%) and precision (RSD 0.2-4.7%) values. Finally, the proposed analytical method was successfully applied to 7 commercially available cosmetic samples including both lipophilic and hydrophilic matrices, such as moisturizing creams, sunscreens, shampoos, liquid hand soaps, and make-ups. Additionally, a laboratory-made cosmetic cream containing the target analyte was prepared and analyzed. The good analytical figures of merit of the proposed method, in addition to its environmentally-friendly characteristics, demonstrates its usefulness to perform the quality control of cosmetic products to ensure the safety of consumers.

**Acknowledgements**
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**FyO-P03**

# DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COSMETICS BY STIR BAR SORPTIVE DISPERSIVE MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Polycyclic aromatic hydrocarbons (PAHs) are a large family of organic compounds formed by the fusion of two or more aromatic rings. They are derived principally from the incomplete combustion of organic matter and fossil fuels, such as coal, petroleum and natural gas [1], and they can be also formed at low temperatures in a slower process during the formation of oil.

In addition to its use as fossil fuel, petroleum is also used as source of raw materials for the manufacture of cosmetic products. Thus, when PAHs are produced during petroleum processing, they could be also present in cosmetics. It is well-known that PAHs are hazardous for human health due to their endocrine disrupting properties and carcinogenic effects. In consequence, the European Regulation on Cosmetic Products [2] forbids the presence of some PAHs. Hence, there is a need to develop sensitive and selective analytical methods to perform adequate quality controls of both raw materials and commercialized cosmetic products, in order to assure the safety of users. Nevertheless, there are not official methods for the identification or determination of these compounds in cosmetic products and the analytical literature regarding to the determination of PAHs in cosmetics is also insufficient.

The aim of this work is to determine the content of different PAHs in cosmetic samples. For this purpose, a microextraction technique developed by our research group, termed stir bar sorptive dispersive microextraction (SBSDME) [3], has been employed as clean-up and preconcentration technique prior to gas chromatography-mass spectrometry (GC-MS). In this case, the extraction is carried out using a neodymium stir bar magnetically coated with a magnetic composite made of $CoFe_2O_4$ magnetic nanoparticles and reduced graphene oxide as extraction device. The main parameters involved in the extraction process were evaluated and optimized, and the method was then applied to the determination of these compounds in different cosmetic samples. This work expands the analytical potential of SBSDME to the determination of compounds in other matrices, such as cosmetics.

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**FYO-P04**

## ARE COSMETIC PRODUCTS SAFE?

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Traditionally, cosmetic products are stable over one specified period of time after aperture (PAO). PAO is usually determined, according to Cosmetic Regulation (EC 1223/2009) [1]. Although, PAO should be scientifically batch to batch evaluated, in the practice it is almost impossible, since the batch is commercialized in a short time period. Currently, the application of the procedures of accelerated stabilisation for pharmaceuticals can not be applicable for cosmetics. For consumer safety it is essential to assure good quality products, this leads to the study of new procedures for stability evaluation. In this concern, the use of analytical markers plays an important role.

The stability of the cosmetic product is related with the presence of preservatives, depending on type and quantity of them. A market study has been done to determine what preservatives were the most used in eye contour care and neck and neckline creams sold in big commercial surfaces. The results have showed that sodium benzoate (SB), potassium sorbate (PS) and phenoxyethanol (PE) have a major presence.

A liquid chromatography with ultraviolet detection method previous a simple extraction procedure has been developed for the simultaneous determination of these three preservatives in cosmetic products.

The separation was successfully achieved for the three compounds using a Poroshell 120 core column (150 x 4.6 mm, 4 µm), a mobile phase containing acetic acid 0.1% and acetonitrile, at a flow rate of 1 ml/min and 45ºC of column temperature, in gradient condition. Retention times of 9.8, 10.2 and 10.5 minutes for PE, SB and PS, respectively were obtained. Chromatographic resolution greater than 1.2 was achieved for all compounds. A simple sample procedure was performed: eye contour care and neck and neckline care creams were treated with methanol in an ultrasonic bath for 30 minutes. The extract was centrifuged for 20 minutes, filtered and injected in the chromatographic system.

Analytical method has been validated following ICH guidelines (Q2A and Q2B) [2]. No interferences were observed in the sample matrix. The concentration response was linear for SB and PS, and polynomial for FE. The r coefficient was >0.98 for all the preservatives. Limit of quantifications of 1.62 (PE), 0.57 (SB) and 0.34 (PS) mg/L were obtained. Intra and inter-day accuracy and precision for the different concentration levels meet the validation criteria for all the compounds.

The described analytical method is being successfully applied to the stability evaluation of cosmetic products for eye contour care and neck and neckline creams sold in big surfaces.

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**FyO-P05**

## WEARABLE SENSOR FOR REAL TIME pH DETERMINATION IN SWEAT

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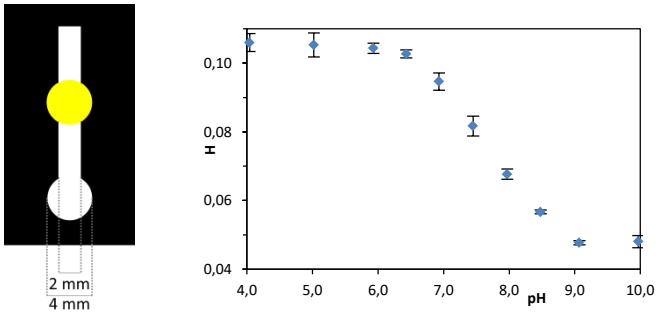
Nowadays, it is more and more common to find devices that permits to everybody carry out analysis of different analytes of interest as glucose in blood or creatinine in urine by themselves, thanks to the development of the Point-of-Care (POC) devices. POC's permit the in situ analysis of the samples, in an easy way, quickly and by the use of a small amount of sample in the sampling area of the device, obtaining result with no need of instrumentation or by the use of a very simple one. In order to match these objectives and make the device useful for everybody in any condition, the WHO has described the ASSURED guidelines for the POC devices[1].
In the recent years, and thanks to the capillary properties of different materials as paper, thread or cloth, the development of the POC devices are turning to a new strategy that implies the inclusion of the POC devices in t-shirts, bracelets or patches obtaining in this way wearables sensors. In this kind of sensor, instead of the addition of the sample in the sampling area, it moves through the device arriving to recognition/transduction area were the property of the sensor changes and can be measured and related to the concentration of the analyte.
In this work, we present a wearable POC that permits the real-time determination of the pH in sweat. For this purpose, we have developed a µCAD (Figure) that contains a pH indicator (4-[4-(2-hydroxyethanesulfonyl)-phenylazo]-2,6-dimethoxyphenol (GJM-534) [2]) covalently immobilized on cotton cloth, which color is going to change from yellow (pH around 6) to pink (pH around 9) depending on the pH. The size and shape of the µCAD (see Figure) was designed taking into account the low flow rate of sweat generated in the wrist when sweating (0.01 µL/min) including a superabsorbent material working as passive pump to avoid the saturation of sample of the µCAD.
The colorimetric device was calibrated using the H parameter from the HSV color space as analytical parameter, obtaining the calibration function and analytical parameters of the device, the reversibility of the µCAD, response time and stability.
Finally, the µCAD was integrated into a bracelet that includes a color detector and a microprocessor that registered the color of the µCAD in real-time and send the information via Bluetooth to a smartphone, obtaining and registering the pH of the sweat while doing exercise.

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**FyO-P06**

## INFLUENCE OF THE RUBBER STOPPERS MOISTURE CONTENT IN STABILITY OF FREEZE-DRIED PRODUCTS

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Freeze-drying process is a usual procedure in pharmaceutical industry for manufacturing solid parenteral products. This process consists on removing the solvent of a solution, commonly water, at low pressure and temperature to increase the shelf life of products. A high residual moisture content (RMC) in the products has adverse effects on their stability, in either chemical (e.g. hydrolysis) and/or physical changes (e.g. collapse). Therefore, RMC is controlled in the freeze-drying process development in order to achieve low RMC levels in the product. At the end of the process, the vial containing the product is closed in a dry atmosphere, using rubber stoppers that provide an adequate barrier against moisture permeation during shelf storage. The influence of this later is especially important according to the literature, due to it is one of the involved in the three potential sources of moisture available for the product during shelf storage [1].

Before a stopper is used for a parenteral product, it is washed, steam sterilized and dried. These processes can increase the moisture content of the rubber stoppers and may have a significant impact on the stability of the lyophilized product, so that it can increase its degradation over time in the presence of water. Therefore, water content in the stoppers must be quantified to understand the critical relationship between the moisture levels of the product and the resulting chemical/physical changes.

Using the coulometric Karl Fisher technique coupled to an oven (KF), an analytical method for determination of the small amounts of water contained in 13 mm diameter grey chlorobutyl rubber stoppers, with 1 leg configuration, has been developed and then validated according the ICH Q2(R1) guideline [2]. Sample presentation, oven temperature, extraction time and flux of carrier gas to transfer the moisture to the cell were the studied parameters.

The influence of the moisture on the stability of the lyophilized was studied from two groups of stoppers, one that had been subjected to the routine steam sterilization process and another that, after this process, was subjected to an extra drying at 105ºC during 48 h.

Product stability was studied under accelerated conditions (40ºC and 75 % of relative humidity) during 6 months. The moisture content of the stoppers was analyzed before the freeze-drying cycle and at 0, 3 and 6 months after lyophilization. In addition, the RMC, assay and impurities of the product were also determined in each control.

The increase of the main impurity with time is in accordance to the decrease in the assay of the product. In both cases, the influence of extra dry stoppers diminishes the effect and, although moisture content of these stoppers increases over time, it is always lower than that of a regular sterilization procedure. All the above suggests that the impurity is related to the hydrolysis of the freeze-dried product by the water released by the rubber stoppers.

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**FyO-P07**

## 3D PRINTED FLUIDIC PLATFORMS WITH COVALENTLY IN SITU POLYMERIZED ORGANIC MONOLITHS FOR AUTOMATED MICRO-SOLID PHASE EXTRACTION

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3D-printing is an incipient technology for the fabrication of novel 3D structures, which lets the building of structures that cannot be easily created by other technologies. Thus, this technology can be defined as an additive manufacturing process that creates a solid physical object from a digital design by adding material layer by layer. Within additive manufacturing techniques, fused deposition modelling (FDM) and stereolithography (SLA) have been widely used in 3D-printing. In particular, the extrusion of heated thermoplastic filaments in FDM allows to build parts layer-by-layer, whereas in SLA, an acrylate-based resin is photopolymerized on a moving support. SLA has some advantages over FDM, including higher printer resolutions, smoother surfaces and avoids the undesirable separation between the successive layers, among others. The promising use of 3D printing has found application in the analytical chemistry area, including the development of entirely new column formats and cartridge/microchip designs. However, 3D-printed structures have a low capacity to generate enough packed material with sufficient surface area in separation procedures adapted to (micro)fluidic platforms. Therefore, its combination with other materials such as porous organic polymers could be an interesting approach to obtain novel dedicated fluidic devices in analytical science.
Monolithic porous organic materials are appealing stationary phases for separation and sample preparation in analytical chemistry owing to the good permeability, easy preparation and surface modification. An interesting advantage of these polymers is that they can be *in situ* prepared within the confines of a mold, which allows their synthesis in practically any tailorable support, avoiding the undesirable use of frits as occurs in particulate materials. However, due to the small surface area of monolithic structures, their combination with other high surface-to-volume ratio materials (namely, nanomaterials, metal-organic frameworks, carbon nanostructures, etc.), offers wide opportunities for the separation or enrichment of small molecules in a wide variety of samples. Particularly, metal nanoparticles, such as gold nanoparticles (AuNPs) are attractive in separation and sample preparation on the basis of its stability, easy surface attachment and specific interactions with aromatic compounds, sulphur moieties and amine derivatives. Indeed, the combination of porous organic monoliths with high surface-to-volume ratio materials have been demonstrated to be useful in analytical chemistry [1]. However, the lack of versatile fluidic devices makes their use in online methodologies, such as (micro)solid-phase extraction (SPE), complicated, because typical configurations are based on disposable cartridges or in-tube arrangements.
The current work is focused on the development of covalently attached *in-situ* porous organic polymers in acrylate-based photopolymerized resins in 3D-SLA printed fluidic platforms. As a proof of concept application, the developed devices (including the monolithic materials) were subsequently modified with AuNPs in order to be used in automatic on-line microSPE of organic emerging contaminants (alkyl esters of 4-hydroxybenzoic acid, bisphenol A and triclosan). Besides, triclosan was determined in human saliva as a front end to liquid chromatographic separations.

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**FyO-P08**

## CASPOFUNGIN DETERMINATION BY HPLC-FLD IN CELL CULTURE MEDIA APPLIED TO *IN VITRO* PK/PD STUDIES

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During the last decades, fungal infections have risen as the first cause of human disease particularly in immunocompromised patients. The high rate of mortality related to invasive fungal infections (often more than 50%) and the increasing resistance of some species of *Candida* to antifungal drugs are a concern worldwide. Indeed, one of the main pharmacological problems nowadays is that the sensitivity pattern of *Candida* and other fungi to antifungal drugs is not well established [1-3].

The emergence of acquired resistance to the most used antifungal family (azoles) has forced specialists to begin using echinocandins as first-line therapy for invasive candidiasis in the neutropenic or immunosuppressed patients [4-6]. Although until now resistance to echinocandin-class drugs remains low in most of *Candida* species, cases of multidrug resistant strains of *Candida glabrata* have been reported [7]. Despite of the introduction of these new antifungal agents, the frequency of invasive fungal infections and resistance to antifungal therapy continues increasing [1].

Due to this fact and based on the reported therapeutic failures, in particular with caspofungin [8], research in the study of the effect of caspofungin drug in *C. glabrata* strains is needed. Considering the cost and the ethical limitation of doing studies in human patients, a two-compartment pharmacokinetic/pharmacodynamic *in vitro* model is going to be used for this aim [9, 10].

These PK/PD studies are based on the correlation of fungal growth with the concentration of antifungal drugs in the matrix. In consequence, the sensitive quantification of the amount of drugs is necessary to carry out in these studies. Being the liquid chromatography the most suitable technique for the analysis of echinocandins, the development and optimization of an on-line sample treatment liquid chromatography method for the quantitative and accurate determination of caspofungin in cell cultures has been developed and validated.

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**FyO-P09**

# STUDY OF THE VARIABILITY OF THE STIR BAR SORPTIVE EXTRACTION IN MULTIRESIDUE ANALYSIS OF POLYMER ADDITIVES BY MEANS OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND PARAFAC DECOMPOSITION

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Stir bar sorptive extraction (SBSE) is a technique of sample preparation that includes extraction and concentration of the analytes in a single step. Although it is based on the same principles as solid phase microextraction (SPME), SBSE exhibits much higher sensitivity and has been successfully applied to monitor traces or ultra-traces around parts per trillion [1]. Twister is the trade name registered by Gerstel for SBSE stir-bars coated with a polydimethylsiloxane (PDMS) layer as sorbent phase.

There are different polymer additives from food contact materials which could be found at low concentration in food. In this work, an UV stabilizer (benzophenone, BP), an antioxidant (butylated hydroxytoluene, BHT), and three plasticizers (diisobutyl phthalate, DiBP; bis(2-ethylhexyl) adipate, DEHA; and diisononyl phthalate, DiNP) are studied by means of SBSE and gas chromatography coupled to mass spectrometry with single quadrupole mass analyser. The control of these compounds is interesting because they are endocrine disruptors which adversely affect hormonal function; twisters are a suitable option to detect them.

Some of the studied compounds, as phthalates, are present in the laboratory environment which makes their analysis harder [2]. In addition, the inherent variability of the twisters make crucial to perform a study of the initial conditions of them. Prior to the first use and when the twisters are stored for some time, they are thermally conditioned. Furthermore, the twisters may be used many times after a suitable reconditioning process which consists of a clean-up thermal desorption step. In this work, the variability of the initial working conditions of the twister is assessed.

A study of the variability with twisters from different batches, conditioned and reconditioned at certain time and temperature conditions, both in the presence and absence of analytes, is performed. Statistical tools, such as ASCA (ANOVA Simultaneous Component Analysis) [3] are used to analyse the effect of the initial physico-chemical state of the twisters on the joint determination of the 6 analytes. The identification and quantification of these analytes are possible using parallel factor analysis (PARAFAC) decomposition because there are coeluents that share *m*/*z* ratios with some of the target compounds and interfere [4].

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**FyO-P10**

# PROCEDURE TO BUILD A SIGNAL TRANSFER SET WITH EXCITATION-EMISSION MATRICES BETWEEN A MASTER FLUORIMETER AND A PORTABLE FLUORIMETER BASED ON LIGHT-EMITTING DIODES

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The transfer of the excitation-emission matrix (EEM) between a portable fluorimeter based on light-emitting diodes (LEDs) and a conventional fluorescence spectrometer based on a xenon source has been reported for the first time in [1] with satisfactory results. The master fluorimeter was a PerkinElmer LS50B Luminescence Spectrometer (Waltham, MA, USA) equipped with a xenon discharge lamp. On the other hand, a portable spectrometer system (StellarNet Inc., Florida, USA) configured by an SL1-LED excitation source, a LED kit (240, 250, 265, 275, 280 and 295 nm), and a high-performance fiber optic spectrometer compact SILVER-Nova Super Range TE Cooled was used. The transfer function proposed in [2] was built with 5 EEM matrices recorded for the same samples in both instruments. Then, for each pair of wavelengths ($\lambda_{exc}$, $\lambda_{em}$), a linear regression was built for the five fluorescence intensities recorded with the master fluorimeter *versus* the ones recorded with the portable instrument. The EEM signal was transferred between both fluorimeters with these regressions.

The aim of this work is the design of a procedure to build *n* mixtures of *p* fluorophores so the *n* EEM matrices would be optimal for the signal transfer. The calibrants used for the signal transfer (coumarin 120, DL-Tyrosine and DL-Tryptophan) are chemically different from the analytes of interest of this work (enrofloxacin and flumequine) which have maximum residue limits set in the EU legislation in force [3]. The procedure is general despite it will be described for *n*=5 and *p*=3.

First, *t* criteria to optimize will be chosen for the selection of 5 ternary mixtures obtaining an application from the concentration space of the five mixtures in the space of the *t* criteria:

$$\left(crit_1, crit_2, ...crit_t\right) = f\left(m_1\left(c_{11}, c_{12}, c_{13}\right), m_2\left(c_{21}, c_{21}, c_{23}\right), ..., m_5\left(c_{t1}, c_{t2}, c_{t3}\right)\right)$$

Different mixtures are obtained when each criterion is optimized. Therefore, the analysis of the viable mixtures has been restricted to the ones obtained with a Pareto front since any other mixture will be worse in at least some of the criteria.

The final purpose of building this transfer function is to perform the fluorescence measurements outside the laboratory with the portable instrument and transfer them to the master instrument. PARAFAC has been used to perform the calibration so the quantification and unequivocal identification were possible as laid down in the regulation [4].

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**FyO-P11**

## *PARENTUCELLIA LATIFOLIA*: A POTENTIAL SOURCE OF LOGANIN IRIDOIDS

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In the present study, we compared the pharmaceutical potential (antioxidant and key enzyme inhibition of clinical relevance) of organic and aqueous extracts of *Parentucellia latifolia* (L.) Caruel subsp. *latifolia* (L.) Caruel, as well as the phytochemical composition. Phytochemical compounds were evaluated by spectrophotometric methods (for total amounts) and HPLC-ESI-DAD-$MS^n$ (for individual compounds). The extracts were screened for antioxidant abilities by *in vitro* assays. Inhibition effects were also investigated against one set of enzymes linked to major health problems. Generally, the methanol (MeOH) and aqueous extracts displayed higher scavenging abilities on radicals and reductive effects when compared with ethyl acetate (EtOAc) extract. On the other hand, the EtOAc extract was the most active inhibitor on cholinesterases (1.81-1.88 mg GALAE/g), amylase (0.70 mmol ACAE/g), glucosidase (2.85 mmol ACAE/g) and lipase (33.24 mg OE/g). The highest TPC was observed in the aqueous extract (25.07 mg GAE/g) while MeOH extract possessed the highest level of TFC (44.15 mg RE/g) and TPAC (3.46 mg CE/g). LC-$MS^n$ metabolite profiling indicated that loganin and its isomers, rutin, and luteolin-*O*-hexoside were the most abundant compounds. Our results suggest that *P. latifolia* may be a valuable source of phyto-agents for the management of noncommunicable diseases.

**FyO-P12**

## BIOMONITORING OF FIFTEEN ENDOCRINE DISRUPTING CHEMICALS IN MALE AND FEMALE HUMAN HAIR BY USING CONTINUOUS SOLID-PHASE EXTRACTION/GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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Endocrine disrupting chemicals (EDCs) comprise a group of compounds that have been extensively examined owing to their potentially harmful effects on human health. The effects of EDCs on the reproductive system have been the subject of much study in recent decades [1]. Human exposure to EDCs, which can be broadly categorized as occupational or environmental, poses a major challenge owing to the structural diversity of these chemicals, which can come from a wide variety of sources — and often at doses below the limits of detection of conventional analytical methods. Animal and *in vitro* studies support the conclusion that EDCs affect the hormone-dependent pathways effecting male and female gonadal development, either through direct interaction with hormone receptors or via epigenetic and cell-cycle regulatory modes of action. Most studies on the effects of EDCs in human populations suggest an association between exposure to these chemicals and reproductive disorders such as infertility, endometriosis, breast cancer, testicular cancer, and poor sperm quality and/or function. Despite some promising discoveries, a causal relationship between reproductive disorders and exposure to specific toxicants remains uncertain owing to the complexity of the clinical protocols used, and of the high variability in the degree of occupational or environmental exposure, measurements and subject sample size. Future studies should focus on a uniform system of examining human populations with regard to exposure to specific EDCs and to their direct effects on the reproductive system.

Bisphenol-A in biological samples can be measured and monitored by using a variety of techniques including gas chromatography–mass spectrometry, enzyme-linked immunosorbent assay, high-performance liquid chromatography with fluorescence detection, liquid chromatography–mass spectrometry and liquid chromatography tandem mass spectrometry (LC–MS/MS). LC–MS/MS is the usual choice for quantifying EDCs and the most suitable for determining alkylphenols, parabens, phenylphenols, bisphenol A, phenylphenol and triclosan with acceptable sensitivity and accuracy [2].

The usefulness of hair to monitor human exposure to environmental contaminants, and its ease of sampling in difficult subjects such as neonates, led us to develop and validate a method for assessing human exposure to alkylphenols, parabens, phenylphenols, bisphenol A, phenylphenol and triclosan by measuring their exposure biomarkers in hair samples from males and females of variable age from 2 to over 70 years. A sample preparation protocol based on solid–liquid extraction and solid-phase extraction (SPE) was developed for this purpose. The conditions for hair denaturation and washing, and solid–liquid extraction, were optimized during the method development process. The target analytes were detected and quantified by gas chromatography coupled to mass spectrometry (GC–MS). Solid-phase extraction of powdered hair provided the highest extraction efficiency and recoveries from 80 to 102%. The resulting limits of detection were in the low ng/g range and precision, as relative standard deviation, was quite good (7.5 %). The proposed method was successfully used to determine EDCs in human hair samples.

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**FyO-P13**

## PHENOLIC PROFILE, ANTIOXIDANT ACTIVITY, AND ENZYME INHIBITORY PROPERTIES OF *LIMONIUM DELICATULUM* AND *LIMONIUM QUESADENSE*

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In this work, we report the phytochemical composition and bioactive potential of methanolic and aqueous extracts of leaves from *Limonium delicatulum* (Girard) Kuntze and *Limonium quesadense* Erben. First of all, the characterization and quantitation of individual phytochemicals was performed with liquid chromatography with diode array and electrospray - tandem mass spectrometry detection. A high percentage of the characterized compounds corresponded to flavonoids, mainly myricetin glycosides.Total phenolics, flavonols, and flavonoids were assayed with conventional methods. Antioxidant and radical scavenging assays (phosphomolybdenum, DPPH, ABTS, CUPRAC, FRAP, metal chelating activity), as well as enzyme inhibitory assays (acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase, glucosidase, and lipase) were carried out to evaluate the potential bioactivity of the mentioned plant species. Methanol extracts presented higher phenolic content and bioactivity than the aqueous extracts for both *Limonium* species, although *L. quesadense* exhibited the most potent activity for most assays.

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**FyO-P14**

# ESTIMACIÓN DEL LÍMITE DE DETECCIÓN EN LA DETERMINACIÓN POR NIR Y CALIBRACIÓN MULTIVARIANTE DE LA HUMEDAD RESIDUAL EN UN FÁRMACO LIOFILIZADO

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Los procedimientos para la validación de métodos analíticos en el ámbito farmacéutico están estrictamente normalizados por los diferentes organismos reguladores. Estos intentan proponer procedimientos científicamente coherentes, aplicables de forma general a diferentes técnicas analíticas, y de implementación simple en los laboratorios industriales. Este último aspecto hace que en algunos casos las propuestas normativas no se ajusten completamente a lo que en el ámbito académico consideraríamos 'el procedimiento más correcto'. Por otra parte, los protocolos propuestos por distintos reguladores para el cálculo de determinados parámetros, como por ejemplo el límite de detección (LOD), pueden diferir significativamente.

En esta comunicación se comparan diferentes aproximaciones para la estimación del LOD en la determinación de la humedad residual (RMC) en un fármaco liofilizado mediante espectroscopia en la región del infrarrojo próximo (NIR) y calibración por regresión de mínimos cuadrados parciales (PLS). Es un ejemplo de especial complejidad dado que se trata de una calibración multivariante, de la determinación de una impureza a baja concentración, y el método de referencia (valoración Karl Fischer) tiene una precisión peor que la del método NIR.

La construcción del modelo PLS-NIR se realizó utilizando una calibración mixta (viales de lotes industriales y de laboratorio) formada por 92 muestras en el intervalo 0,15%-1,47 % RMC, y un conjunto de 25 viales para su validación. Una vez validado, se ha aplicado para la determinación de RMC en aprox. 2000 viales industriales.

Se comparan los resultados obtenidos utilizando los procedimientos para la estimación del LOD propuestos por ICH[1], Farmacopea Europea[2], Ortiz et al[3] (aproximación pseudounivariante), utilizando tanto regresión clásica por mínimos cuadrados como regresión ortogonal, y la estimación del rango de valores de LOD (min y máx) en una calibración PLS descrita por Allegrini y Olivieri[4]. Se ha estudiado también como afecta el número de muestras en el conjunto de calibración y la distancia de su centroide al origen de coordenadas. De acuerdo con los resultados obtenidos, se recomienda el uso de la ecuación propuesta por ICH y regresión ortogonal cuando el número de muestras de calibración es grande y su centroide próximo al LOD. Si este no es el caso, entonces la mejor alternativa parece ser la denominada 'aproximación pseudounivariante' utilizando, también, regresión ortogonal. Se recomienda un mínimo de 20 muestras en el conjunto de calibración.

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**FyO-P15**

## EVALUACIÓN DE LA EXPOSICIÓN DÉRMICA A CONTAMINANTES ATMOSFÉRICOS: BENCENO, TOLUENO, ETILBENCENO Y XILENOS

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Se consideran contaminantes dañinos o perjudiciales del aire aquellos que causan un efecto adverso sobre la salud o el medioambiente. La Agencia de Protección Medioambiental de los Estados Unidos (US EPA) ha creado una lista con más de 150 contaminantes, que incluye sustancias que causan cáncer y generan efectos adversos neurológicos, respiratorios y reproductivos. Uno de los contaminantes más extendido en el aire es el benceno, y aunque las fuentes de estos contaminantes son generalmente locales, están omnipresentes. El benceno es un compuesto orgánico que se encuentra en el aire como resultado de emisiones originadas en la combustión de carbón y petróleo, evaporación de combustible en gasolineras, emisiones de los vehículos, humo de cigarrillos, incendios… [1]. Se clasifica como cancerígeno basado en estudios que demuestran el aumento de la incidencia de ciertos tipos de leucemia en adultos expuestos durante su jornada laboral al benceno [2]. El benceno se suele determinar de forma conjunta con otros compuestos similares como el tolueno, los isómeros del xileno y el etilbenceno, conocidos como BTEX, que pueden ser peligrosos para la salud humana.

El objetivo de este estudio se centra en evaluar la exposición dérmica a diferentes contaminantes atmosféricos, empleando el BTEX como ejemplo representativo. Para ello, se ha desarrollado una cámara de simulación que permite generar una atmósfera con una concentración conocida y constante de contaminantes atmosféricos, como son los BTEX, para realizar estudios de permeabilidad/exposición dérmica en su interior. La cámara de simulación consiste en una campana cerrada de laboratorio, modelo HZ08252 de Bruker, formada por una base metálica y paredes y techo de polimetilmetacrilato, con un volumen interno de 640 L, en la que se introduce un flujo constante de aire limpio y seco, que evapora en un conector tipo T, un flujo de disolución de patrones de BTEX proporcionado por una bomba isocrática de un instrumento de cromatografía líquida (LC) HP 1050 de la marca Hewlett-Packard (Palo Alto, Estados Unidos). La concentración de los VOCs en el interior de la cámara de simulación se ajusta mediante el control de la concentración y flujo de la disolución de BTEX y el flujo de aire seco.

Los estudios de permeabilidad/exposición dérmica se realizan mediante una celda similar a la celda de Franz, que consta de un compartimento donde se encuentra la disolución receptora (agua al 0,9% NaCl), y en cuya superficie se sitúa una membrana simulante de piel (silicona con espesores de 0.5-3 mm o Strat-TM) de manera que ésta queda totalmente en contacto con la disolución receptora por una cara, y expuesta a los contaminantes por la otra durante diferentes tiempos de exposición.

La concentración de BTEX en la disolución receptora se determina mediante un procedimiento basado en cromatografía de gases – espectrometría de masas empleando un inyector de espacio de cabeza (HS-GC-MS). Con los resultados obtenidos en estos ensayos de migración/exposición se ha calculado el flujo molecular (flujo de analito que pasa por unidad de tiempo a través de una superficie de área unidad perpendicular a la dirección del gradiente de concentración), y el tiempo de retardo (tiempo que el analito tarda en difundir a través de la membrana) de los BTEX a través de las diferentes membranas.

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**FyO-P16**

# PERFIL DE AGREGACIÓN MEDIANTE CROMATOGRAFÍA DE EXCLUSIÓN MOLECULAR –(SE)HPLC-DAD– DE LA PROTEÍNA DE FUSIÓN AFLIBERCEPT

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Las proteínas de fusión son una clase emergente de biofármacos, empleadas en enfermedades de gran prevalencia. Se sintetizan a partir de la traducción de dos o más genes independientes que se unen entre sí de manera artificial, combinando funciones o actividades de interés. Aflibercept (AFL) es una proteína de fusión recombinante de aproximadamente 96.9 kDa de parte proteica, con alrededor del 15% de glicosilación, confiriéndole a la molécula un tamaño global de 115 kDa aprox. Contiene fusionados el dominio 2 de VEGFR1 (receptor transmembrana de crecimiento endotelial 1) y el dominio 3 de VEGFR 2 (receptor transmembrana de crecimiento endotelial 2); estos dos dominios están fusionados a su vez a la región constante de una inmunoglobulina IgG1. AFL es el principio activo de los medicamentos Eylea® y Zaltrap®, el primero empleado para el tratamiento de la degeneración macular en la retina del ojo y el segundo para el tratamiento del cáncer de colon metastático.

La formación de agregados en los medicamentos biotecnológicos es un atributo crítico de la calidad. En disolución, la exposición de los residuos de aminoácidos hidrofóbicos con el medio acuoso provoca la interacción entre entidades de proteína y puede provocar agregación. La estructura dinámica de las proteínas hace que tengan una tendencia natural a formar agregados naturales; sin embargo, existen numerosos factores ambientales a los que el biofármaco puede estar sometido durante toda su vida útil que pueden originar procesos de agregación no naturales. Esto representa graves problemas de seguridad debido a la respuesta inmune grave que pueden producir; por lo tanto, la agregación debe ser controlada desde el desarrollo de la proteína terapéutica hasta su administración al paciente.

La técnica analítica por excelencia que se usa para estudiar el perfil de agregados de pequeño orden de proteína es la cromatografía liquida de exclusión molecular (SEC). Esta técnica presenta la gran ventaja de ser muy reproducible en la separación y detección de monómeros y dímeros, siendo por tanto ideal para la detección del inicio del proceso de agregación. Además, dadas las características de las fases móviles empleadas, el análisis se realiza en el estado natural conformacional de la proteína. El empleo de detectores de diodos en fila, facilita el análisis comparado de los espectros de UV de la proteína y de sus estados de agregación. En este trabajo se presenta un estudio del perfil de agregación (SE)HPLC-DAD característico del medicamento Zaltrap® (AFL). El método cromatográfico empleado hace uso de una fase móvil compuesta por tampón fosfato a pH 7 y la separación se lleva a cabo en una columna específica de separación por tamaños de anticuerpos monoclonales. Además del perfil cromatográfico característico de agregación, el método se validó mediante estudios de degradación acelerada que sirvieron también para obtener información del comportamiento de esta proteína de fusión cuando se somete a factores ambientales tales como aumento de temperatura y ciclos de congelación descongelación.

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**FyO-P17**

# IDENTIFICACIÓN DE PRÁCTICAS PASTORILES EN LA CUEVA DE *EL MIRADOR* (SIERRA DE ATAPUERCA, BURGOS) MEDIANTE EL ANÁLISIS DE ESTEROLES, FITOESTEROLES, ÁCIDOS BILIARES Y HORMONAS

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La caracterización de residuos/sedimentos orgánicos prehistóricos es una nueva vía de investigación que, mediante la identificación de su origen, naturaleza y composición, pretende aportar datos científicos sobre diversos aspectos relacionados con la domesticación animal, las paleodietas, la identificación de contenidos de recipientes arqueológicos, o la habitabilidad de asentamientos [1].

Este trabajo se centra en el estudio de sedimentos orgánicos, denominados *fumiers*, provenientes de excavaciones realizadas en rediles localizados en la cueva de *El Mirador* (Sierra de Atapuerca, Burgos, España). El uso de la cueva como redil se ha documentado durante el Neolítico (entre 7300 y 5300 años cal BP) y la Edad del Bronce (entre 3700 y 3000 años cal BP). Estos sedimentos son acumulaciones derivadas de actividades antrópicas, generadas por la práctica periódica de quemar los excrementos de los rumiantes estabulados en la cueva. La quema en montón de estos restos genera capas o facies de diferente coloración, dependiendo del grado de combustión o de la ausencia de ésta (en general, total/ blanca o gris, parcial/negra y marrón/ no combustionada). Estas capas, sobre todo las negras y las marrones, poseen una buena conservación, lo que permite la caracterización de compuestos orgánicos que nos pueden ayudar a identificar especies de animales estabulados y costumbres pastoriles [2].

Para determinar el origen animal de los residuos/sedimentos y las prácticas pastoriles se ha desarrollado un método para el análisis conjunto de ácidos biliares, esteroles, fitoesteroles y hormonas mediante LC-MS.

La extracción de los analitos se ha realizado asistida por microondas con 25 mL de una mezcla de DCM:MeOH (2:1, v/v). Tras un proceso de centrifugado, secado y saponificación del extracto, se realiza una segunda etapa de extracción líquido-líquido para su posterior análisis mediante LC-MS.

Los resultados obtenidos para la recuperación, límites de detección y efecto matriz, han sido satisfactorios en todos los casos. Este método ha sido aplicado a 33 muestras de dos de los sectores (100 y 200) de la cueva de El Mirador.

Para determinar el tipo de animal estabulado se han utilizado relaciones entre los esteroles y fitoesteroeles y los ácidos biliares [3]. Mientras que la relaciones entre esteroles y fitoesteroles en algunos casos son contradictorias, en el caso de los ácidos biliares, en todos los casos la identificación de los restos corresponde a rumiantes.

Para identificar prácticas pastoriles, se ha utilizado la relación entre hormonas y ácidos biliares. En este caso se ha observado un mayor valor en capas del sector 200 lo que podría estar relacionado con la separación de hembras embarazadas, recién paridas y sus crías del resto del rebaño. Esta última hipótesis debe ser contrastada con los resultados de otros estudios realizados en los mismos niveles, principalmente con los del análisis taxonómico de los restos óseos y con los de la micromorfología de suelos, actualmente en curso.

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**FyO-P18**

# SIMULTANEOUS DETERMINATION OF Ca, K, Mg, Fe, Cu, Zn, Se, Mn and Mo BY ED-XRF IN PHARMACEUTICAL PRODUCTS

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A direct method for the mineral profile determination in pharmaceutical products by energy dispersive X-ray fluorescence (ED-XRF) was developed. For the validation of the data obtained by this technique, these results were compared with a perfectly verified technique such as inductively coupled plasma optical emission spectrometry (ICP-OES).

For the direct measurement of the samples by ED-XRF, a 13 mm of diameter and 2-3 mm of thickness pellets were made corresponding to 0.8 g of sample. For the determination of mineral profile by ICP-OES a previously microwave assisted acid digestion was need.

The internal calibrations of the ED-XRF (GeoChem and Low Density) can not quantify correctly the mineral profile in any type of pharmaceutical products. For this reason, external calibrations were carried out. Inorganic salts were employed to perform the external calibrations, knowing the exact concentration of each analyte in all the cases. For calibration equations, the counts per second obtained by ED-XRF were represented in front of the concentration of the element in the pellets. The concentrations for all samples obtained by external calibration were compared with data by reference method ICP-OES. Also it was compared the concentration indicated in the label package with the concentrations obtained by ICP-OES.

Good calibrations were obtained for Ca, K, Fe, Mg, Cu, Zn, Se, Mn and Mo with $R^2$ values of 0.993, 0.998, 0.990, 0.990, 0.993, 0.98, 0.9998, 0.996 and 0.96, respectively. Concentration values calculated with the external calibrations offer accurate results obtaining a determination coefficient of 0.80 in front of reference method. Concentrations obtained by reference method agreed with the concentrations indicated in the label package of the pharmaceutical products with a determination coefficient of 0.992.

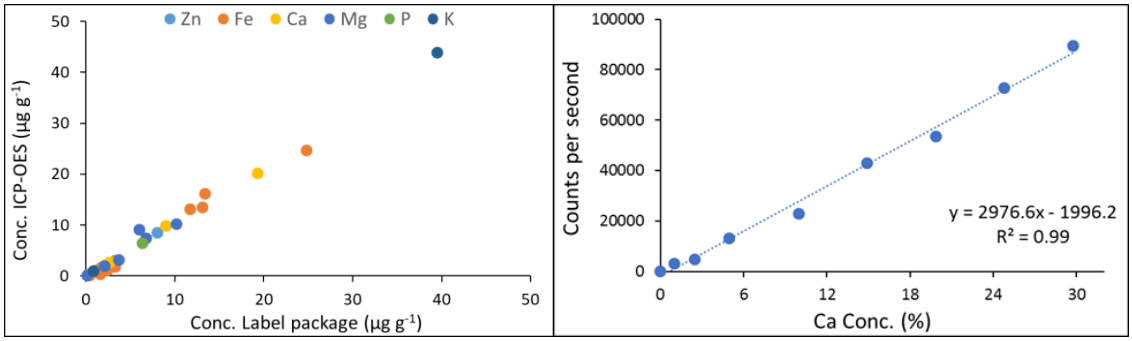


Figure 1. Comparison between concentration obtained by ICP-OES and the values indicated in the label package (in the left) and calibration performed for Ca employing ED-XRF signal in front of the concentration (in the right).

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**FyO-P19**

## COMPARISON OF TRANSMISSION AND REFLECTANCE NIR PROCEDURES FOR THE DETERMINATION OF ACENOCOUMAROL IN LOW DOSE SINTROM TABLETS

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Narrow therapeutic index (NTI) drugs contain a small dose range where the drug produces a beneficial effect without causing severe or potentially fatal difficulties. Oral anticoagulants are widely known for their NTI. Sintrom ® (API, acenocoumarol) is the most used anticoagulant in Spain. This drug requires regular and frequent blood control due to its tight therapeutic window, the high variability on individual response and its multiple interactions with other drugs, even food. The accurate determination of the dose taken by the patient is mandatory.

Nowadays, NIR spectroscopy can be considered as a well-established analytical technique in the pharmaceutical world. Usual applications includes API determination and uniformity of dosages control of solid preparations. Until now, the standard procedure for acquiring NIR spectra has been in reflectance mode. However, under these conditions, only a few mm of the tablet is actually being sampled. As a consequence, the use of transmission NIR spectroscopy has been proposed as a safer alternative, since the whole tablet is being measured.

This communication has two main objectives: to develop a NIR procedure to quantify the API content in a low dose medicament (Sintrom 1, 1 mg/tablet) and to perform a critical comparison of the results obtained using NIR reflectance and transmission modes.

Partial Least Square (PLS) calibration models were developed and validated using tablets from six different production batches and ninety laboratory-made samples Different spectral pretreatments and spectral ranges were assayed. The calibration sample set encompasses an API concentration range of 70-130% of the nominal concentration; the effect of compaction pressure was considered by preparing calibration samples compacted at three different pressures: 120, 140 or 160 MPa. All samples were also analyzed using a reference HPLC procedure.

In both acquisition modes, the NIR results obtained showed no significant differences with HPLC values. However, the model obtained using reflectance mode produced small differences in API concentration as a function of the tablet face irradiated, while transmission results were not affected by this fact. Also, the precision in the transmission mode was slightly better than in reflectance.

**FyO-P20**

# DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PARACETAMOL, ACETYLSALICYLIC ACID AND CAFFEINE IN ANALGESICS

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Methods used in pharmaceutical analysis must be validated to demonstrate that they are sufficiently accurate, specific, sensitive and precise to conform to the regulatory requirements of the pharmaceutical industry, thus ensuring safety and quality of pharmaceutical products. Validation is a formal and systematic way to assess that the analytical procedure is suitable for its intended purpose, produces reliable and accurate results and also fulfils the requirements of regulatory bodies [1].

Acetylsalicylic acid (AAS) and paracetamol (PCM) are used as analgesic and antipyretic agents. They may appear associated with caffeine (CFN) in many pharmaceutical formulations. These compounds can be simultaneously determined in tablets and other formulations using RP-HPLC with UV/visible detection [2]. Measurements were made with an Agilent Technologies series 1200 HPLC equipped with a RP C18 column and a variable wavelength UV/visible detector.

Prior to method validation, the chromatographic conditions were optimized using a Taguchi experimental design to identify the most influencing factors and their optimal levels. Six factors at three levels (composition of methanol, acetonitrile and 0.01 M phosphate buffer in the mobile phase, pH, flow rate and detection wavelength) and one factor at two levels (column temperature) were assigned to the columns of a $L_{18}(2^1 \times 3^7)$ orthogonal array. Saccharose was used as a noise factor to simulate the effect of the excipient in order to increase robustness of the analytical method. ANOVA of the peak areas for the three compounds indicated that the most influencing factor was wavelength, but the composition and pH of the mobile phase was the most influencing factors on peak resolution. Saccharose didn't affect either the peak areas or the retention times. The optimized HPLC conditions used in further experiments were: temperature, 25 ºC; composition of the mobile phase MeOH:AcN:Phosphate buffer, 30:5:25; pH, 2.7; flow rate, 1 $mL \cdot min^{-1}$), wavelength, 230, 246 and 273 nm for AAS, PCM y CFN respectively.

The validation parameters to be estimated, according to ICH guidelines, include accuracy, precision, specificity and limit of detection, limit of quantitation, linearity, range and robustness [1]. Limits of detection ranged from 0.3 to 0.4 $mg \cdot L^{-1}$. Linear range extended up to 80 $mg \cdot L^{-1}$. Repeatabilities and reproducibilities were better than 2% and 10%, respectively. Trueness was evaluated for AAS and PCM through participation in a proficiency test; method bias was lower than 5% for the three analytes. The measurement uncertainty was evaluated from the validation results combined with the quantification of other uncertainty sources by the GUM approach [3].

The optimized RP-HPLC method is simple, rapid, sensitive and accurate and therefore suitable for the routine analysis of acetylsalicylic acid, paracetamol and caffeine in analgesic formulations. It was applied to the determination of these drugs in commercial formulations sold in Spain.

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**FyO-P21**

## ANÁLISIS DE CANNABINOIDES SINTÉTICOS POR ESPECTROSCOPÍA INFRARROJA CON REFLECTANCIA TOTAL ATENUADA MEDIANTE PELÍCULA SECA

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La Nuevas Substancias Psicoactivas (NPS), o nuevas drogas de diseño, han sido definidas por la Oficina sobre Drogas y Crimen de las Naciones Unidas (UNODC) como sustancias de abuso, tanto de forma pura como en preparados, que no están sujetas al control internacional, pero que pueden suponer una amenaza para la salud pública [1]. Los cannabinoides sintéticos son NPS capaces de interaccionar con los receptores cannabinoides CB1 o CB2.

Las mezclas herbales que incluyen cannabinoides sintéticos aparecieron en el mercado bajo la denominación “Spice”, y pueden ser adquiridos en Internet o en tiendas especializadas. Aunque se anuncian como una mezcla de hierbas sin aditivos sintéticos y no aptos para el consumo humano, cuando se fuman presentan efectos similares a los producidos por el cannabis. A finales del año 2008, se notificaron al Centro de Monitorización Europeo de Drogas y Adicción (EMCDDA) los primeros casos de presencia de cannabinoides sintéticos en mezclas herbales tipo “Spice” [2].

Teniendo en cuenta los datos del Sistema de alerta temprana de la Unión Europea, el pasado año 2016-2017, se reportaron más de 32.000 incautaciones de cannabinoides sintéticos, con un peso de 1.4 toneladas [3]. El aumento del tráfico y consumo de este tipo de sustancias destaca la importancia del desarrollo de métodos rápidos de *screening* para su identificación preliminar. Las metodologías propuestas en la literatura científica para la determinación de cannabinoides sintéticos en mezclas herbales incluyen la cromatografía de gases y de líquidos (ambas técnicas con detección mediante espectrometría de masas) y el análisis directo en tiempo real con un espectrómetro de masas (DART-MS) entre otros.

La espectroscopia infrarroja con reflectancia total atenuada (IR-ATR) es una de las técnicas recomendadas por la UNODC para la identificación de cannabinoides sintéticos en mezclas herbales [4]. En este estudio se plantea la posibilidad de emplear esta técnica como método rápido de screening, con un mínimo tratamiento de muestra, para la identificación de cannabinoides sintéticos en muestras de preparados herbales y desarrollar una metodología apropiada para la cuantificación de los cannabinoides identificados.

El procedimiento propuesto implica pesar 25 mg de muestra y extraer los cannabinoides de la superficie de las hojas con 500 µL de cloroformo, agitando la mezcla de forma manual durante 1 minuto. A continuación se extrae el sobrenadante y se depositan 5 µL del extracto sobre el cristal ATR, procediendo a la evaporación del disolvente con una corriente de aire tangencial. La identificación de los cannabinoides sintéticos se realiza mediante la comparación del espectro obtenido con los la librería obtenida de la página web SWGdrug que incluye 590 sustancias. Una vez identificado/s, se procede a cuantificar el/los analito/s mediante interpolación del área de pico característico en la recta de calibrado correspondiente, obtenida a partir de los espectros de las disoluciones de patrones de cannabinoides sintéticos depositados en el cristal ATR y medidos de igual forma que las muestras.

Empleando el procedimiento desarrollado se identificaron y cuantificaron cannabinoides sintéticos (UR-144, UR-144 + 5F-AKB48, JWH-019 + JWH-203) en muestras incautadas, con concentraciones entre 4 y 53 % p/p, proporcionando resultados comparables con el método de referencia empleado basado en cromatografía de gases – espectrometría de masas.

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**FyO-P22**

## ESTADO NUTRICIONAL DE COBRE EN UNA SERIE DE PACIENTES CON ENFERMEDADES CRÓNICAS.

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**Introducción**: El cobre es un micronutriente esencial en los seres humanos y tanto su exceso como su deficiencia producen severas consecuencias patológicas [1].
**Objetivo**: Investigar el estado nutricional de cobre en una serie de pacientes infanto-juveniles con enfermedades crónica de diferente etiología.
**Material y métodos:** Se realizó un estudio transversal en cien pacientes entre 1 a 31 años de edad, con enfermedades crónicas de diferente etiología; remitidos consecutivamente para su valoración nutricional a la Unidad de Nutrición Pediátrica del Hospital Clínico Universitario de Valladolid. Los pacientes se clasificaron de acuerdo al sexo, grupos de edad por Tanner, diagnóstico de patología de base y estado de nutrición por índice de masa corporal (IMC: kg/cm$^2$). Se evaluó el perfil bioquímico por métodos estándar y el nivel de cobre (CSC: µg/dL) por AAS. Los puntos de corte empleados para diagnosticar hipo e hipercupremia fueron de <70 µg/dL y >150 µg/dL, respectivamente. El nivel significativo fue $p<0.05$. Para el análisis estadístico se usó el programa SPSS/PC (IBM Corp., Armonk, NY, USA).
**Resultados:** La media IMC (-0.19±2 kg/cm$^2$) fue normal, el 31% tenía desnutrición y el 22% obesidad. La media CSC fue de 117.1±28.3 µg/dL (IC95% 111.4-122.7 µg/dL), rango de 20-194 µg/dL. Los niños tuvieron una media CSC significativamente mayor (127.8 ± 31 µg/dL) que los adolescentes (104.6 ± 19.7 µg/dL) ($p=0.000$). En cambio no hubo diferencias significativas (ANOVA, $p = 0.891$) por grupo de enfermedad crónica. El 13% presentó hipocupremia y el 11% hipercupremia. El grupo con enfermedad renal presento el mayor número de casos con hipocupremia, y el de enfermedades varias y sindrómicas el de hipercupremia. Hubo una correlación inversa y significativamente con la edad ($r=-0.29$, $p=0.003$). El riesgo de presentar hipocupremia en nuestra serie fue 6 veces mayor en los pacientes desnutridos [OR=6.6 (95% CI 1.9-23.7), $p=0.003$] que sin desnutrición. En cambio, el riesgo de presentar hipercupremia fue 12 veces mayor en los pacientes menores de 10 años [OR=12.8 (95% CI 11.6-104.5), que en el resto de la serie, y 4 veces mayor en los pacientes con déficit de prealbúmina [OR=4.87 (95% CI 1.2-20.3), $p=0.041$] que entre los pacientes con prealbúmina normal.
**Discusión**: El cobre es un importante micronutriente y numerosas enzimas son cobre dependientes. Sus funciones se encuentran relacionadas principalmente con la formación de tejido conectivo, metabolismo del hierro, desarrollo del sistema nervioso central y función cardiovascular (metabolismo del colesterol) [1]. Aunque la deficiencia de cobre clínicamente evidente es relativamente infrecuente en humanos, las manifestaciones clínicas más comunes son anemia, neutropenia y anormalidades óseas que incluyen fracturas [2]. Si bien existen pocos estudios sobre niveles de cobre, en concordancia con la literatura el riesgo de hipocupremia fue mayor en los pacientes desnutridos [1-3]. La toxicidad crónica de cobre no ocurre normalmente en humanos debido al sistema de transporte que regula su absorción y excreción [3]. Sin embargo, llama poderosamente la atención que el grupo de niños menores de 10 años y aquellos con déficit de prealbúmina (un marcador de desnutrición) presentaran mayor riesgo de hipercupremia.
**Conclusiones**: En nuestra serie, el nivel sérico de cobre se relacionó inversamente a la edad, y el mayor riesgo de presentar niveles anormales de cobre con el estado de desnutrición. Se sugiere que el nivel sérico de cobre debería evaluarse en pacientes con enfermedad crónica en riesgo de desnutrición.
**Palabras clave**: cobre sérico, enfermedad crónica, hipocupremia, hipercupremia.

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*
## *Valladolid 18-19 julio 2019*

## *COMUNICACIONES PÓSTER*

## *MEDIOAMBIENTE*

**MAB-P01**

## SOLID-PHASE EXTRACTION UPLC-MS/MS METHOD DEVELOPMENT FOR THE MONITORING OF ANTIBIOTICS IN THE POCTEFA RIVER WATERS

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Contamination of natural waters with antibiotics has become a subject of global concern, because of the potential dangerous consequences to environmental and human health. Indeed, the excessive and inappropriate use of antibiotics on humans and animals and their subsequent release into the environment results in emergence, dissemination, and persistence of antibiotic resistance that threatens their efficiency. As a result, the identification, characterization and monitoring of these compounds in the environment are crucial to assess their dynamics and potential toxicological effects on ecosystems.

The POCTEFA territory covering the Spanish Communities of Aragon, Catalonia, Basque Country and Navarre, and the French regions of New Aquitaine and Occitania is characterized by intense animal farming; livestock farms are its main rural economic engine at both sides of the border. The growing concern about potential contamination of this area with veterinary antibiotics stimulated a wide range study funded by OUTBIOTICS Project* involving 40 sampling points covering the Ebro, Cantabrian and Adour-Garonne basins.

The analytical method has been developed to control a wide range of antibiotics at the levels encountered in the environmental water samples. The procedure consists of 10-fold solid-phase extraction preconcentration of antibiotics followed by their separation by reversed-phase Ultra Performance Liquid Chromatography (UPLC) and tandem MS detection. The presentation reports quanitative results obtained for a number of target compounds chosen on the basis of their use in the animal farming in the POCTEFA region. The most abundant species found were fluoroquinolones present in 70% of sampling points studied. The detection limits achieved for most of the species are in the range of 5-100 ng/l.

Moreover, the use of the QExactive hybrid quadrupole-Orbitrap mass spectrometer combining high-performance quadrupole precursor selection with high-resolution, accurate-mass (HR/AM) Orbitrap™ detection allowed the exploratory analysis providing data on other contaminants and their metabolites.

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**MAB-P02**

## DETERMINATION OF BENZOTRIAZOLE ULTRAVIOLET STABILIZERS IN SEAWEED SAMPLES

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Emerging compounds employed in pharmaceuticals, detergents or personal care products (PCPs) are reaching the coastal environments through the effluents from wastewaters treatment plants. Some compounds like benzotriazole ultraviolet stabilizers (BUVSs) added in sunscreens, cosmetics or dyes can also enter directly in marine ecosystems via bathing or aquatic sports.

Because European Union considers a priority the sustainable use of our oceans to maintain and consolidate Europe as the world's leading tourist destination [1] it is necessary to know and control the presence of these pollutants in the environment.

BUVSs have been produced since the 1980s [2] and used in a wide variety of products like paints, plastics, detergents or contact lenses. However they are also currently used in many PCPs like sunscreen lotions, soaps, shampoos, toothpastes, make-up, etc.

Besides persistent and toxic in plants [3] and animals [4], BUVSs could be bioaccumulative, so it is essential to study their presence in the environment. Thereby, marine organisms like seaweeds have been used as bioindicators of contamination in marine environment since they could accumulate pollutants in their tissues.

We have developed a novel method based on microwave assisted extraction (MAE) and ultra-high-performance liquid chromatography with diode array (UHPLC-DAD) and mass spectrometry confirmation (UHPLC–MS/MS) to determine six BUVSs (UV P, UV 326, UV 327, UV 328, UV 329 and UV 360) in seaweeds.

The optimised method provided recoveries from 69.5 to 92.3%, with intra-day and inter-day precision values lower than 10% for the most of compounds. The obtained limits of detection were in the ranges 1.79-4.58 and 0.89-1.76 $ng \cdot g^{-1}$ dry weight (dw) for UHPLC-DAD and UHPLC-MS/MS, respectively.

Then it was applied for the analysis of twelve seaweed species of Las Canteras beach (Gran Canaria, Spain), sampled during four months. One of the target compounds, UV 360, was measured in five species belonged to Ochrophyta and Rhodophyta Phylums in concentrations between 42.54 and 114.7 $ng \cdot g^{-1}$ (dw), the highest concentrations being found in the rhodophyte *Asparagopsis taxiformis*. These results suggest that seaweeds could be suitable as bioindicators of the presence of these compounds in the environment.

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**MAB_P03**

# DETERMINATION OF STEROID HORMONES IN MILK SAMPLES USING NOVEL FABRIC PHASE SORPTIVE EXTRACTION (FPSE)

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Milk is a fundamental food in a balance diet which is consumed daily. Moreover, dairy products are consumed from early stages of life. For these reasons, it is absolutely necessary to ensure the quality of this products. In fact, nowadays exist a large regulation about milk and dairy products quality both in the origin of these products and during the product processing. Nevertheless, livestock is exposed to many micropollutants as pesticides, veterinary drugs, hormones or even plasticizers. These compounds could be absorbed by animals and finally be in dairy products, producing a decrease in the product quality [1].

The extraction of these micropollutants from biological samples as milk is a real challenge because this type of samples presents a lot of interferences as lipids and proteins which produce a significant loss in the extraction efficiency. For these reasons it is usually common the application of several pretreatment steps to prepare the sample before extraction procedures. However, these steps produce the increase of use of organic solvents as well as the overall analysis times.

Fabric phase sorptive extraction (FPSE) is a novel microextraction technique for liquid samples developed by Kabir and Furton in 2014 [2] which integrates the advantages of sol–gel derived. The proposed FPSE method has several advantages such as minimum use of organic solvents, short extraction times and high preconcentration factors [3].

In this work three different fabric media has been tested to extract 15 steroid hormones from raw milk samples. The fabric media tested has been sol-gel silica carbowax 20M, poly(ethylene glycol) 300 and poly(tetrahydrofuran) 250. For the three fabric media, all the variables involved in the extraction process were studied following a $2^4$ experimental design. The variables studied were sample volume, extraction and desorption times and desorption volume. The experimental design performed showed a good extraction efficiency for steroid hormones with high $\log K_{OW}$ using sol-gel silica carbowax 20M media. To determine the different hormones, ultra-performance liquid chromatography tandem mass spectrometry was used. The developed methodology presents a great selectivity, repeatability and sensitivity.

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**MAB-P04**

## MONITORING OF PHARMACEUTICAL RESIDUES IN WASTEWATER FROM URBAN AND RURAL AREAS OF GRAN CANARIA ISLAND (SPAIN)

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The presence of pharmaceutical residues in the environment is a concerning issue for the scientific community because the concentrations of this type of compounds have been continuously increasing in the last decades. In fact, some of these compounds have been included in some legislations as the Watch List of the Water Framework Directive of Water of the European Commission because of the harmful effects of these compounds to aquatic ecosystems [1].

Wastewaters have revealed as the main sources of pharmaceutical residues because many times the wastewater treatment plants (WWTPs) are not designed to eliminate these compounds. The elimination of pharmaceutical residues is a real challenge because these compounds present a wide range of physicochemical properties and the treatment processes are not fully effective for all types of pharmaceuticals [2].

Moreover, in rural and isolated areas, this problem is most severe because very often it is not possible to build traditional water treatment facilities. To overcome this important drawback, natural WWTPs have revealed as a great solution because they are low-energy consumption facilities which use the slope of the ground and plants to purify wastewaters [3]. This means that the environmental impact of human development in rural and small communities could be managed and controlled.

In this work, 11 pharmaceutical compounds have been analyzed in a traditional WWTP which treats the wastewater of a high-density populated area of Gran Canaria and in a natural WWTPs which treats wastewater from a small village of Gran Canaria island (Spain) and consists in a different types of constructed wetland arranged in series. Samples from different treatments of both WWTPs were taken throughout the process in order to evaluate the efficiency of the purifying treatments for the elimination of pharmaceutical residues. It was observed that the concentrations were lower as the purification treatments progressed. In fact, 10 of 11 compounds were detected in the inlet wastewater at concentrations up to 100 $\mu g \cdot L^{-1}$, while the concentrations at the wastewater effluent were in the range of low $\mu g \cdot L^{-1}$.

This work is one of the activities of Project ADAPTaRES co-funded by the European Union program INTERREG MAC, by means of the European Regional Development Fund. The project has 16 partners from the Canary Islands, Madeira, and Cape Verde and its main objective is to promote adaptation to climate change, prevention and guarantee of resistance to specific risks such as drought through the promotion of efficient water use and reuse in Macaronesia.

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**MAB-P05**

# DEGRADATION OF CIPROFLOXACIN IN WATER USING VISIBLE LIGHT AND DIFFERENT HYBRID NANOMATERIALS AS PHOTOCATALYSTS AND STUDY OF DEGRADATION BY-PRODUCTS

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In recent decades, drugs have become a group of emerging pollutants for the aquatic environment. This has become an important issue because its possible potential effects are unknown both for the human being (development of antibiotic resistance) and for the aquatic environment. This problem has been accentuated by the waste produced by the pharmaceutical industries and by the excess of drugs consumed by modern society.

In order to solve this problem, new technologies for the treatment of water that allow their reuse have become very important. These include heterogeneous photocatalysis, which uses catalysts such as $TiO_2$ and UV light (4% of solar radiation) for the degradation of drugs up to $CO_2$ and $H_2O$ [1]. However, in recent years the research is focused on the use of visible light present in solar radiation. For this purpose, broad-spectrum catalysts, such as graphitic carbon nitride (g-$C_3N_4$), or plasmonic photocatalysis are used. The plasmonic photocatalysis leverages the absorption of visible radiation by gold or silver nanoparticles supported on nanostructured semiconductor or insulating materials, allowing an improvement in the photocatalytic activity of the nanohybrid materials.

In this communication, the results obtained in the degradations of one of the most consumed antibiotics, ciprofloxacin, are presented. In the degradations, low power LED visible light was used (less energetic than UV light, which means an energy saving) and catalysts containing $TiO_2$ doped with g-$C_3N_4$ and others derived from the formers with different percentages of gold and silver (bimetallic nanoparticles) were synthesized and characterized. In addition, the possible mechanism of degradation was studied thanks to the use of scavengers that capture the radicals responsible for the degradation in the reaction medium.

Moreover, the identification of degradation by-products is an important issue because these compounds may be more toxic than ciprofloxacin. Ultra-performance liquid chromatography coupled to high resolution mass spectrometry is used for the first time to propose tentative identification of ciprofloxacin degradation by-product based on exact mass and $MS^2$ fragment data. The mass accuracy provided by the quadrupole-time of flight mass spectrometer used has allowed to refute the identification proposed in literature for some of the by-products.

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**MAB-P06**

## DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF TECHNOLOGY CRITICAL ELEMENTS IN SEAWEED SAMPLES BY ICP-MS

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Technology Critical Elements (TCEs) are increasing their use in the last years due to their industrial applications; therefore, they might cause environmental pollution problems. Seaweed are a source of essential minerals but, they can also concentrate and bioaccumulate these non-essential elements from the surrounding seawater. Recently, seaweed are being very used in the pharmaceutical industry, in cosmetics and in animal and human nutrition. The interest in the analysis of the elemental composition of edible seaweed has also increased in recent years, due to the rise in their consumption by the western population. As a consequence, it is necessary to develop simple and fast methods for TCEs determination in seaweed to monitor the levels of pollution and ensure food security.

The objective of this study is the development of a method for TCEs determination in edible seaweed after microwave acid digestion with $HNO_3$ (69% v/v) and $H_2O_2$ (33% v/v). The digested samples were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The measurements were performed in KED mode (collision cell with He) and applying mathematical corrections to minimize the possible polyatomic interferences.

The calibration was carried out using the standard addition method in a concentration range from 0 to 100 µg $L^{-1}$ for TCEs determination. A Certificate Reference Material (BCR®-670, Aquatic Plant) was used for the selection of the analytical isotopes and the flow of helium in the collision gas cell of ICP-MS. The analytical characteristics of the method (limits of detection and quantification, reproducibility, analytical recovery, and accuracy) were evaluated. A good agreement was obtained between the experimental values and the certificated values for the elements present in the CRM (La, Pr, Nd, Eu, Sm, Tb, Gd, Dy, Ho, Er, Yb, Lu, Sc, Y).

Finally, the method was applied to TCEs determination in several types of seaweed samples from the Galician coast commercialized in Spain.

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**MB-P07**

# DETERMINACIÓN DE ELEMENTOS CRÍTICOS TECNOLÓGICOS EN PESCADOS Y MOLUSCOS MEDIANTE ICP-MS

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La concentración de elementos críticos tecnológicos (TCEs) en el medio ambiente ha aumentado en los últimos años debido a su gran número de aplicaciones industriales (nuevas tecnologías), medicina etc. La presencia de estos elementos en el medio marino hace que se acumule en los organismos acuáticos, pasando por los distintos eslabones de la cadena trófica hasta llegar al ser humano a través de su alimentación. Los pescados y moluscos son organismos marinos importantes desde el punto de vista alimenticio, pero también pueden ser utilizados como bioindicadores de la contaminación.

El objetivo de este estudio es el desarrollo de un método analítico para la determinación de elementos críticos tecnológicos (TCEs), elementos del grupo del platino (PGEs) y elementos de las tierras raras (REEs) en muestras de pescados y moluscos. La preparación de la muestra se realizó mediante digestión ácida asistida por microondas utilizando ácido nítrico (69% v/v) y $H_2O_2$.(33% v/v). El contenido de los elementos fue determinado en los digeridos mediante espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS), trabajando en modo colisión utilizando He y ecuaciones matemáticas para la corrección de las interferencias. La selección de los diferentes isótopos empleados para la determinación de cada elemento, así como el flujo de He se realizó utilizando un material de referencia de tejido de mejillón (BCR-668). La calibración fue realizada utilizando el método de adiciones estándar en un intervalo de concentraciones entre 0 y 100 µg $L^{-1}$. Los límites de detección obtenidos se encuentran en un rango de concentraciones de 0.03 ng $g^{-1}$ (Lu) y 2.21 ng $g^{-1}$ (Te). El método propuesto fue aplicado para la determinación de estos elementos en muestras de pescados y moluscos.

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**MAB-P08**

# DESARROLLO Y OPTIMIZACIÓN DE UNA METODOLOGÍA DE ANÁLISIS DE LOS 15 COMPUESTOS INCLUIDOS EN LA LISTA DE OBSERVACIÓN DE LA DECISIÓN 2018/840/EU

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El desarrollo de la legislación y las normativas con respecto a la presencia de contaminantes en el agua en Europa ha sido constante en los últimos años. La Unión Europea publicó en el año 2000 la Directiva 2000/60/EC para establecer un marco de acción comunitaria de regulación de la calidad del agua. A partir de esta regulación, diversas directivas europeas han legislado sobre cuales son los contaminantes cuyos valores (Normas de Calidad Ambiental, NCA) no pueden ser sobrepasados en las masas de aguas europeas para asegurar la calidad de las mismas. La última Directiva fue publicada en 2013 (Directiva 2013/39/EU) y en ella se incluyen un total de 45 compuestos considerados contaminantes prioritarios. En estas directivas se propone además, la creación de una lista de observación de una serie de compuestos no regulados, que pudieran tener un posible efecto medioambiental, de manera que una monitorización de dichos compuestos se hiciera necesaria para ulteriores consideraciones de legislación sobre ellos.

En base a ello, en 2015 se publica la primera lista de observación (Decisión 2015/495), en la que se incluye un total de 17 compuestos a monitorizar en las aguas superficiales. Tras recabar datos de investigaciones realizadas en los estados miembros de la Unión Europea, la comisión de medio ambiente modificó dicha lista, retirando 5 compuestos de los inicialmente propuestos e incluyendo otros 3 (Decisión 2018/840). Entre los compuestos incluidos en esta última lista de observación se encuentran hormonas (17α-etinilestradiol (EE2), 17β-estradiol (E2) y estrona (E1)); antibióticos (azitromicina, eritromicina, claritromicina, amoxicilina y ciprofloxacino) y pesticidas (metiocarb, imidacloprid, tiacloprid, tiametoxam, clotianidina, acetamiprid y metaflumizona) [1].

Aunque existen algunos métodos analíticos que permiten la determinación de los compuestos de la Decisión 2015/495/EU, aun no se han desarrollado métodos que permitan la determinación fiable y simultanea de los 15 compuestos incluidos en la Decisión 2018/840. En este trabajo se propone por primera vez un método analítico que permite su determinación simultanea en aguas superficiales. El método se basa en la extracción de los contaminantes mediante extracción en fase sólida y la posterior separación y determinación empleando cromatografía líquida con detector de espectrometría de masas de triple cuadrupolo. Se realizó la optimización de las etapas de extracción, separación cromatográfica y determinación mediante espectrometría de masas. Una vez optimizada, la metodología se validó demostrando su aplicabilidad en muestras de agua con unas óptimas propiedades analíticas principales (por ejemplo, unos límites de detección iguales o inferiores a los dictados por la Decisión 2018/840/EU y con unos valores de precisión y exactitud cercanos a los obtenidos en anteriores publicaciones [2]) y complementarias (por ejemplo, reducido tiempo de análisis).

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**MAB-P09**

# DETERMINACIÓN MULTIRESIDUO DE DISRUPTORES ENDOCRINOS EN LODO DIGERIDO, COMPOST Y SUELO MEDIANTE UAE-dSPE y LC-MS/MS

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Los compuestos perfluorados, tensioactivos, plastificantes, conservantes, biocidas y filtros UV son algunos de los contaminantes emergentes que mayor preocupación generan por su actividad como disruptores endocrinos. Estos compuestos se encuentran presentes en productos de cuidado personal y en productos químicos de uso doméstico o industrial etc. lo que hace que lleguen de manera continuada a las estaciones depuradoras de aguas residuales urbanas donde pueden quedar retenidos en los lodos de depuradora. Dichos lodos, una vez tratados por digestión o compostaje, pueden ser aplicados como fertilizantes en suelos agrícolas de acuerdo a la directiva europea 86/278/CEE. No obstante, hasta la fecha, los únicos contaminantes químicos que se encuentran regulados para dicha aplicación son siete metales pesados, no existiendo valores límite para compuestos orgánicos. Por otro lado, las metodologías analíticas para la determinación de dichos compuestos en matrices ambientales se han centrado en matrices líquidas como aguas residuales y superficiales mientras que apenas se han descrito metodologías para su determinación en matrices sólidas, como suelos y lodos, probablemente por la complejidad de dichas matrices [1].

Por ello, el objetivo del presente trabajo fue la optimización y validación de una metodología analítica para la determinación simultánea de 23 disruptores endocrinos en lodo digerido, compost y suelo. Los compuestos objeto de estudio fueron seis compuestos perfluorados (cinco ácidos perfluorocarboxílicos y el ácido perfluorooctanosulfónico), el plastificante bisfenol A, cuatro tensioactivos aniónicos (alquilsulfatos sódicos), cuatro conservantes (parabenos), dos antimicrobianos (triclosan y triclocarban) y seis filtros solares (benzofenonas). El tratamiento de muestra consistió en extracción asistida por ultrasonidos (UAE) con 3 mL de metanol:ácido acético (95:5, v/v) durante 7 min y limpieza del extracto mediante extracción en fase sólida dispersiva (d-SPE) con 0.8 g de $C_{18}$. La determinación analítica se realizó mediante cromatografía líquida acoplada a espectrometría de masas de triple cuadrupolo (LC-MS/MS) [2]. Para la optimización del método se empleó un diseño experimental Box-Behnken debido a las diferentes propiedades físico-químicas de los compuestos de interés y al alto número de variables a optimizar (disolvente de extracción, tiempo de extracción, número de extracciones, tipo y cantidad de sorbente para la limpieza por d-SPE). El método se validó para los tres tipos de matrices en términos de límites de detección y cuantificación, exactitud y precisión. La exactitud del método, expresada como recuperación relativa, se encontró en el rango entre 70-120% para la mayoría de los compuestos y matrices mientras que la precisión, expresada como desviación estándar relativa, fue inferior al 21%. Los límites de detección se encontraron en el rango comprendido entre 0.01-6.2 ng $g^{-1}$, referidos a materia seca. La aplicabilidad del método fue corroborada mediante su aplicación a lodos digeridos, compostados y suelos. EL compuesto que se encontró con mayor frecuencia y a amyor nivel de concentración fue el dodecilsulfato sódico.

**Agradecimientos**
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**MAB-P10**

# MÉTODO ANALÍTICO DE RUTINA PARA LA DETERMINACIÓN DE METABOLITOS DE PRINCIPIOS ACTIVOS FAMACOLÓGICOS Y PARABENOS EN AGUAS POTABLES Y RESIDUALES

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A las estaciones depuradoras de aguas residuales llegan cada día enormes cantidades de residuos que necesitan ser tratados y, a ser posible, eliminados en su totalidad para evitar que estos sean vertidos a las aguas superficiales y originen un peligro para el medio ambiente. En los últimos años, se ha puesto un especial énfasis en una serie de contaminantes, denominados emergentes, cuyo uso y tratamiento, una vez llegan al medio ambiente, carecen de una legislación específica. La presencia de estos contaminantes emergentes en matrices ambientales de todo tipo, desde aguas residuales hasta en seres vivos, como los peces, ha suscitado una gran preocupación y, es por ello, que han aumentado exponencialmente en los últimos años los estudios publicados centrados en la determinación y distribución de estos compuestos en la forma en la que se consumen (productos de partida) y en matrices acuosas como las aguas residuales, superficiales o potables. Muy escasos aún son los estudios donde se aborda la identificación y cuantificación de los metabolitos o productos de degradación derivados de este tipo de contaminantes. Algunos estudios afirman que existen productos de degradación cuya toxicidad y, por tanto, riesgo que estos suponen al medio ambiente son similares o incluso mayor que sus productos de partida. Con el fin de poder ofrecer una mayor información sobre posibles patrones de consumo en el caso de los principios activos farmacológicos, la exposición humana a productos de cuidado personal, en particular a los parabenos, y poder evaluar cómo afecta un contaminante emergente al medio ambiente, tanto en la forma en la que se consume como a través de los metabolitos de estos, se ha desarrollado un método para su determinación simultánea, pudiendo servir como análisis de rutina en aguas residuales y aguas potables. La extracción se llevó a cabo mediante una extracción en fase sólida a través de cartuchos OASIS HLB. Para la separación y determinación de los compuestos objeto de estudio se hizo uso de una cromatografía líquida de alta resolución acoplada a espectrometría de masas, consiguiendo la determinación satisfactoria de todos los compuestos con una sola inyección y un tiempo de análisis cromatográfico de 30 min. Los límites de detección del método obtenidos se sitúan dentro del siguiente rango: desde 0.3 hasta 10 ng $L^{-1}$ para aguas residuales y desde 0.14 a 8.3 ng $L^{-1}$ para aguas potables. En el caso de los límites de cuantificación, estos presentan valores situados entre 1.0 y 33 ng $L^{-1}$ en aguas residuales y desde 0.5 y 28 ng $L^{-1}$ en muestras de agua potable. Los valores de exactitud del método se encuentran entre el 66 y el 120% en aguas residuales y entre el 86 y el 120% en aguas potables con unos valores de precisión por debajo del 17% en todos los casos y en ambas matrices. El método validado y desarrollado se aplicó a diferentes muestras de aguas residuales y potables. Ningún compuesto analizado, ni como producto de partida ni como producto de degradación, fue detectado en las muestras de agua potable analizadas mientras que todos ellos fueron detectados en las muestras de aguas residuales. Las concentraciones encontradas de algunos productos de degradación se encontraban a niveles similares o más altos que sus productos de partida. La concentración más alta encontrada en agua residual (68 µg $L^{-1}$) se corresponde con el ácido 4-hidroxibenzoico, precisamente, uno de los productos de degradación analizados.

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**MAB-P11**

## DETECTION OF PESTICIDE RESIDUES ACCUMULATED IN BEES BY NANOFLOW LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY

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Bees have been an integral part of agriculture for many centuries for the pollination of crops. In fact, approximately 35% of crops depend directly on pollinators. Several studies have proven the role of pesticides in bee deaths and colony collapse disorder. It has been reported that pesticide residues can be accumulated in the pollen and nectar of treated plants, thus posing a potential risk to honey bees.

The development of analytical methods, which can determine pesticide residues in these types of matrices at very low concentrations, has acquired significant relevance across the globe. On the other hand, to get how pesticide residues can affect bees it is necessary to develop analytical methods, which could detect these compounds in a specific part (abdomen, thorax or head) from one single specimen.

In this article, a nanoflow liquid chromatography system coupled to high resolution mass spectrometry (nanoflow LC/ESI Q-Orbitrap-MS) has been applied for the development of a multiresidue pesticide method for the determination of 162 multiclass pesticides in specific part of honeybee samples (c.a. abdomen, head or thorax). The reduced flow rate provided an enhancement in sensitivity and a strong reduction of matrix effects, thus only a quick and simple ultrasound assisted extraction sample treatment was needed. Satisfactory results were obtained for all tested analytes with concentration levels lower than 0.5 ng $g^{-1}$ in all cases, thus being acceptable for monitoring purposes. Matrix effect was negligible for 94% of compounds. Extraction recoveries were ranged from 70% to 105%, being in agreement with SANTE guideline. Finally, the applicability of the method was demonstrated, by successful application to the analysis of incurred honeybee samples.

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**MAB-P12**

## EXTENDING THE IONIZATION COVERAGE OF LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY FOR PESTICIDE ANALYSIS THROUGH DIELECTRIC BARRIER DISCHARGE IONIZATION

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Depending on the particular chemical class, the determination of some of the pesticides might be carried out by either GC-MS or by LC-MS. Among the large amount of pesticide classes, some of them are thermolabile (so they might be degraded in the GC injection portal) but they do not have the right moieties to be efficiently ionized by electrospray.

As alternative to current commercial sources, new ionization sources based on dielectric barrier discharge ionization (DBDI) have been reported. DBDI has gained attraction in recent years as a versatile ionization method available in different formats (ambient ionization probes, GC-MS, LC-MS or CE-MS interfaces), suitable for many applications including ambient mass spectrometry imaging, environmental analysis, biological and pharmaceutical and food safety. The (dielectric barrier) discharge is typically formed between two electrodes, with at least one dielectric layer which separates the electrode from the plasma. DBDI sources feature different ionization mechanisms that include electron capture and proton transfer.

In this work, we report on a thorough evaluation of DBDI as ionization interface for LC-MS for pesticide analysis in food. It reveals attractive advantages over ESI and APCI provided its singular ionization mechanism versatility. Over 100 pesticides across a wide range of physicochemical properties were selected and the results were compared with both electrospray and atmospheric pressure chemical ionization (APCI) sources. Matrix effects, analyte coverage, sensitivity and salt adduct formation were evaluated with the three different approaches. The ability of DBDI to efficiently ionize challenging compounds such as captan, o-phenylphenol, bromophos ethyl, dazomet, and dinocap is also demonstrated.

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**MAB-P13**

# DETERMINACIÓN DE URANIO POR DIFERENTES TÉCNICAS EN SUELOS NO PERTURBADOS DE ZACATECAS, MÉXICO

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*Introducción*

Los niveles de radionúclidos en suelos suelen ser bastante constantes. El uranio tiene una gran importancia dentro de la protección radiológica y la salud publica debido a su radiotoxicidad y quimiotoxicidad [1][2]. La Agencia Internacional de Energía Atómica (IAEA) y la Organización Mundial de la Salud (OMS) entre otros organismos, recomiendan el monitoreo constante del medioambiente de radionúclidos naturales y antropogénicos. Para este trabajo, se han analizado 33 muestras de suelo superficial de diferentes zonas del Estado de Zacatecas [3] que es uno de los 31 estados que, junto con la Ciudad de México, conforman los Estados Unidos Mexicanos, ubicado en la región centro-norte del país.

El objetivo de este trabajo consiste en caracterizar los suelos, determinar la concentración de uranio por diferentes técnicas analíticas, comprobar alteraciones en la relación isotópica $^{238}U/^{235}U$, proponer un valor de referencia en la concentración de uranio en suelo y comprobar una posible alteración en la concentración de uranio en la zona de Villa de Cos.

*Materiales y métodos*.

Se han utilizado para este trabajo las técnicas XRD y XRF para caracterización de las muestras de suelo que requieren un tratamiento mínimo. Las técnicas ICP-OES, ICP-MS y SF-ICP-MS requirieron de la digestión del suelo por microondas para posteriormente leerse en el instrumento previas diluciones adecuadas. La técnica radioanalítica de espectrometría alfa demanda de la digestión total por microondas, separación radioquímica, electrodeposición y lectura en el instrumento durante 300 000 segundos [4].

*Resultados*

Se han caracterizado las muestras por XRF y XRD obteniéndose Si, Al, Fe, K, Na y Ca como los principales componentes mayoritarios en forma de óxidos; y de Sr, Zr y Ba como los componentes minoritarios principales. Se detecto la presencia de cristales de halita (NaCl) en la zona de Villa de Cos. Los resultados obtenidos por ICP-OES no fueron confiables debido a las interferencias espectrales arrojando resultados muy altos. ICP-MS dio resultados muy confiables y rápido con una media de concentración de 2.3 mg $Kg^{-1}$, la otra técnica analítica SF-ICP-MS arrojo un promedio de 2.6 mg $Kg^{-1}$ con una relación isotópica $^{238}U/^{235}U$ promedio de 138.86 siendo lo normal $n(^{238}U)/n(^{235}U) = 137.794 \pm 0.027\ 2\sigma$ [5]. Espectrometría alfa es la técnica estándar para medir actínidos, pero con altos costes y tiempos de medición. Los resultados promedio obtenidos fueron de 2.9 mg $Kg^{-1}$. No fue posible determinar la relación isotópica $^{238}U/^{235}U$ por espectrometría alfa, debido a que el tiempo de medición de 300 000 segundos es insuficiente para una buena estadística de conteo de $^{235}U$.

Los resultados generales de uranio por todas las técnicas empleadas coinciden en un incremento o anomalía en la concentración de uranio (29.7 mg $Kg^{-1}$) en la zona de Villa de Cos con respecto al promedio general de los resultados en el resto de las muestras analizadas en este trabajo y a los valores promedio reportados en la literatura de otras zonas del planeta.

*Conclusiones*

La concentración de uranio en suelo de la zonas muestreadas es normal (2.8 mg $Kg^{-1}$ es el promedio mundial) a excepción de la Zona de Villa de Cos, que presenta una acumulación anómala. La relación isotópica $^{238}U/^{235}U$ no demuestra un empobrecimiento o enriquecimiento de $^{235}U$ en el área. Se propone un valor de referencia para esta área de 2.1 mg $Kg^{-1}$ de uranio.

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**MAB-P14**

# ESTUDIO DE NUEVOS MATERIALES PARA LA DESCONTAMINACIÓN DE AGUAS NATURALES

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La aparición de contaminantes prioritarios y emergentes en agua es un tema de imperiosa actualidad que ha llevado a la comunidad científica a investigar nuevas soluciones y alternativas para su eliminación [1]. Se han ensayado una gran variedad de procesos tecnológicos en este sentido, entre ellos los basados de oxidación avanzada y el uso de membranas, habiéndose obtenido resultados dispares en cuanto a su rendimiento para ciertas familias de compuestos y con un inconveniente común en relación a su viabilidad técnico-económica. El uso de materiales adsorbentes, por el contrario, cuenta con las ventajas de su bajo coste, su fácil manipulación y su menor impacto ambiental. Por el momento, su principal desventaja es su rendimiento selectivo para determinados compuestos y la dificultad de su regeneración [2]. Entre la amplia variedad de materiales adsorbentes empleados, están adquiriendo especial relevancia las arcillas sintéticas o naturales y éstas modificadas orgánicamente por su alta capacidad de intercambio catiónico, su alta superficie específica, su capacidad de hinchamiento y su microporosidad, propiedades que influyen determinantemente en su capacidad de adsorción [3, 4].

Los objetivos de este trabajo fueron preparar y caracterizar dos materiales funcionales, ambos obtenidos mediante reacción de intercambio catiónico entre arcillas del tipo silicatos laminares y alquilaminas primarias. Se utilizó para ello una montmorillonita natural (Mt), una mica sintética expansible de alta carga (Na-mica-4) y octadecilamina como surfactante, a partir de las cuales se obtuvieron las correspondientes organioarcillas (C18-Mt y C18-mica-4). El potencial uso como adsorbentes en aguas que contienen contaminantes emergentes como el ibuprofeno fue estudiado.

Ambos materiales fueron caracterizados por difracción de rayos X, potencial Z, espectroscopia IR-FT y análisis termogravimétrico antes y después de los ensayos de adsorción. Todos estos ensayos pusieron de manifiesto que la eliminación de ibuprofeno está fuertemente ligada a la estructura y las características de la superficie de cada organoarcilla. Se consiguió una alta adsorción de ibuprofeno (hasta el 100%) en menos de 5 y 60 minutos para la $C_{18}$-Mt y la $C_{18}$-Mica-4, respectivamente. El grado de adsorción de ambas no se vio afectado por la concentración de ibuprofeno en el rango 0,05-80 mg $L^{-1}$ para la $C_{18}$-Mt y en el rango 0,05-40 mg $L^{-1}$ en el caso de la $C_{18}$-Mica-4, ni tampoco por el pH de la muestra en el rango de 2 a 9, sin embargo, disminuyó a valores de pH extremadamente ácidos (pH 1) y básicos (pH 12). Los ensayos realizados confirmaron la eficacia de estos materiales para la eliminación de ibuprofeno del agua.

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**MAB-P15**

# ANALYSIS OF EMERGING POLLUTANTS IN WASTEWATERS BY USING SCREEN-PRINTED ELECTRODES

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Chemical contamination of surface waters from both natural and anthropogenic sources continuously increases as the human way of life improves. Poor water quality not only constitutes a threat to human health and the environment in multiple ways, but also makes water unfit for different uses and purposes and hence reduces the water resources availability. This coupled to water scarcity caused by over-use and uneven distribution of water resources in both time and space, which are exacerbated by climate change, present major challenges in securing enough water of good quality to meet human, environmental, social and economic needs to support sustainable development of countries.

In this context, the treatment of municipal wastewaters to reuse them in agriculture becomes a key process in the Mediterranean countries and other arid and semi-arid regions, which are confronting increasing water shortages [1]. However, a careful control must be done to assure that contaminants present in wastewaters are effectively removed along the depuration process, so that they are not present in the reclaimed water nor in the vegetables grown with that.

This is especially important for emerging contaminants, *i.e.*, these which have appeared recently due to the use of new products and whose toxicity is still under study. In order to develop safe and effective regulations for these compounds, extensive studies are still required to evaluate their consumption, fate, absorption rate by organisms, target tissues and toxicity. In these studies, liquid chromatography coupled to mass spectrometry (LC-MS) is the most powerful analytical technique available due to its high selectivity, sensitivity, precision and accuracy. However, it suffers from high cost, low portability and the need of specialized staff to carry out the analyses.

Electrochemical sensors could be a good complement for LC-MS measurements in these situations were cheap and fast screening, *in situ* analysis or on-line monitoring are required, *e.g.*, in the control of wastewater depuration process. In this sense, voltammetric measurements using commercial screen-printed electrodes have shown to be very convenient and versatile for these organic pollutants susceptible to be oxidised or reduced [2]. Among these, we can mention UV filters, corrosion inhibitors and many families of drugs [3].

In this work, we evaluate the suitability of differential pulse voltammetry (DPV) using carbon-based screen-printed electrodes to determine benzotriazole (extensively used as corrosion inhibitor) and several electroactive drugs usually present in wastewaters, as a first step to develop a screening and monitoring strategy for wastewater treatment based on electrochemical sensors.

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**MAB-P16**

## ACTIVE SILICA FROM RICE STRAW FOR AVOIDING NITRATES OF THE COMPREHENSIVE WATER CYCLE: A PROOF OF CONCEPT

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Rice is one of the most important cereals and its cultivation is increasing [1], however, the large quantity of waste generated (rice straw) during its production is usually a major problem that demands more ecofriendly solutions than uncontrolled waste burnings or waste abandonment. In this sense, it is interesting to note that ash from rice straw contains relatively high amounts of silica [2]. On the other hand, it is necessary to bear in mind that there are many vulnerable zones in Europe in relation to the nitrate (Council Directive 91/676/EEC; Brussels, 4.5.2018 COM(2018) 257 final). This work included in the LIFELibernitrate project [3] demonstrates that the active silica obtained from rice straw [4] (95 % efficiency) can be useful for eliminating 30 % of nitrate of the comprehensible water cycle in several planned scenarios according to the project. The obtained silica was characterized by several techniques and a continuous flow system scalable to real situations (between 76 dm/h and 152 dm/h) was developed for studying the nitrate absorption. Good results are achieved in reference to the proposed amount of rice straw proposed in the project. The regeneration of the active silica is also studied. In conclusion, a successful functionalization of silica extracted from a waste product like rice straw is reported and the reduction of nitrates is demonstrated.

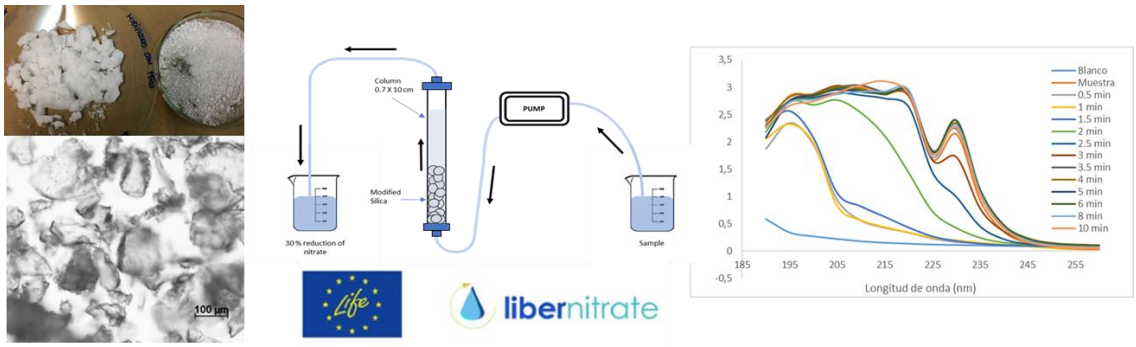


Figure 1. Active silica and its use as adsorbent of nitrates in waters.

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**MAB-P17**

# DETERMINACIÓN Y CUANTIFICACIÓN "*IN VIVO*" DE COMPUESTOS VOLÁTILES DE SELENIO PRODUCIDOS POR *ESCHERICHIA COLI* TRAS SU EXPOSICIÓN A Cs-SeNPs

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Las nanopartículas de selenio (SeNPs) se consideran un nuevo compuesto de selenio que se caracterizan por sus propiedades antioxidantes y menor toxicidad comparada con las especies tanto orgánicas como inorgánicas de este elemento [1]. El metabolismo de compuestos inorgánicos de selenio en bacterias es un área de investigación activa [2], sin embargo, el metabolismo del Se en forma de SeNPs ha sido menos explorado [3]. Las SeNPs poseen propiedades antimicrobianas y pueden ser consideradas como una alternativa a la aplicación de las nanoparticulas de plata, de mayor toxicidad. Sin embargo, las transformaciones de estas nanoparticulas en su interacción con sistemas biológicos como bacterias ha sido poco estudiada.

En este trabajo se ha evaluado, con fines de comparación, la producción de compuestos volátiles de selenio tras la exposición de *E. coli* a selenito sódico y SeNPs modificadas con chitosán (Cs-SeNPs) mediante microextracción en fase sólida en espacio en cabeza y cromatografía de gases acoplada a espectrometría de masas (HS-SPME-GC/MS). Tras comprobar mediante microscopía electrónica de transmisión que las Cs-SeNPs eran estables en el medio de cultivo de la bacteria, ésta fue crecida durante 24 h a 37ºC y 120 rpm en presencia de 1 y 2 mg Se $L^{-1}$ tanto en forma de selenito como de Cs-SeNPs. A continuación, para realizar la cuantificación de las especies volátiles de selenio se llevó a cabo una doble extracción. En primer lugar, las fibras de carboxen/polidimetilsiloxano fueron expuestas durante 26 min al espacio en cabeza de la bacteria, después y sobre esa misma fibra se llevó a cabo la extracción de dimetilsulfuro deuterado ($d^6$- DMS), empleado como patrón interno, en las condiciones de crecimiento de *E. coli*. Finalmente, se llevó a cabo la desorción térmica en el puerto de inyección a 300ºC para su posterior análisis GC/MS

Las especies volátiles identificadas fueron dimetilselenio (DMSe) y dimetildiselenio (DMDSe), independientemente de la fuente de selenio aplicada. La primera de ellas no pudo cuantificarse ya que la señal obtenida estaba por debajo del límite de detección para las dos concentraciones estudiadas. Sin embargo, para el DMDSe se obtuvieron concentraciones máximas del orden de 6 y 8 µg $L^{-1}$ cuando la bacteria fue expuesta a 1 y 2 mg Se $L^{-1}$ en forma de nanopartículas, respectivamente y de 5 µg $L^{-1}$ cuando se expuso a 1 y 2 mg Se $L^{-1}$ en forma de $Na_2SeO_3$. Finalmente, se hizo uso del análisis multifactorial para comprobar la existencia de diferencias significativas entre los cantidades aplicadas y la fuente de selenio empleada.

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**MAB-P18**

## SIMULTANEOUS DETERMINATION OF SHORT AND LONG-CHAIN PERFLUOROALKYLATED SUBSTANCES IN SURFACE WATERS

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Polyfluoroalkylated substances (PFAS) are man-made chemicals that have been in use since the 1950s and are ubiquitous in the environment. Analysis of PFAS has typically been focused on perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOS) and other long-chain PFAS which can be easily determined by reversed phase (RP) liquid chromatography (LC). Short-chain PFAS, such as perfluorobutanoic sulphonic acid (PFBS), have been introduced as alternatives to long-chain PFAS as these shorter chain length substances do not bioaccumulate [1]. Reports on environmental levels of short-chain PFAS are relatively scarce which may be due to the chromatographic challenge of these polar, poorly retained water soluble short-chain PFAS using a reversed phase LC method.

Thus, the aim of this work is the simultaneous determination of short-chain and long-chain PFAS, including C2-C18 perfluoroalkyl carboxylic acids and C1-C10 perfluoroalkyl sulphonates, in water samples. This task is challenging due to the wide range of physico-chemical properties. The method involves solid-phase extraction (SPE) using a mixed-mode with weak anion-exchange functionality (Oasis WAX) cartridge and LC tandem mass-spectrometry (LC-MS/MS) using weak anion-exchange functionality (Acclaim WAX, 3 µm; 3 mm × 50 mm).

A chromatographic separation of C2-C18 perfluoroalkyl carboxylic acids and C1-C10 perfluoroalkyl sulphonates was successfully achieved using mixed-mode chromatography and the value of the method was evidenced by application to river water samples

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**MAB-P19**

# DESARROLLOS ANALÍTICOS PARA LA EVALUACIÓN DE PESTICIDAS EN COLMENAS DE APIS MELLIFERA

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En los últimos años existe una gran preocupación por el marcado descenso en el número de poblaciones de abejas a nivel mundial. Es evidente el carácter multifactorial de este proceso, donde los pesticidas tienen un peso importante. Esto ha provocado un creciente interés por evaluar el estado de las colmenas y conocer cómo los pesticidas son introducidos en las mismas. Además del uso de tratamientos veterinarios para el control de *Varroa* y otros parásitos, la presencia de pesticidas en la colmena es consecuencia del polen transportado por las abejas desde los cultivos agrícolas. El desplazamiento medio de la abeja es de aproximadamente 8 kilómetros alrededor de la colmena, por lo que se puede considerar el uso del polen una medida de *biomonitoring* de pesticidas en el medio ambiente. El análisis del polen tiene especial complejidad debido a la naturaleza de la matriz y su alto contenido en proteínas, lo que hace necesario el uso de procedimientos de lavado para obtener recuperaciones aceptables.

El proyecto INSIGNIA* (*citizen science investigation for pesticides in apicultural products*) tiene entre sus objetivos el análisis de estas muestras de polen en una amplia red europea de 16 países. El presente trabajo ha consistido en el desarrollo y validación de un método para el análisis simultáneo de 231 residuos de pesticidas en polen, así como el estudio de su estabilidad en la matriz. El procedimiento de extracción consiste en un método QuEChERS modificado que incluye una etapa de *freezing-out* para eliminar los componentes proteicos y lipídicos. La validación se ha estudiado en términos de recuperación, repetibilidad y linealidad a dos niveles de concentración (0,01 mg/kg y 0,05 mg/kg), empleando métodos de GC-QqQ-MS/MS y LC-QqQ-MS/MS. Este método ha sido aplicado a muestras de polen de entorno ecológico del proyecto INSIGNIA, encontrando concentraciones de algunos pesticidas en el rango de 1-4 µg/kg gracias a los bajos límites de cuantificación y la posibilidad de contaminación cruzada del entorno de las colmenas.

Para los estudios de estabilidad de los pesticidas en el polen, se fortificaron blancos de muestra y se almacenaron durante 14 días a temperatura ambiente (20 ºC) o bien en congelador (-7 ºC). Se realizaron análisis en el día 0, 8 y 14 para estudiar la influencia de la temperatura en la estabilidad de los pesticidas, encontrando que un 8% de los mismos (chlordane, clofentezin, dichlorvos, dicrotophos, fluazifop-p-butyl, fluopyram, flusilazole, heptenophos, methamidophos, mevinphos, monocrotophos, napropamide, ntempyram, omethoate, paclobutrazol, pyridate, tetrachlorvinphos, tolylfluanid) se degradan transcurridas dos semanas a temperatura ambiente, mientras que en congelador no sufren degradación. Por otro lado, un 1% de los pesticidas (oxyfluorfen, picoxystrobin, pyrifenox) se degradaron tanto a temperatura ambiente como en el congelador, mientras que el 91% restante (211 compuestos) no vieron afectadas sus concentraciones tras 14 días, con independencia de las condiciones de almacenamiento. Estos resultados muestran que, aunque un almacenamiento y transporte de las muestras de polen en condiciones de frío es recomendable para asegurar la estabilidad de los pesticidas, no se trata de un factor esencial ya que la temperatura sólo afecta a un número reducido de compuestos.

*Pilot study on environmental monitoring of pesticide use through honeybees. Proyecto INSIGNIA" (PP-1-1-2018). Financiado por la Unión Europea (3rd Health Programme).

**MAB-P20**

## SIMULTANEOUS ANALYSIS OF PHTHALATES AND POLYCYCLIC AROMATIC HYDROCARBONS IN PM10 SAMPLES COLLECTED IN AN ATLANTIC SUBURBAN AREA

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Polycyclic aromatic hydrocarbons (PAHs, basically generated by incomplete combustion processes) and phthalate esters (PAEs, widely used, especially as plasticizers) are well-known organic pollutants. Both kinds of compounds are widely found in environment, including in atmospheric particulate matter (PM), and some of them are considered toxic.

The objective of this study was to examine the concentration and seasonal variations of PAHs and PAEs in $PM_{10}$. Analytical methodologies which allow a simultaneous organic extraction and chromatographic separation of the two pollutant classes were employed. Besides, aqueous extraction of PAEs by an in-tube solid phase microextraction method coupled to liquid chromatography [1] was performed in order to compare the concentration and distribution of PAEs in both phases.

Fourteen samples of $PM_{10}$ (24h) were collected at the University Institute of Environment (IUMA, Liáns, Oleiros) of the University of A Coruña. The sampling point is located in a suburban area in Northwest Spain, with a significant influence of Atlantic Ocean. The analyzed samples have been selected from a wider set covering one whole year [2] taking into account their values for several parameters determined during general characterization. The 16 EPA priority PAHs and 8 PAEs have been simultaneously analyzed by liquid chromatography after dichloromethane extraction.

Estimated concentrations of PAHs were in the range <LOD-468 pg/m$^3$, with ΣPAHs = 472-2152 pg/m$^3$ and benzo[b]fluoranthene and benzo[ghi]perylene as the predominant PAHs. The estimated levels of PAEs were between values below detection limit and 29 ng/m$^3$ ($\Sigma_{org}$PAEs = 0.5-42 ng/m$^3$) in the organic extract, whereas in the aqueous fraction they were in the range <MDL-42 ng/m$^3$ ($\Sigma_{aq}$PAEs = 8-53 ng/m$^3$). Different phthalates were extracted with each solvent, with diethylphthalate being only extracted with water and diisononylphthalate only with dichloromethane. Dibutylphthalate (sum of di-n-butyl- and diisobutylphthalate), better extracted with water, was the major in the aqueous fraction, and diisononylphthalate in the organic fraction for most of the samples. Correlations with other parameters have been explored and seasonal variations have been also identified, with higher levels of phthalates in the warm period, and higher levels of PAHs in the cold period due to domestic heating.

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**MAB-P21**

## COLORIMETRIC SENSOR BASED ON POLYDIMETHYLSILOXANE-SALICYLATE/NITROPRUSSIDE COMPOSITE FOR ANALYSIS OF AMMONIUM ION IN RAINWATER

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Colorimetric sensors are useful in the environmental field due to their simplicity, rapid visualization, sensibility, cost-effectiveness and, possibility of the in-situ analysis. The role of ammonium ion in the atmosphere can be understood if the different compounds of nitrogen, its emission sources and the nitrogen cycle are taken into account. Traffic, combustion of fueoil and industrial and agricultural activities are its main anthropogenic sources. The ion ammonium is in rainwater due to the scavenging of the ammonia in gas phase or in the particulate material present in the clouds.

The objective of this work is the development of a sensor based on Berthelot´s reaction which allows analyzing the levels of ammonium concentration during the rainfall events. Two types of polydimethylsiloxane (PDMS) devices were prepared entrapping inside PDMS the reagents involved in the derivatization reaction of the ammonium ion. One of them contained thymol and nitroprusside (Prieto-Blanco et al., 2015 and 2019). In the other sensor the thymol was replaced for sodium salicylate. Small units were prepared in the form of discs, using half a disc for each analysis. The derivatization takes place when the reagents are released into a solution containing the sample. The formed derivative can be measured using UV-visible spectrometry or visual inspection. Better detection and quantification limits (0.03 and 0.09 µg/mL) and lower concentration and volume of reagents were obtained with the salicylate devices than with thymol devices.

The method was applied to two types of sampling, one of them, a fractionated and short-term samplings and another type of longer duration. In the former case, it was not observed a decrease of ammonium concentration during the events. Therefore, the rainout process could have more contribution than washout process. The rain scavenged the ammonia in gas phase and ammonium in particulate material inside the clouds. In the longer duration samplings, a relationship between sampling time and ammonium concentration was found. For long sampling time (approximately 40h) the measured ammonium concentration was around 20 µM. This fact may be due to losses by evaporation. The optimized method allows studying the rainout and washout processes for the ammonium ion using a low sample volume (500 µL) and minimizing toxic reagents and waste generation.

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**MAB-P22**

# ESTUDIO PRELIMINAR DEL COMPORTAMIENTO DE UN BIOSENSOR ENZIMÁTICO BASADO EN ELECTRODOS IMPRESOS DE CARBONO CON NANOTUBOS DE CARBONO Y NANOPARTÍCULAS DE ORO PARA LA DETECCIÓN DE BISFENOL A EN AGUA

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El bisfenol A (BPA) es una de las materias primas clave utilizadas en la producción de resinas epoxídicas y polímeros de policarbonato, que tienen aplicación en la industria química tales como botellas de agua potable, biberones y envases de alimentos [1]. Este compuesto es de especial interés debido a su actividad estrogénica [2], la probabilidad de causar varios tipos de cáncer [3], así como enfermedades cardíacas y obesidad [4]. En la bibliografía se ha reportado que el BPA ha sido detectado en aguas residuales industriales, aguas subterráneas, aguas superficiales e incluso agua potable [5,6].

En la bibliografía se encuentran descritas diversas metodologías analíticas para la determinación de BPA, entre las que cabe mencionar HPLC, LC-MS, GC-MS y ELISA [1-3]. Los biosensores electroquímicos son unos dispositivos con un diseño sencillo, flexible y con grandes posibilidades de miniaturización, por lo que pueden producirse en masa a muy bajo coste. La utilización de biosensores electroquímicos enzimáticos basados en tirosinasa es una metodología que se ha reportado en la bibliografía [4]. Sin embargo, no se han encontrado referencias sobre el desarrollo de este tipo de sensores utilizando como soporte electrodos impresos para la detección in-situ de BPA en agua.

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 nanotubos de carbono y nanopartículas de oro, para la detección de BPA en muestras ambientales. Para ello, se ha llevado a cabo, en primer lugar, la optimización de los parámetros químicos e instrumentales involucrados en la detección (concentración de enzima, el pH y el potencial aplicado). Una vez optimizadas dichas variables, cuyos valores óptimos fueron 500 U para la concentración de tirosinasa, pH 6,4 y un potencial aplicado de -0,1 V, se llevó a cabo la calibración del biosensor analizando por triplicado disoluciones estándar de BPA en el rango de concentraciones entre 0 y 30 µM obteniendo un coeficiente de correlación aceptable y un LOD de 3,1 µM. Asimismo, se estudió la repetibilidad de la medida obteniendo un resultado del 5% (RSD). Teniendo en cuenta que el biosensor es enzimático, las constantes cinéticas del proceso enzimático fueron también estudiadas, resultando una cinética que se aparta ligeramente del modelo de Michaelis-Menten, con una constante de Hill calculada de 1,74, lo que indica una unión cooperativa del sustrato al centro catalítico de la enzima. Finalmente, se ha explorado la aplicabilidad del método en muestras de agua potable y en muestras de aguas naturales, resultando recuperaciones bajas de BPA que indican que los parámetros químicos involucrados en el proceso de detección tienen que ser aplicados para evitar la probable desactivación de la enzima cuando el medio de aparta de la idealidad. En este proyecto, se presentan los resultados obtenidos de una primera exploración del biosensor enzimático, aunque serían precisas optimizaciones adicionales del método para conseguir una adecuada aplicabilidad en muestras reales.

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**MAB-P23**

# EMANACIÓN DE TORÓN DE MATERIALES DE CONSTRUCCIÓN Y RIESGOS RADIOLÓGICOS ASOCIADOS

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La humanidad está expuesta a la inhalación de gases radioactivos. Uno de ellos es el gas $^{220}$Rn (torón), que es producto de desintegración de la cadena radiactiva del $^{232}$Th. El torio se encuentra de forma natural en el ambiente en suelos, rocas y por tanto también en los materiales empleados en la construcción de viviendas. Su descendiente gaseoso, el torón puede escapar del material y pasar al aire que se respira. El estudio del torón ha ganado en importancia en los últimos años a pesar de su corto periodo de semidesintegración (10.6 h) debido a su demostrada contribución en el desarrollo de cáncer de pulmón [1], como consecuencia de sus emisiones radiactivas así como las de sus descendientes [2], principalmente las radiaciones alfa, beta y gamma.

En este trabajo, se han estudiado las propiedades de emanación de algunos de los materiales de construcción comúnmente usados en la Península Ibérica. Así en primer lugar se ha determinado la tasa de exhalación y seguidamente el factor de emanación mediante la estimación previa del contenido en $^{232}$Th de los materiales. Finalmente se ha calculado la dosis radiológica que recibe un individuo como consecuencia de la inhalación de torón en el interior de una vivienda construida con dichos materiales.

El procedimiento experimental consiste en la utilización de un método activo que emplea un monitor continuo de torón (marca RTM1688-2 de SARAD). La muestra se coloca en una cámara de acumulación acoplada en continuo a dicho monitor. Los gases emanados son recogidos por el monitor mediante un circuito cerrado que circula con un flujo de aire a un ritmo de 0.30 L/min. Para cada muestra el tiempo total de medida fue de 10 días en intervalos de 2 horas. El límite de detección fue de 5 Bq/m$^3$.

Entre los resultados se puede destacar que la tasa de exhalación varió entre 0.0008 a 0.0468 Bq·kg$^{-1}$·h$^{-1}$ para los materiales cerámicos y para el circonio respectivamente. El factor de emanación del torón osciló entre el 0.4 al 29.1 % para los materiales cerámicos y la madera, respectivamente. Por último la dosis anual efectiva varió entre 2.5 y 142 µSv/año para los materiales cerámicos y el circonio respectivamente. Estos valores se encuentran dentro del rango típico de valores encontrados en otros países [2].

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**MAB-P24**

# DETERMINACIÓN DE ELEMENTOS TRAZA EN MUESTRAS DE SUELO MEDIANTE EXTRACCIÓN ULTRASÓNICA ASISTIDA POR SONORREACTOR E ICP-MS

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La presencia de elementos traza en suelos es de gran importancia debido a los efectos tóxicos a largo plazo que éstos pueden causar tras su acceso al organismo por inhalación, o a través de la cadena alimentaria [1,2]. Frente a las técnicas convencionales de pretratamiento de las muestras de suelo para la determinación de elementos traza basadas en la digestión ácida simple o asistida por microondas [3], en los últimos años la energía de ultrasonidos (US) se ha convertido en una importante herramienta de pretratamiento de muestras biológicas y ambientales para la extracción de elementos, empleándose baños de US [4], sondas [5] y sonorreactores [6], con recuperaciones comparables a las obtenidas en las técnicas clásicas en condiciones experimentales más favorables [7]. El objetivo principal de este trabajo es la optimización de una metodología de digestión ácida eficiente, rápida, miniaturizada y de bajo coste para la extracción de elementos traza de interés ambiental (As, Cd, Cu, Pb, Zn) en muestras de suelo, basándonos en el empleo de la energía de ultrasonidos asistida por sonorreactor, y posterior análisis por ICP-MS. El empleo del sonorreactor combina las ventajas de las sondas y de los baños de US, ya que permite miniaturizar y acelerar el proceso, extrayendo varias muestras simultáneamente.

La optimización de las condiciones de extracción se llevó a cabo en muestras de material de referencia certificado "SRM 2710a Montana I Soil", utilizando un diseño Box-Behnken (BBD) junto con una metodología de superficie de respuesta (RSM) para el diseño experimental. Bajo las condiciones óptimas, se exploró la aplicabilidad del método a mediciones de As, Cd, Cu, Pb y Zn en un conjunto de muestras de suelo recogidas en el área industrial de Puchuncaví-Ventanas, Chile [8]. Todas las muestras se extrajeron en paralelo mediante digestión ácida estándar según un protocolo validado [5] y se analizaron mediante un protocolo ICP-MS igualmente validado [5]. Las condiciones de extracción óptimas obtenidas fueron un medio del 83.3 % $HNO_3$, un tiempo de sonicación de 562.5 segundos, una amplitud del 96.6 % y una relación muestra/disolvente de 0.18 mg/mL. Se obtuvo una correlación satisfactoria entre la digestión ácida estándar y la extracción asistida por sonorreactor para todos los elementos en las muestras reales ($R^2 \approx 0.9$). Las recuperaciones fueron superiores al 110 % para Cu, Cd y Pb, mientras que para Zn y As fueron del 92 % y 63 %, respectivamente. En conclusión, el método de digestión ácida asistido por sonorreactor de ultrasonidos ha resultado una herramienta de extracción eficaz para la determinación de As, Cd, Cu, Pb y Zn en muestras de suelo industrial.

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**MAB-P25**

# DETERMINACIÓN DE $NO_2$ POR CROMATOGRAFIA IÓNICA EN CAPTADORES PASIVOS Y SU EVALUACIÓN EN EL AIRE AMBIENTE DE BARCELONA

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## Introducción

La Agència de Salut Pública de Barcelona, como centro de análisis de la red de vigilancia de la contaminación atmosférica de Catalunya, desarrolla evaluaciones de alta resolución de la calidad del aire en Barcelona para conocer los niveles y la evolución anual de los contaminantes críticos de la calidad en tramos de calle y áreas pequeñas.

Más allá de la gestión y evaluación de los datos de las estaciones fijas, estas evaluaciones realizadas a pequeña escala permiten la comparación de acciones mitigadoras de la contaminación del aire y pueden dar soporte a la toma de decisiones en la planificación municipal. Estas medidas reales pueden permitir estimar con mayor precisión la exposición ambiental de la población (por lugar de residencia).

## Objetivos

-Desarrollar una metodología para la determinación de $NO_2$ en aire ambiente mediante soportes de muestreo de captación pasiva y posterior determinación por cromatografía iónica con detección de conductividad.

-Medir la contaminación atmosférica de $NO_2$ a través de métodos de referencia y complementarios (captadores pasivos) en puntos alejados de las estaciones fijas.

- Disponer de un mapa de calidad del aire de alta resolución para NO2 por tramos de calle.

## Material y métodos

Las evaluaciones de los niveles de contaminación a escala pequeña se realizan con una unidad móvil de control atmosférico dotada de analizadores de acuerdo con los métodos de referencia de las directivas europeas de $NO_2$ i mediciones complementarias con difusores pasivos de $NO_2$, con muestreos por triplicado y tiempos de exposición mínimos de 7 días. El límite de cuantificación establecido es de 1,5 ppb.

Los resultados obtenidos mediante el muestreo pasivo se corrigen con los valores de referencia obtenidos en las estaciones de control atmosférico fijas.

## Resultados

La metodología desarrollada ha permitido realizar evaluaciones a pequeña escala durante el período 2017-2019 en los proyectos siguientes:

1. Evaluación de la calidad del aire en diferentes distritos de la ciudad: distrito de Sant Andreu y de Ciutat Vella.
2. Evaluación de la calidad del aire en proyectos de reordenación urbana y pacificación de zonas de la ciudad (supermanzanas): Sant Antoni, Horta-Guinardó y Eixample.
3. Los valores de precisión (< 15% entre triplicados) y exactitud (90-110%) obtenidos durante la validación del método son satisfactorios.

## Conclusiones

Los resultados obtenidos mediante los métodos de captación pasiva permiten disponer de datos fiables de los niveles de contaminación con una alta resolución territorial y a pequeña escala.

Con esta información se puede dar una respuesta válida a nivel de las actuaciones de planificación de ciudad y de comunicación e información a la población del impacto de la contaminación sobre la salud.

**MAB-P26**

# DETERMINATION OF NATURAL URANIUM AND THORIUM BY VARIOUS ANALYTICAL TECHNIQUES IN SOILS OF ZACATECAS STATE (MEXICO)

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Radionuclides with a long half-life such as uranium ($^{238}U$) and thorium ($^{232}Th$) represent today, along with other toxic trace elements, an important category of inorganic pollutants to be monitored because of their environmental hazards. Therefore, the knowledge of the amount of radioactivity in different environmental matrices, such as topsoils, has been a concern in many countries and has led to its determination in order to know the average background activity of these natural radionuclides in different geographical areas.

Besides the problems derived of the low concentrations (activities) of these radionuclides, their determination in soils is further complicated because a total mineralization of the samples, with nitric, hydrochloric and hydrofluoric acids, must be carried out previously to the application of the analytical technique chosen for their final determination. This leads to variable chemical recoveries and uncertainties that increase if additional separation or pre-concentration steps are necessary.

There are several analytical techniques proposed to determine uranium and thorium in the liquid extracts: Alpha-spectrometry (α-spec), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma double focusing sector field mass spectrometry (ICP-SF-MS) and X-ray Fluorescence (XRF). Each technique has advantages and shortcomings relative to interferences, precision, accuracy and cost, but their global suitability must also take into consideration whether each detection technique is overly complex or requires extensive and laborious pre-determination steps, as is the case with α-spec.

In this communication, we describe the results found when the analytical techniques listed above were applied to 32 top-soils sampled in undisturbed areas of the State of Zacatecas (Mexico), known for its large deposits of silver and other minerals, but with scarce and very old information about actinides contents. The objective is to find the more suitable analytical technique for this type of samples, optimizing the procedures and selecting the most appropriate methodologies, taking into account analytical parameters such as accuracy and time of analysis.

In order to assess if there are statistically significant differences among the results provided by the various analytical techniques, parametric (t-Student test) and/or non-parametric (sign test and Wilcoxon signed-rank test) paired-samples tests were applied No significant differences were found for ICP-MS and α-spec results, for both radionuclides, uranium and thorium. Although α-spec is the most widely used technique, it requires a laborious and time-consuming procedure involving the use of ion-exchange resins and a electrolysis pre-concentration previous to the determination, whereas ICP-MS can be used directly after the mineralization step. Therefore the use of ICP-MS can be proposed as an alternative to α-spec.

Finally, and after elimination of outlier samples, the average background concentrations of uranium and thorium in undisturbed top-soils of Zacatecas state, were stablished as 2.1 mg Kg-1 and 10.75 mg Kg-1, respectively, being these values similar to those found in similar areas of Mexico.

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**MAB-P27**

## CHEMICAL AND MINERALOGICAL CHARACTERIZATION OF UNDISTURBED SOILS FROM ZACATECAS (MEXICO)

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Toxic trace elements, also known as heavy metals, 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. Soils can act as temporary reservoirs from which those elements could be released back, and the real environmental risk must consider the total concentrations as well as their mobility/availability.

Whereas total concentrations can be determined by non-destructive analytical techniques, such as Neutron Activation Analysis (NAA) or X-ray fluorescence (XRF), the available/mobilizable (also known as pseudo-total) contents are usually determined after a non-total digestion of the soil sample with concentrated acids. This is the approach followed by US-EPA 3051A norm, the *de facto* standard, that proposes a microwave assisted acid digestion with concentrated nitric or/and hydrochloric acids. The elements present in the resulting liquid extracts, are then determined by using a technique adequate for trace analysis, such as Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The analytical results (total or available/mobilizable contents) can be studied by univariate (element to element) or by multivariate statistical techniques, such as Cluster Analysis (CA) or Principal Component Analysis (PCA), that allow to find relationships amongst soils and/or trace elements, not available at a first glance by the univariate approach.

In this communication, we describe the results of a survey carried out to assess the contents of toxic trace elements in 25 undisturbed top-soils of Zacatecas (Mexico), an area known for its large deposits of silver and other minerals, but with scarce and very old information toxic trace elements. The 25 samples were initially characterized by X-Ray Diffractometry (XRD), finding that most of the top-soils had a composition based on quartz and plagioclases, except for a few calcite-based samples.

The investigated elements were As, Cd, Cr, Cu, Ni, Pb and Zn. Total concentrations were determined with a Bruker S8 Tiger XRF spectrometer. For the available/mobilizable elements, the EPA-3051A was carried out on a Milestone Ethos Plus microwave, analyzing the resulting extracts with an Agilent 7500 ICP/MS spectrometer.

The average total contents of As, Cr and Ni ranged between 40-60 mg/kg 24-39 mg/kg; Cu, Pb and Zn between 130-180 mg/kg whereas Cd was negligible. On the other side, the average available/mobilizable contents of As, Cr and Ni were 24-39 mg/kg; Cu and Pb 70-100 mg/kg; Zn was around 180 mg/kg and Cd around 2 mg/kg. In all the cases, the soils were classified as suitable for 'industrial' and 'residential' uses, according to the NOM-147-SEMARNAT/SSA1-2004 Mexican norm.

PCA and AC allowed to separate the information due to the samples from that corresponding to the analyzed elements. Three different toxic trace element associations were found for both total and pseudo-total concentrations: Cr-Ni, As-Cu-Zn y Cd-Pb. In the case of the top-soil samples, both PCA and AC located some samples with higher contents in the studied elements.

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**MAB-P28**

# MICROEXTRACCIÓN DISPERSIVA EN FASE LÍQUIDA CON LÍQUIDOS IÓNICOS PARA LA DETERMINACIÓN DE CLOROBENCENOS EN MUESTRAS MEDIOAMBIENTALES POR CROMATOGRAFÍA DE GASES-ESPECTROMETRÍA DE MASAS

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Los clorobencenos (CBs) son liberados al medio ambiente a consecuencia de la actividad industrial o como resultado directo de su empleo en productos de limpieza, desodorantes, insecticidas, herbicidas o defoliantes. Dado que los procedimientos habituales de tratamiento de aguas residuales no eliminan de forma completa estos compuestos, una fracción significativa de los mismos contamina las aguas naturales, pudiendo depositarse sobre la materia sólida suspendida y sobre los sedimentos. Asimismo, las aplicaciones en agricultura originan su presencia en suelos agrícolas. Los CBs son peligrosos para la salud y han sido catalogados por la Agencia de Protección Ambiental estadounidense como contaminantes prioritarios [1,2].

Esta comunicación presenta la optimización, validación y aplicación de un procedimiento analítico para la determinación de diez CBs (1,4-diclorobenceno (1,4-DCB), 1,3-diclorobenceno (1,3-DCB), 1,2-diclorobenceno (1,2-DCB), 1,3,5-triclorobenceno (1,3,5-TCB), 1,2,4-triclorobenceno (1,2,4-TCB), 1,2,3-triclorobenceno (1,2,3-TCB), 1,2,4,5-tetraclorobenceno (1,2,4,5-TeCB), 1,2,3,5-tetraclorobenceno (1,2,3,5-TeCB), pentaclorobenceno (PCB) y hexaclorobenceno (HCB)) en aguas de distinta procedencia y suelos agrícolas. El procedimiento propuesto se basa en la preconcentración de los analitos mediante microextracción dispersiva en fase líquida (DLPME) empleando como fase extractante un líquido iónico (IL) generado *in situ*. Los extractos preconcentrados se analizaron mediante cromatografía de gases con espectrometría de masas (GC-MS). La incompatibilidad del IL con el sistema cromatográfico se solventó empleando desorción térmica desde microviales, esto es, calentando la fase enriquecida en la unidad de desorción térmica (TDU), lo que permite liberar los analitos desde el IL y su atrapamiento en el módulo de enfriamiento de la TDU, antes de ser transferidos a la columna analítica.

El análisis de los suelos implicó una etapa de extracción asistida por ultrasonidos (UAE), liberando los CBs desde la matriz sólida hasta una disolución reguladora de acetato de pH 4. La adición de los componentes iónicos del IL (275 µL de una disolución 1 M de cloruro de 1-hexil-3-metilimidazol ($[C_6MIm]Cl$) y 310 µL de disolución 1 M de bis(trifluorometil)sulfonilimida de litio ($Li[NTf_2]$) por separado, genera la fase extractante ya dispersa en el seno de la fase acuosa, pudiendo así omitirse el empleo de un disolvente orgánico como agente dispersante. Para el análisis de aguas, las muestras (10 mL) fueron directamente sometidas a la etapa IL-DLPME. La aplicación de diseños centrales compuestos (CCD) y el método ortogonal Taguchi permitió una fácil selección de las condiciones óptimas tanto de la etapa de preconcentración como de inyección a través de la TDU.

Debido a la presencia de efecto matriz, la cuantificación en muestras de suelos se llevó a cabo mediante adiciones estándar, mientras que para las aguas se emplearon patrones acuosos. Se encontraron niveles de contaminación comprendidos entre 0.03 y 2.7 ng/mL, dependiendo del analito, en las cuatro muestras de agua analizadas. Los suelos mostraron niveles comprendidos entre 0.8 y 50 ng/g, que correspondieron a 1,3,5-TCB y 1,2-DCB, respectivamente. El método IL-DLPME combinado con TD-GC-MS fue validado a través de estudios de recuperación y mediante el análisis de un material de referencia certificado (CRM143 BNAs-Sandy Loam 1), confirmándose su exactitud.

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**MAB-P29**

# ESPECIACIÓN DE PLATA ELEMENTAL Y PLATA (I) MEDIANTE MICROEXTRACCIÓN DISPERSIVA EN FASE SÓLIDA MAGNÉTICA Y ESPECTROMETRÍA DE ABSORCIÓN ATÓMICA CON CALENTAMIENTO ELECTROTÉRMICO

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Se han propuesto muchos procedimientos para la síntesis de nanopartículas basadas en óxidos de hierro [1]. La técnica de coprecipitación es probablemente la forma más simple y asequible de conseguir partículas magnéticas de esta naturaleza. La precipitación completa del óxido ocurre a pH comprendido entre 8 y 14 con una estequiometría de 2:1 ($Fe^{3+}/Fe^{2+}$) en un ambiente no oxidante. Varios grupos de investigación han señalado que si se emplean ultrasonidos en la síntesis de óxidos metálicos se tienen ventajas, como una distribución de tamaño más uniforme, mayor área superficial, menor tiempo de reacción y mejor pureza de fase sólida. Así, los óxidos de hierro se han sintetizado en presencia de ultrasonidos [2] desde distintos medios y, en un intento de simplificar al máximo la obtención de nanopartículas magnéticas de ferrita se ha propuesto la combinación del procedimiento de coprecipitación con ultrasonidos en atmósfera abierta [3]. En este caso, la óptima formación de las nanopartículas se consigue rápidamente para una relación $Fe^{2+}/Fe^{3+}$ 2:1, lo que resulta completamente distinto a lo recomendado en el método de coprecipitación, en el que la relación óptima es de 1:2. Las nanopartículas formadas no muestran magnetismo en ausencia de un campo magnético externo, pero si lo hacen en su presencia, lo que permite la separación de la fase sólida en presencia de otra líquida sin necesidad de filtración y/o centrifugación. La capacidad de retención de estas partículas está relacionada con su carga superficial, que es nula a un pH 7,9, aproximadamente. En medios más ácidos la carga es positiva.

En este trabajo se optimiza un procedimiento para la síntesis rápida de nanopartículas de ferrita en condiciones experimentales muy sencillas. Estas partículas, recién sintetizadas, muestran una gran capacidad de adsorción de especies inorgánicas. Tras la optimización de las condiciones experimentales se propone un procedimiento para la especiación de Ag(I) y Ag(0) en diversos tipos de muestras mediante la medida final de la plata con Espectrometría de Absorción Atómica Electrotérmica (ETAAS).

Las partículas magnéticas de ferrita se obtienen añadiendo un pequeño volumen de $NH_4OH$ concentrado a una mezcla 0,02 M de Fe(II) y 0,01 M de Fe(III) a 60 °C. Se aplica ultrasonidos durante 4 min y se separan de la fase acuosa con ayuda de un imán. Tras lavar con agua, las partículas así obtenidas se emplean directamente

Para conseguir la adsorción de Ag(I) en las partículas de ferrita se ajusta el pH de la muestra (10 mL) a 8 y se añade la disolución a las partículas recién obtenidas. La adsorción es inmediata. Se separa la fase sólida con ayuda de un imán, y se lava con agua. El residuo sólido se trata con un microvolumen de una disolución mezcla de ácidos nítrico y fluorhídrico y se mide la señal de la plata en el espectrómetro de ETAAS. El contenido en Ag(0) se obtiene en un segundo experimento tras tratamiento con $H_2O_2$.

El procedimiento optimizado se ha aplicado a la especiación de plata en muestras de agua de diversa procedencia, lixiviados de bayetas, estropajos, apósitos de enfermería y muestras de ensaladas embolsadas. Para comprobar la fiabilidad de los resultados obtenidos, se analizaron también tres materiales de referencia estándar SRM-1640a, TM-23.4 y TM-25.4 que corresponden a muestras de agua de distinta procedencia.

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**MAB-P30**

## A NOVEL SETUP FOR THE SEPARATION OF MICROPLASTICS FROM MARINE SEDIMENTS

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Microplastic, considered a new class of persistent environmental pollutants, has attracted great interest in the lastest years. Microplastics, defined as plastic particles between 20 µm and 5 mm in size, can originate from two sources, direct manufactured products that contain microplastics (such as facial scrubs and toothpaste), called primary microplastics, and fragments produced from larger pieces of plastic through photo-oxidation, mechanical action and/or biodegradation (called secondary microplastics)[1]. Notably, it was found that the majority of microplastics are secondary microplastics and their abundance in water would increase due to the continuous input of plastic materials from different origins into the environment, leading to continuous formation of secondary microplastics[2].

Microplastic particles have invaded the aquatic environment and are distributed widely in freshwaters and oceans, including water and sediments, reaching even the deep sea. Once microplastics reach the marine environment, they can be hazardous for marine organisms, including phytoplankton, zooplankton, bivalves (prunes, mussels, etc.), marine mammals, turtles, seabirds and fish. Microplastics can not only cause physical damage to marine organisms due to contact, absorption or ingestion, but also provide a potential pathway of exposure to organic pollutants for marine organisms[1,3]. Consequently, microplastics have recently been identified as an important global emerging problem that affects marine organisms.

The aim of this work is to improve the separation of plastic particles from aquatic sediments. For this purpose, we developed a small-scale novel density separation setup, made of glass, and compared the viability of different high-density salt solutions. We tested the efficiency of the separation setup by artificially spiking sediments with known quantities of microplastics of different specific density (polyethylene, polystyrene, polypropilene, polyvinyl chloride, polyethylene terephthalate and nylon 6,6).

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**MAB-P31**

# DETERMINACIÓN SIMULTÁNEA DE LOS PRODUCTOS DE DEGRADACIÓN PRINCIPALES DE PRINCIPIOS ACTIVOS FARMACOLÓGICOS Y PARABENOS, MEDIANTE EXTRACCIÓN SELECTIVA POR LÍQUIDOS PRESURIZADOS Y DETERMINACIÓN POR LC-MS/MS, EN LODOS DE DEPURADORA, COMPOST Y SUELOS

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Las prácticas de reutilización de lodos de depuradora en suelos agrícolas implican la asignación de un valor económico a este producto de la depuración, y resuelven parcialmente algunos problemas de la agricultura en relación con el bajo contenido en materia orgánica de los suelos en países de climas mediterráneos. Por otro lado, requieren una evaluación continua de los riesgos asociados a la presencia de ciertas sustancias químicas y agentes biológicos presentes en lodos de depuradora que pueden ser perjudiciales. Los avances científicos de los últimos años han suscitado una gran preocupación debido a la gran variedad de compuestos orgánicos presentes en esta matriz. Dentro de estos, los contaminantes emergentes son probablemente los que más interés han generado recientemente pues, además de carecer de una legislación específica, se sospecha de sus efectos perjudiciales para el medio y se ha demostrado que su presencia en lodos de depuradora es constante e inevitable a través de la excreta humana, al no completarse su degradación en la mayoría de las actuales estaciones depuradoras de aguas residuales urbanas. La mayoría de los estudios publicados se han centrado en la determinación y distribución de estos compuestos en la forma en la que se consumen (productos de partida), en sus principales fuentes (las aguas residuales urbanas) y en sus principales destinos directos (los ríos). Muy escasos aún y, en su caso, muy orientados a la contaminación de aguas, son los estudios que han abordado en profundidad la identificación y cuantificación de los productos de degradación de esos contaminantes emergentes en lodos y en suelos agrícolas tratados con lodos. Para una evaluación más completa de los efectos que origina al medio ambiente un determinado contaminante emergente y teniendo en cuenta la existencia de estudios que demuestran que algunos productos de degradación pueden ser tan tóxicos o incluso más que su producto de partida, el objetivo de este trabajo ha sido desarrollar y validar un método analítico que permitiera la determinación simultánea, tanto de un grupo de principios activos farmacológicos y de parabenos como de los productos de degradación principales derivados de estos en muestras de lodos digeridos anaerobicamente, compost y suelos. Para ello, se desarrolló un método de extracción selectiva mediante líquidos presurizados (SPLE), con el fin de lograr la extracción de todos los compuestos objeto de estudio junto con una etapa de clean-up en un solo paso. La determinación se llevó a cabo a través de cromatografía líquida de alta resolución acoplada a espectrometría de masas (LC-MS/MS). La metodología permite la determinación satisfactoria de todos los contaminantes en una sola inyección, con un tiempo de análisis cromatográfico de 30 minutos. Los límites de detección y cuantificación del método se encuentran dentro de los siguientes rangos: 0.04 ng $g^{-1}$ a 4.31 ng $g^{-1}$ en lodos digeridos; 0.04 ng $g^{-1}$ a 3.91 ng $g^{-1}$ en compost; 0.04 ng $g^{-1}$ a 2.17 ng $g^{-1}$ en muestras de suelo. La precisión, expresada en valores de porcentaje de desviación estándar relativa, fue inferior al 21% en todos los casos y los valores de exactitud variaron del 60 al 120% en más del 98% de los casos. Esta metodología desarrollada y validada se aplicó a los tres tipos de matrices sólidas ambientales mencionadas anteriormente. Dieciseis compuestos fueron detectados en muestras de lodos digeridos, aunque solo en ocho fue posible su cuantificación, tanto en lodos digeridos como en muestras de compost. Las concentraciones en lodos digeridos (desde 9 hasta 1881 ng $g^{-1}$) fueron entre 3 y 8.5 veces más altas que las encontradas en compost (desde 1.4 hasta 115 ng $g^{-1}$). En el caso de los suelos, sólo un compuesto fue cuantificado, a una concentracion de hasta 30 ng $g^{-1}$.

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**MAB-P32**

# DISRUPTORES ENDOCRINOS EN PROCESOS DE ESTABILIZACION DE LODOS DE DEPURADORA: DETERMINACIÓN, DISTRIBUCIÓN Y EVALUACIÓN DE RIESGOS

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El desarrollo de nuestra sociedad ha traído consigo nuevos problemas de contaminación ambiental y con ello, posibles efectos sobre la salud humana y la biosfera. El desarrollo de nuevos productos recurrentes en nuestra vida cotidiana como productos de cuidado personal, productos químicos de uso industrial, plásticos, textiles, etc., han dado lugar a la descarga continua al medioambiente de nuevas sustancias contaminantes, muchas de ellas consideradas como disruptores endocrinos, capaces de alterar el equilibrio hormonal y la regulación del desarrollo embrionario, provocando así alteraciones en el crecimiento, desarrollo, reproducción y comportamiento de los organismos vivos.
Estas sustancias llegan a las estaciones depuradoras de aguas residuales (EDARs) a través de los sistemas de alcantarillado y una vez en las EDARs, pueden quedar retenidas en los lodos generados como subproducto del proceso de depuración. Estos lodos son tratados mediante procedimientos de estabilización y deshidratación antes de su destino final. En España, la valorización agrícola supone un 82% de los lodos generados y, por tanto, la aplicación directa sobre los terrenos agrícolas. Uno de los principales problemas de la aplicación de estos lodos a suelos agrícolas es la presencia en estos de sustancias contaminantes, las cuales pueden contaminar no solo aguas superficiales o subterráneas sino, además, pueden ser absorbidos por los cultivos, incorporándose así a la cadena trófica [1].

El conocimiento del comportamiento de estas sustancias en los diferentes procesos de estabilización de lodos es de gran importancia para evaluar la eficacia de dichos tratamientos en la reducción de los posibles riesgos ambientales. Esto pone de manifiesto la necesidad de metodologías analíticas fiables que permitan la determinación de estos disruptores endocrinos en las diferentes tipologías de lodos de depuradora y tras la aplicación de estos lodos a los suelos agrícolas.

En este trabajo se empleó una metodología analítica, previamente optimizada y validada [2], para la determinación de 23 contaminantes disruptores endocrinos en lodos procedentes de diferentes procesos de estabilización (digestión anaerobia, digestión aerobia, compostaje y lagunaje). Se evaluó la eficacia de estos tratamientos en la eliminación de estos contaminantes y los posibles riesgos ambientales tras la aplicación de los lodos a campos de cultivo. Los surfactantes, seguidos de los biocidas y de los filtros UV fueron los contaminantes disruptores endocrinos que se encontraron a concentraciones más elevadas con concentraciones entre 512-1673 ng/g. La digestión anaeróbica resultó el proceso de estabilización de lodos que mejor elimina los contaminantes estudiados.

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**MAB-P33**

## DETERMINACIÓN ISOTOPICA DE URANIO EN MICROORGANISMOS

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La energía nuclear es una de las principales fuentes de generación de energía a nivel mundial [1]. Sin embargo, durante las diferentes etapas del ciclo del combustible nuclear se producen una serie de desechos que contienen radionucleidos potencialmente dañinos para la salud humana y para el medioambiente, siendo el uranio uno de los más abundantes.

Otra etapa crítica es el proceso de enriquecimiento isotópico de uranio, dado que su eficiencia es baja y se generan gran cantidad de residuos radiactivos. Una alternativa a los tratamientos clásicos puede ser la utilización de métodos biológicos que se basen en el potencial de algunos microorganismos para capturar selectivamente un isótopo, produciendo por tanto un fraccionamiento isotópico.

En el presente trabajo se ha explorado la posibilidad de eliminación y fraccionamiento isotópico de uranio utilizando algas genéticamente mejoradas (Chlamydomonas Chlorophyta y ChISPGI) con capacidad para capturar y bioacumular este elemento. Para ello, se cultivaron estas algas modificando una serie de factores (tiempo de exposición, concentración y composición isotópica), y posteriormente por centrifugación se separó el sobrenadante del pellet o residuo obtenido.

El procedimiento analítico realizado constó de dos fases: digestión ácida con calor en vaso abierto de PTFE, y separación radioquímica. En la primera fase, los sobrenadantes y los pellets fueron sometidos a una digestión ácida añadiendo agua oxigenada suprapur y ácido nítrico bidestilado, evaporando hasta sequedad para la posterior redisolución del residuo obtenido en ácido nítrico 4%. Una vez atacadas las muestras, fue necesario llevar a cabo una segunda fase de separación radioquímica sobre una alícuota de cada disolución de digestión, para disminuir la presencia de elementos mayoritarios en la muestra, y por tanto poder minimizar la incertidumbre de la medida de la relación isotópica $^{235}U/^{238}U$. Para ello se utilizó una columna comercial UTEVA [2,3], con un acondicionamiento previo con ácido clorhídrico 6M y ácido nítrico 0.1M. A continuación se cargó la muestra obtenida por digestión ácida y, después de una fase de lavado, se realizó la elución de uranio con ácido clorhídrico 0.02M.

El uranio total se determinó mediante un espectrómetro de Masas con Plasma de Acomplamiento Inducido modelo Thermo-Fisher iCAP-RQ, con sistema analizador cuadrupolo (Q-ICPMS). Las medidas de las relaciones isotópicas $^{235}U/^{238}U$ se llevaron a cabo con un espectrómetro de masas con fuente de plasma Thermo-Fisher Element 2 XR con sistema analizador compuesto por un sector magnético y un sector electroestático (HR-ICPMS), y el factor de discriminación instrumental se calculó utilizando como patrón isotópico de uranio el material de referencia certificada IRMM-053 (Institute for Reference Materials and Measurements).

El fraccionamiento isotópico obtenido fue de un 10.45% ([(Rmedidat=12días/Rmedidat=0días)-1]*100), por lo se pudo confirmar que el alga es capaz de realizar una captura isotópica selectiva positiva durante el proceso de bioacumulación, y por lo tanto producir un enriquecimiento del isótopo $^{235}U$.

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**MAB-P34**

# INFLUENCE OF MICROALGAL BIOMASS TREATMENT FOR THE RECOVERY OF NUTRIENTS ON THE PRODUCTION OF DEGRADATION BYPRODUCTS

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Microalgae-bacteria symbiotic systems are promising methods in residual water purification field. Microalgae use light as energy source and consume $CO_2$ in photosynthesis while purifying wastewaters with high area productivities, enhanced by symbiotic growing with bacterial aggregates. Biomass produced can be subsequently utilized as source of carbohydrates, proteins and lipids for further exploitation in the production of biofuels, biogas, animal feed or fertilizers. The process is also used to extract other high added value products like pharmaceutical or pigments. To reduce the costs of microalgae cultivation is necessary to enhance valorization of extracted components of the biomass.

The principal difficulty in nutrient recovery from biomass is cell wall disruption. For this purpose, several pretreatments have been used in order to maximize nutrient extraction while avoiding the degradation of the released compounds by either chemical or cell metabolism processes, which result in the production of inhibitory byproducts that can reduce the quantity and quality of the target products.

Different processes based on physical or chemical pretreatment followed by enzymatic hydrolysis were applied to microalgal biomass to evaluate its effects on the production of a variety of inhibitory compounds. Algal-bacterial biomass was grown in a photo-bioreactor treating pig manure residual wastewaters. Seven pretreatments at two different operational conditions were performed: acid (HCl), alkali (NaOH), oxidative ($H_2O_2$), steam explosion, ultrasound and bead mill. Enzymatic hydrolysis was performed on the whole pretreated suspension. Samples of liquid fractions after pretreatment and after enzymatic hydrolysis were taken for further analysis. Monosaccharides and degradation byproducts were quantified by HPLC using a Bio-Rad HPX-87H ion-exclusion column installed on a Waters e2695 separation module. A refractive index detector (Waters 2414) was used to quantify the concentration of monosaccharides and degradation byproducts such as methanol, xylitol, glycerol, ethanol and acetone, while oxalic, formic, acetic, lactic, butyric, succinic and levulinic acids, furfural and HMF were measured with a photodiode detector (Waters 2998) at 210 nm [1].

Multivariate analysis of the results using Principal Component Analysis, PCA, and Hierarchical Cluster Analysis, HCA, was performed to expose the correlation between the inhibitory byproducts and the treatments applied. It was found that much more degradation byproducts were released by chemical treatments, while physical pretreatments produced negligible amounts of degradation compounds. Enzymatic hydrolysis generated slightly more degradation byproducts in physically pretreated samples, due to lower previous pretreatment degradation or cell metabolism degradation. In order to further investigate the mechanisms and sources of degradation byproducts, experiments using pure materials containing carbohydrates, proteins and lipids are being performed.

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**MAB-P35**

# ESTUDIO COMPARATIVO DE MÉTODOS DE EXTRACCIÓN DE MICROPLÁSTICOS EN MUESTRAS DE PLANCTON MARINAS

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La caracterización de los microplásticos existentes en muestras del medio ambiente marino resulta esencial para conocer su impacto y establecer acciones para combatir este tipo de contaminación de los océanos. Sin embargo, aún no existen protocolos estandarizados ni métodos de referencia para determinarlos en las diversas matrices ambientales. Entre ellas, el plancton, es una de las menos estudiadas y se ha reportado que el zooplancton puede llegar a ingerirlos [1,2]. A su vez, los peces también podrían incorporarlos en su organismo, bien sea por confusión en su alimentación o por ingestión de zooplancton [3]. Estudiar esta matriz es, pues, de capital importancia, puesto que es el primer eslabón de la cadena trófica.

La estrategia más adoptada para tratar muestras biológicas consiste en digerir la materia orgánica, filtrar e identificar los microplásticos (en general con microscopía FTIR). La destrucción de la materia orgánica se basa habitualmente en digestiones enzimáticas y/o tratamientos alcalinos (como KOH), y/o tratamientos oxidantes ($H_2O_2$). Pero estos dos últimos aún presentan muchas dudas respeto a si preserva la integridad de los polímeros a recuperar.

En el presente estudio se comparan dos métodos para el tratamiento de muestras de plancton: un tratamiento alcalino basado en KOH al 10% y otro enzimático combinando proteasa y lipasa. En ambos se ha realizado un último paso con $H_2O_2$ para completar la destrucción de la materia orgánica. Para ello se han generado muestras representativas de plancton, sobrecargadas con microplásticos (rango 70-300 µm). Se ha evaluado la destrucción de la materia orgánica y la recuperación analítica para cada tipo de plástico (PP, PS, PE, PET, PA y PVC).

La caracterización combina la lupa binocular y microscopio FTIR (en modo reflectancia). Para ahorrar tiempo en la búsqueda de los microplásticos con el microscopio FTIR, se han diseñado unas placas de aluminio con oquedades para el emplazamiento de las partículas a estudiar.

Como validación adicional, el protocolo enzimático se aplicó a muestras de un ejercicio interlaboratorio en el marco del proyecto Baseman JPI-Oceans, con resultados excelentes.

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**MAB-P36**

# EFFECT OF FERTILIZATION PRACTICES ON THE CONCENTRATION OF TRACE ELEMENTS IN AGRICULTURAL SOILS

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Soil plays an important role in agricultural production, as it participates in the growth and nutrition of plants. Background concentrations of potentially toxic trace elements (PTTEs) in soil depend in great extent on the bedrock material geology, but agricultural practices, industrial emissions, atmospheric deposition or irrigation with contaminated water can cause accumulation of these elements. Of special concern is the accumulation of PTTEs in agricultural soils due to application of liquid and solid manure, compost or inorganic fertilizers [1,2].

The effect of organic and inorganic fertilizers on agricultural soil in relation to available and total P contents, organic matter and a variety of elements including As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn has been investigated. Soil samples were collected from two different locations, Agustoni and Anguil, in La Pampa (Argentina). At each location, two neighbouring and controlled plots were fertilized with organic or inorganic fertilizer for several years to grow experimental crops, mainly soya. Soil samples were collected at two depths (0-5 cm and 5-20 cm). The 46 samples collected were dried, ground, sieved (particle size 0.2 mm) and sent to the University of Valladolid. Samples were characterized by X-ray diffraction (XRD), finding silica and plagioclase as major phases, with the Agustoni samples showing less compositional homogeneity than the Anguil samples. Organic matter was determined by redox titrimetry (Walkley-Black method) and available phosphorous by the molybdenum blue spectrophotometric method in sodium hydrogencarbonate extracts. Trace elements were extracted with nitric acid in a microwave oven (US-EPA-3051 method) and determined by ICP-OES or ICP-MS. The concentrations of the different elements did not exceed the limit values in soils for agricultural use, according to the Argentinian regulations [3].

Analysis of Variance (ANOVA) and boxplots, applied separately to the results from the two locations, demonstrated a significant effect of soil depth on organic matter and total P contents, while the fertilizer used affected significantly the contents of As, Cu, Ni and Pb. A joint interpretation of the results from both locations by Principal Component Analysis and Hierarchical Cluster Analysis showed two separated groups of variables, the first including Zn, Cr, Ni, Cd, P and extractable P, and the second one clustering very closely the rest of elements. Samples from the two locations were grouped separately, but no clustering of samples by type fertilizer or soil depth was observed, thus suggesting that the bedrock geology has a much more pronounced effect on the contents of the investigated variables in the soils than the fertilizer applied.

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*
## *Valladolid 18-19 julio 2019*

## *COMUNICACIONES PÓSTER*

## *NANO-BIO-ÓMICAS*

**NBO-P01**

# SÍNTESIS DE NUEVOS NANOCLÚSTERES METÁLICOS FLUORESCENTES Y SU APLICACIÓN COMO MARCAS PARA LA DETECCIÓN BIMODAL DE PROTEÍNAS ESPECÍFICAS EN SUERO HUMANO

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Avances recientes han permitido la síntesis sencilla de nanoclústeres (NCs) metálicos fluorescentes de diámetro inferior a 3 nm, constituyéndolos como una nueva clase ultrapequeña y biocompatible de fluoróforos para su uso como marcas biológicas. Dado su pequeño tamaño, los NCs pueden utilizarse para marcar anticuerpos sin apenas afectar al reconocimiento antigénico. Además, los NCs metálicos pueden ser combinados con otras modalidades de detección como la espectrometría de masas elemental, permitiendo la detección con gran amplificación de biomarcadores. Así mismo, mediante la bioconjugación de NCs de diferentes metales con anticuerpos seleccionados, será posible realizar análisis simultáneos de diferentes biomoléculas. La disponibilidad de NCs de distintos metales es clave para el desarrollo de estos ensayos simultáneos. Hasta el momento se han usado principalmente oro y plata [1] [2] para la síntesis de estas partículas. Por ello, actualmente es de especial interés la búsqueda de nuevas rutas para la síntesis de NCs fluorescentes de otros metales.

En este contexto, se propone la investigación de la síntesis en medio acuoso de NCs de platino e iridio con pequeños ligandos tiolados como estabilizadores. El platino y el iridio son metales nobles, no tóxicos y biocompatibles. En nuestros estudios, la síntesis de estos NCs metálicos ha sido optimizada en términos de emisión fluorescente, baja dispersión de tamaños, rendimiento de la síntesis y estabilidad de los NCs. Para ello se han empleado medidas de microscopía electrónica de transmisión, medidas espectroscópicas y espectrometría de masas.

Los PtNCs e IrNCs muestran picos de fluorescencia característicos (620 y 430 nm respectivamente). De esta forma, constituyen marcas de detección bimodal: mientras que la fluorescencia permite la optimización de la metodología de forma rápida y barata, la espectrometría de masas podría ofrecer mejores límites de detección gracias a la amplificación de la señal debida a los cientos de átomos de Pt e Ir por NC.

Como prueba de concepto, los PtNCs fueron empleados para la determinación de immunoglobulina E (IgE) en suero humano mediante fluorescencia y espectrometría de masas. Para ello, en una primera etapa se bioconjugó el anticuerpo Anti H IgE con los PtNCs siguiendo el método de la carbodiimida y este bioconjugado fue utilizado en un inmunoensayo competitivo. Se consiguió un límite de detección (LD) extremadamente bajo en las medidas con ICP-MS (0,08 $ng \cdot mL^{-1}$) gracias a la alta amplificación conseguida (aprox. 1300 átomos de platino por anticuerpo de reconocimiento). El LD obtenido por fluorescencia fue algo mayor (0.6 $ng \cdot mL^{-1}$), pero aun suficiente para muchas aplicaciones. Finalmente, la aplicabilidad de la metodología desarrollada se demostró a través de la determinación de IgE en cinco sueros y la validación de los resultados se llevó a cabo con éxito para las mismas muestras con un kit ELISA comercial.

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**NBO-P02**

## MULTILAYER GRAPHENE MEMBRANES AS EFFICIENT PRECONCENTRATION PLATFORMS FOR MULTIELEMENTAL ANALYSIS BY TOTAL REFLECTION X-RAY FLUORESCENCE

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The presence of trace metal ions in waters represents an important environmental risk due to their toxicity and bioaccumulation ability. Thus, European Directives provided maximum contaminant levels in drinking waters of 1 µg/L for Hg, 20 µg/L for Ni, 10 µg/L for As, 10 µg/L for Se and 5 µg/L for Pb [1]. In order to perform the analytical control of these and other metals and metalloids, a wide number of nanomaterials (NMs) have been recently proposed as novel sorptive platforms for trace metal analysis by total reflection X-ray fluorescence spectrometry (TXRF), especially in water samples [2]. Among these NMs, carbon-based NMs are especially suitable for TXRF due to the reduced background in comparison with metallic NMs at high concentration that overlap several spectral lines of target analytes. Graphene and carbon nanotubes (CNTs) fulfill this requirement. However, the synthesis of graphene seems simpler and does not require metallic catalysts, typically used in CNTs synthesis. Additionally, graphene has a high surface area, adequate for solid-phase extraction (SPE). Typical methods using graphene-based sorbents involve graphene nanosheets that need different operations such as elution, centrifugation, filtration or decantation before analyte measurement. However, multilayer graphene membranes reported for the first time by our group are easy to handle and do not require an isolation step after extraction. Moreover, graphene membranes can be directly analyzed by TXRF after placing them onto quartz sample carriers. We have reported the use of graphene membranes for direct immersion thin film microextraction (DI-TFME) of Cr(VI) [3] and headspace thin film microextraction (HS-TFM) of an organomanganese compound [4]. This work reports on the implementation of multilayer graphene membranes for microextraction of metal chelates prior to TXRF [5]. The strategy consists of forming neutral metal chelates with ammonium pyrrolidine dithiocarbamate (APDC) of Co(II), Ni(II), As(III), Se(IV), Au(III), Hg(II), Pb(II) and Bi(III) in aqueous solution, with further sorption onto the graphene membrane floating onto the sample. Hydrophobic interactions between carbon-based ring structures of graphene and chelates facilitate the extraction. Multielemental sorption of neutral APDC chelates was performed under optimal conditions, *i.e.* reduction time for the synthesis of graphene membranes, 8 min; pH 3; 0.04% (w/v) APDC concentration; 50 mL sample volume; 900 rpm stirring rate; 10 min extraction time; room temperature conditions. Detection limits ranged from 0.2 to 0.6 µg/L, which were below the maximum contaminant levels in waters established by the European Directives. Method validation was successfully performed against two certified reference materials (*i.e.* QC1014 and QC1187). Different types of water samples were analyzed and suitable recoveries were obtained for non-saline waters, with the exception of Bi(III), for which low recoveries were achieved in all samples. The method is simple, sensitive and solvent free, being suitable for the simultaneous determination of metal ions at trace level in waters.

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**NBO-P03**

## NITROGEN AND SULPHUR CO-DOPED CARBON DOTS AS TURN-ON FLUORESCENT SENSORS FOR THE DETECTION OF PERIODATE ANION

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Carbon dots (CDs), nanomaterials with sizes ranging from 2 to 10 nm synthesized from natural non-toxic precursors such carbohydrates, have emerged as one of the most promising fluorescent nanoprobes because of their great advantages including low toxicity, good biocompatibility, high aqueous stability and easy functionalization [1]. In the last years, hydrothermal synthesis of CDs using green carbon sources, *e.g.* fruit peels and fruit juices [2,3], have been tried in order to achieve cost–effective synthetic procedures with low environmental impact. However, the reaction time and temperature involved in hydrothermal processes remain a drawback. Additionally, analytical assays based on fluorescent CDs typically consist of two stages, *i.e.* firstly, synthesis and purification of fluorescent CDs and secondly, detection of the target analyte.

Herein, we report an *in situ* strategy based on the integration of synthesis of CDs and sensing within a single step, thus simplifying the procedure and shortening the time required to accomplish the assay. Following this strategy, highly blue-fluorescent nitrogen (N) and sulphur (S) co–doped CDs were synthesized by photochemical oxidation of broccoli aqueous extracts [4]. The use of a natural precursor rich in proteins and glucosinolates (natural S–linked glucosides) facilitated the efficient N and S co–doping of the CDs during the photochemical reaction without the need for further post–synthetic treatments. N and S co–doping allows more active sites in the CDs surface resulting in an enhancement of their luminescent properties [5]. Monodisperse CDs (~8 nm average size) with up–conversion fluorescence properties and a quantum yield of 22% were obtained.

Furthermore, CDs fluorescence enhancement (turn–on) was observed when periodate anion ($IO_4^-$) was added to the reaction medium. Periodate is a highly oxidizing agent that can promote the scission of simple polysaccharides (glycol cleavage) such as glucose, fructose or sucrose, thereby acting as a catalyst during carbohydrates oxidation [6]. Thus, when CDs were synthesized in the presence of $IO_4^-$, oxidation of the carbohydrates was favoured yielding CDs with smaller size that might promote the confinement of emissive energy traps leading to a fluorescence enhancement. The increase in fluorescence intensity caused by the presence of $IO_4^-$ in the reaction mixture was the basis for a fast, green, low cost and sensitive fluorescent assay for detecting this oxidizing agent in wastewater. The detection limit was 19 µM $IO_4^-$ and the repeatability expressed as the relative standard deviation was 3.2% (N=5). Besides, recovery studies performed with synthetic wastewaters showed results in the range of 90–97%, hence indicating the reliability of the fluorescent assay.

The described approach does not require large and tedious procedures for purification of CDs and the synthesis time is remarkably shortened as compared to conventional hydrothermal methods. Besides, the involvement of other oxidizing species and inhibitors of the photochemical reaction could expand the scope of this assay.

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**NBO-P04**

## SINGLE PARTICLE ICP-MS COMO MÉTODO DE SCREENING PARA LA DETECCIÓN DE NANOPARTÍCULAS

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La detección de partículas individuales mediante espectrometría de masas con plasmas de acoplamiento inductivo (SP-ICP-MS) es una metodología que permite obtener información sobre la concentración en número y distribución de tamaños de nanopartículas, a niveles de concentración por debajo de 1000 partículas por mL y tamaños de 10-20 nm, permitiendo diferenciar las formas particuladas de un elemento de sus formas disueltas. Los métodos de screening para la detección de partículas no se basan únicamente en la identificación de un analito en base a su composición química, como sucede con analitos convencionales, sino que también se requiere información relacionada con su tamaño para identificar a las partículas como tales. SP-ICP-MS es una técnica basada en el conteo de partículas y presenta unas prestaciones adecuadas para ser utilizada como método de screening para la detección rápida de partículas, habiendo sido utilizada esporádicamente con estos fines, aunque utilizando metodologías no validadas.

SP-ICP-MS se basa en el análisis de suspensiones de partículas muy diluidas ($10^7$-$10^8$ $L^{-1}$) y en la adquisición de datos a frecuencias muy altas (>100 Hz) durante cortos períodos de tiempo (minutos). Las señales obtenidas consisten en barridos de tiempo compuestos de una línea base, debida a la señal de fondo o a la presencia de especies disueltas del elemento monitorizado, y pulsos producidos por las propias partículas. La identificación de partículas en dichos barridos implica la discriminación entre las lecturas procedentes de las partículas y de la línea base, mediante la aplicación de un valor umbral definido a partir de un múltiplo de la desviación estándar de la línea base. Los criterios de discriminación fueron estudiados en base al concepto de valor crítico desarrollado por Currie [1] con el fin de minimizar el número falsos positivos (lecturas de línea base consideradas como partículas). Por otro lado, a partir de los barridos de blancos procesados adecuadamente, se pueden establecer límites de decisión, basados en estadística de conteo, que permiten identificar la presencia de partículas por encima de un determinado tamaño.

Los objetivos de este trabajo han sido la evaluación y validación de SP-ICP-MS como método de screening para la detección rápida de nanopartículas. La metodología estudiada se ha aplicado a la detección de nanopartículas de plata y oro en alimentos procedente de aditivos autorizados. Con la instrumentación utilizada se obtuvo un límite de decisión de $10^5$ partículas por litro, pudiendo detectar la presencia de partículas de plata y oro por encima de 25-30 nm. Las prestaciones analíticas como método de screening fueron: sensibilidad 80%, especificidad 97%, frecuencia de falsos positivos 3%, frecuencia de falsos negativos 20% e intervalo de incertidumbre $6x10^4$-$3x10^5$ $L^{-1}$.

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**NBO-P05**

## ELECTROANALYTICAL TECHNIQUES FOR THE CHARACTERIZATION OF NANOMATERIALS


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Nanomaterials are increasingly used in industrial, biomedical and consumer products applications [1]. Among them, silver nanoparticles (AgNPs) have probably been the most studied and with more applications due to their antimicrobial properties. However, at the same time, their release into the environment is becoming a major concern due to its negative impact on living organisms [2], and for this reason, in the European Union the legislation is becoming more restrictive.

The development of nanotechnology must be accompanied by studies that make possible to know the effects that these materials will have both on man and on the environment. Therefore, it is increasingly necessary to develop powerful analytical techniques to determine and characterize nanomaterials in different media. The electroanalytical techniques open new doors for the selective detection of NPs and their ions present in the same solution, without the need for complex previous treatments of the samples studied [3].

In this work, the size of AgNPs was determined in commercial samples of food supplements and parapharmacy products (Wellness®, Biovedik® and High-Stability®) using Voltametry of immobilized (nano) particles (VInP) and nanoparticle impact chronocoulombimetry (CRCInP). The results of these techniques were compared with the determination of sizes by scanning electron microscopy of field emission (FESEM) and transmission electron microscopy (TEM).

The mean sizes that were obtained with VInP were 27±3 nm. (Wellness®), 18±1 nm. (Biovedik®) and 47±6 nm. (High Stability®). Using the CRCInP, the average sizes for the Wellness® were 10±3 nm., Biovedik® 14±3 nm., and in the High-Stability® two populations with average sizes of 16±3 nm and 27±3 nm. were observed. The statistical analysis (t-test of two samples, $p < 0.05$) showed significant differences between the results obtained by the VInP and CRCInP techniques.

The study by FESEM of the samples Biovedik® and High-Stability® showed the existence of nanoparticles with an average size of 19±6 nm. and 20±10 nm., respectively. The SEM measurements of the Wellness® sample showed AgNPs of a size of 9±8 nm. Comparing the results of electron microscopy with the two electroanalytical techniques, more accurate results are obtained using CRCInP with respect to VInP. In addition, the CRCInP allows obtaining a distribution of sizes of each one of the samples in a single experience.

The value of the average size obtained for the Wellness® and High-Stability® samples with VInP was approximately double that obtained by CRCInP and TEM / FESEM. This result is consistent considering the agglomeration of the nanoparticles during the immobilization of the sample on the surface of the electrode, so that the effect of agglomeration on unstabilized samples is the main disadvantage of the VInP method for characterizing the sizes of the AgNPs. However, the average size of the AgNPs in the Biovedik® sample was 18±1 nm. by VInP, 14±3 nm. by CRCInP, and 19±6 nm. with FESEM. This shows that for samples stabilized against the agglomeration of NPs, VInP can be a quick and simple method, as well as useful, for the estimation of AgNPs sizes.


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**NBO-P06**

## SINGLE CELL ANALYSIS WITH ICP-TQ-MS DETECTION TO CHARACTERIZE SELENIZED YEAST

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Selenium is an essential trace element for humans that is present in proteins and enzymes, which play critical roles in very relevant processes such as the antioxidant defence. Additionally, at high doses it has been used in cancer prevention treatments. Thus, adequate dietary intake of Se is essential and can be complemented using selenium supplements. One of the most commonly used supplements is based on selenized yeast (Se-yeast) that is produced by growing different strains of *Saccharomyces cerevisiae* on Se-containing media and can accumulate up to 3000 µg $g^{-1}$ of Se [1]. In this regard, the optimization of Se incorporation during Se-yeast production and the characterization of the amount of Se and its chemical forms present in the final products require the development of suitable analytical methods.

In this work, we propose the development and optimization of an analytical methodology based on single cell inductively coupled plasma mass spectrometry (SC-ICP-MS). The instrument was fitted with a microflow nebulizer and a total consumption spray chamber to permit individual cell introduction with high transport efficiency. Additionally, the use of triple-quadrupole detection, makes possible the monitoring of constitutive elements like phosphorus useful as cell markers. Analytical figures of merit are presented, and the methodology is applied to the analysis of this food supplements in order to determine important parameters like the percentage of selenium-containing cells and the Se amount per cell. Complementary techniques such as HPLC-ICP-MS and TEM were used for the identification of the different Se forms present in the yeast samples.

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**NBO-P07**

## SEARCHING FOR PREDICTIVE BIOMARKERS OF PANCREATIC CANCER IN SERUM VOLATOLOME. A PRELIMINARY STUDY

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Pancreatic adenocarcinoma (PAC) is the third most frequent cause of cancer mortality in Spain, and the specific survival by PAC has not changed significantly in the last 40 years, regardless of the stage of the disease. Patients with advanced disease continue to have a 5-year survival of 2% or less. An obstacle in the fight against PAC is that its early symptoms are varied and non-specific, leading to late detection of cancer. Therefore, it would be of great interest to find biomarkers that allow early diagnosis.

Volatile organic compounds (VOCs) are the final products of cell metabolism and can be detected in both breath and body fluids. VOCs can reflect metabolic changes in response to external factors and intrinsic factors such as inflammation, necrosis, alteration of the microbiota, and of course cancer.

In this work we present the preliminary results of a study in which the volatolomic profile of the blood serum of 20 PAC patients and 20 healthy volunteers from La Rioja is compared. The analysis of these serum samples was performed by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC-MS). GC-MS has been already used in metabolomic studies on pancreatic cancer both alone [1,2] and in combination with other metabolomic platforms such as LC-GC and $^{1}$H-NMR [3]. In this work, it is the first time that it is proposed to be coupled to HS-SPME for the identification of PAC biomarkers, although the use of this technique has been previously reported in another type of cancer such as melanoma [4].

Using the NIST library, a series of volatile organic compounds (VOCs) have been tentatively identified in the serum samples. It is worth noting that higher levels of VOCs were found in healthy individuals than in PAC patients.

Machine learning algorithms were used to select five chromatographic peaks that allow to differentiate between cancer patients and healthy individuals (ROC curves with AUC> 0.7). The best predictive results are obtained with the combination of two of them (peaks at 2.87 and 16.77 min) with an AUC value of 0.8631 comparable to those of the two tumor biomarkers used for the diagnosis of pancreatic adenocarcinoma, carbohydrate antigen 19-9 (CA 19-9) with AUC 0.8546 and carcinoembryonic antigen (CEA) with AUC 0.9327.

Mass spectrum searching of these peaks in the NIST library provided tentative identifications, with match factors around 800, corresponding to short chain alkanes, alcohols and esters (4-10 carbon atoms).

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**NBO-P08**

# LIQUID CHROMATOGRAPHY WITH UV/VS AND MS DETECTION FOR THE DETERMINATION OF EPIGENETIC EVENTS IN DNA AS CONSEQUENCE OF NANOPARTICLES EXPOSURE.

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Incorporation of engineered nanomaterials into industrial and biomedical application is increasing fast and so are the potential risks associated with them. Physical and chemical properties of materials with particles sizes in the nanometric scale are different compared to those observed at larger scales. One of those changes is related to the coefficient of diffusion which ensures that their main transport mechanism in the environment is air diffusion. Because of that the effect of this kind of nanoparticles in human health have attracted interest in the last few years from a safety point of view. [1]

In the case of titanium dioxide ($TiO_2$) a normal-size particles (>100 nm) are biologically classified as inert. However, due to the introduction of nanotechnology, the use of $TiO_2$ nanoparticles (<100 nm) has been increased and it is used in an extensive range of products, such as consume [2], cosmetics or biomedical. It's true that the health effect of these nanoparticles is still quite unknown but the epigenetic modifications that they can cause in biological systems start to be a subject of research interest. In this regard, recent experiments have identified changes in DNA methylation (mainly cytosine methylation) after the exposure to $TiO_2$ nanoparticles.[3] Cytosine methylation of genomic DNA plays an essential role in many important biological processes, such as genomic imprinting, tumorigenesis, gene regulation and retrotransposon silencing. Aberration of DNA methylation has detrimental effects on development, including embryonic lethality, cancer and genome instability. It is of critical importance that DNA methylation patterns be stably inherited over many cell divisions.

In the present work we aim to develop an analytical method that permits the simultaneous determination of methylcytosine and hydroxymethyl cytosine as main epigenetic events in DNA to further evaluate the effect of $TiO_2$ nanoparticles. This is done by using reversed phase liquid chromatography coupled to two different detection systems: UV/VIS and MS (Q-TOF). Analytical figures of merit of both separation-detection systems as well as potential interferences arising from the different abundances of the two epigenetic events with respect with the unmodified nucleobase in DNA samples will be addressed.

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**NBO-P09**

# CANCER CELL TARGETING AND THERAPEUTIC DELIVERY OF SILVER NANOPARTICLES BY MESOPOROUS SILICA NANOCARRIERS: INSIGHTS INTO THE ACTION MECHANISMS USING QUANTITATIVE PROTEOMICS

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Recently, silver nanoparticles (AgNPs) are receiving considerable attention as potential anticancer therapeutic agents [1]. However, their use is limited by their tendency to aggregate, as well as by the different mechanisms of toxicity that can induce in the organism, since their administration could also cause cytotoxic effects on healthy cells. Thus, the design of a delivery nanosystem able to selectively target cancer cells is necessary. In this way, mesoporous silica nanoparticles (MSNs) are a promising tool as a drug delivery system due to its high biocompatibility and intrinsically large drug loading capacity in their tuneable mesopore arrangement. Furthermore, thanks to their high density of silanol groups which can be chemically modified, MSNs present a high versatility in terms of surface modification to introduce organic molecules such as targeting ligands or stealth moieties [2]. In this line, cancer tissues and cells overexpress different surface receptors, like the transferrin receptor (TfR), that can be used for selecting specific targeting ligands [3]. Recently, we have used MSNs as nanovehicles decorated with transferrin (Tf) to provide a nanoplatform for the nucleation and immobilization of AgNPs (MSNs-Tf-AgNPs). We have performed the physico-chemical characterization of the nanosystem and evaluated their therapeutic potential in cancer treatment using bioanalytical strategies [4].

In the present communication, we will describe the use of flow cytometry to evaluate the selectivity of the designed nanosystem towards cancer cells. We have compared the cellular internalization of the nanosystem into HepG2 cells that overexpress TfR, with the internalization into MC3T3-E1 cells, which display a normal expression of the same receptor. Then, the cellular localization of the nanosystem inside the cells have been studied by transmission electron microscopy (TEM). In addition, with the aim of elucidating the biomolecular mechanisms by which the proposed nanosystem exerts its anticancer action, we have performed a SILAC-based quantitative proteomic experiment aimed to the identification of differentially expressed nuclear proteins in cells exposed to the nanosystem. This quantitative proteomic experiment has been further validated through the analysis of gene expression by qPCR. This approach has allowed for the identification of key proteins and transcripts involved in cell cycle regulation, cell proliferation and DNA damage. Thus, the present nanosystem offers the possibility of a targeted therapy using reduced doses of silver nanoparticles as the cytotoxic agent.

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**NBO-P10**

## EVALUATION OF THE POTENTIAL OF RHODIUM NANOPARTICLES IN PHOTODYNAMIC THERAPY FOR CANCER

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Nowadays, a variety of nanomaterials are emerging as promising tools for cancer therapeutic and diagnostic applications. In this way, the potential of metallic nanoparticles such as AgNPs, AuNPs or SeNPs, have already been demonstrated. The nanometric scale of these nanoparticles provides them with a multitude of unique physicochemical properties, including the ability to absorb incident electromagnetic radiation. This radiation makes the free electrons of the metal to collectively resonate at a certain wavelength in a process called surface plasmon resonance (SPR). Then, absorbed energy can be converted into heat by means of what is known as photothermal conversion, dissipating through particles-medium interfaces at a rate dependent on the medium, the particle size and the light source, and increasing the temperature only in its immediate surroundings. In these terms, metal NPs may serve as light-activated heaters in the nanoscale. Some studies have revealed that the activation of metallic NPs via near infrared (NIR) laser light, which is a non-ionizing tissue penetrating radiation, is a promising non-invasive and selective tumour treatment [1-3].

Although several Rh complexes have been studied as potential therapeutic agents [4], the potential of RhNPs in photodynamic therapy for cancer has not yet been studied. Therefore, and with the aim of evaluating such potential, the synthesis of RhNPs of different sizes have been optimized. The different synthesized RhNPs have been analytical characterized by electron transmission microscopy (TEM-EDX) and X-ray diffraction (XRD), in order to determine their size and morphology, as well as to confirm their composition and crystallinity. In addition, a functional biological characterization has also been carried out using different cellular lines as *in vitro* model. Several bioanalytical assays have been used to evaluate the cellular viability upon exposure at different concentrations and irradiation times, the cell cycle profile and the induction of apoptosis, among others.

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**NBO-P11**

# DETERMINACIÓN EN CONTINUO DE ROJO ALLURA EN BEBIDAS CARBONATADAS POR INHIBICIÓN DE LA FLUORESCENCIA DE DOTS DE $MoS_2$

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Los materiales bidimensionales, como el grafeno y más recientemente los dicalcogenuros de metales de transición (TMDs), han sido de gran interés debido a sus excepcionales propiedades. Estos nanomateriales ($MX_2$), con una estructura tipo sándwich en la que el metal y los átomos calcógenos se unen por enlaces covalentes, son fácilmente exfoliables ya que las láminas adyacentes interaccionan mediante fuerzas de Van der Waals [1]. A partir de estas láminas y, tras un tratamiento térmico adecuado, se obtienen nanopuntos o dots.

A diferencia de las nanoláminas de TMDs, los dots presentan marcada fotoluminiscencia que puede ser inhibida debido a su interacción con determinados compuestos capaces de absorber su energía de emisión [2]. Este hecho se puede utilizar para el desarrollo de metodologías de análisis para la cuantificación de analitos que inhiban su señal.

En este trabajo se presenta una metodología para la determinación de colorantes alimentarios sintéticos mediante la inhibición de la emisión de fluorescencia de nanopuntos de TMDs. Estos aditivos proporcionan color y mejoran las características organolépticas de los alimentos, aunque como suelen contener grupos azo (R-N=N-R') y anillos aromáticos, suponen riesgos para la salud [3]. Por ello, la ley regula las cantidades máximas permitidas en alimentos, siendo de gran importancia su determinación y cuantificación.

La figura 1A muestra la inhibición de la fluorescencia de nanopuntos de $MoS_2$ en presencia de Rojo Allura. Gracias a esta interacción, se ha desarrollado un método analítico para la determinación del colorante mediante análisis por inyección en flujo (FIA). La figura 1B muestra los fiagramas de la interacción de $MoS_2$-Dots con concentraciones crecientes de analito.

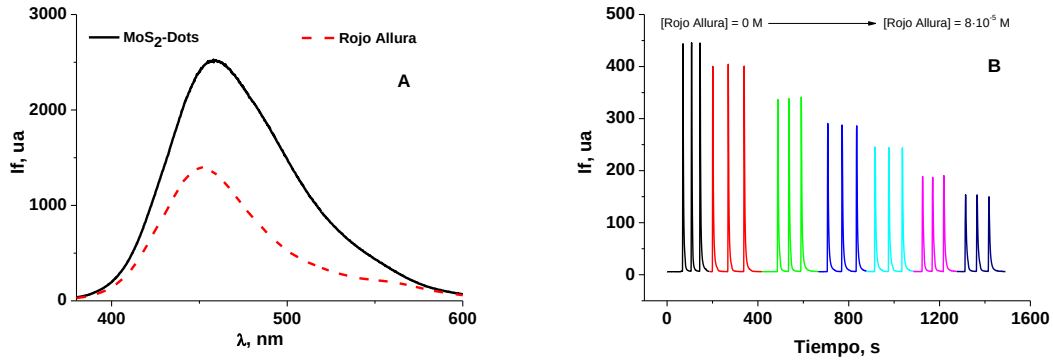


Figura 1. A) Espectros de fluorescencia de dots de $MoS_2$ en ausencia y presencia del colorante alimentario Rojo Allura. B) Fiagramas con detección fluorescente de dots de $MoS_2$ en presencia de concentraciones crecientes de Rojo Allura.

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**NBO-P12**

## MOLECULARLY IMPRINTED POLYMERS INDUCED BY MAGNETIC FIELD POLYMERIZATION

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The synthesis of molecularly imprinted polymers (MIPs) in different formats has attracted a great interest in recent years, with special emphasis in the development well-organized nanostructures. Thanks to their improved properties, these structures are especially suited for some applications, such as sensing, drug delivery or even analytical separations, when compared with the commonly applied bulky configurations. Up to now, different techniques have been applied to the synthesis of nano and micro-MIP features including, electrical deposition, photolithography, microstereolithography, mechanical microspotting, soft-lithography, e-beam or electrospining.
Nanocomposite MIPs are defined as materials where the inclusion of an inorganic material provides additional properties, such as magnetic susceptibility, luminescence or conductivity [1]. The incorporation of superparamagnetic iron oxide cores, quantum dots microcrystals or metallic nanoparticles, among others, has broadened up the applicability of MIPs to different fields. Thereby the use of magnetic MIPs is particularly promising because the use of magnetic fields for the removal of the nanocomposites from the solutions avoids the need of tedious separation steps, such as centrifugation or filtration, in analytical separations
In this work [2], we present a novel approach for the preparation of molecularly imprinted polymer (MIP) coatings directly onto magnetic nanoparticles (MNPs) by using alternating magnetic fields (AMFs) to trigger the polymerization reaction. MIPs were synthetized using rhodamine 123 (R123) as model template molecule, methacrylic acid (MAA) as functional monomer and trimethylolpropanetrimethacrylate (TRIM) as cross-linker. Different parameters were optimized such as, the amount of oxide nanoparticles and the composition of the pre-polymerization mixture. Under the optimized conditions, it was possible to carry out the thermal polymerization of a thin MIP shell on each MNP core by using electromagnetic heating without altering the properties of the recognition layer. The thickness of the polymerized MIP layer grafted onto the MNPs was fine-tuned by adjusting the dose of electromagnetic field (101.4 kHz, total power dissipation = 105 W). The resulting magnetic MIP nanoparticles (MNP-MIPs) were characterized by ATR and X-ray diffraction (XRD) spectroscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS).

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**NBO-P13**

# IMPROVING THE IDENTIFICATION COVERAGE IN METABOLOMICS ANALYSIS OF PIG FECAL SAMPLES BY CHROMATOGRAPHIC TECHNIQUES COUPLED TO MASS SPECTROMETRY IN HIGH RESOLUTION MODE: INFLUENCE OF SAMPLE PREPARATION

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Pig feces is an interesting biological sample to be implemented in metabolomics experiments by virtue of the information that can be deduced from the interaction between host and microbiome. However, pig fecal samples have received scant attention, especially in untargeted metabolomic studies. In this research, an analytical strategy was planned to maximize the identification coverage of metabolites found in pig fecal samples. For this purpose, two analytical platforms such as LC–QTOF MS/MS and GC–TOF/MS were combined to evaluate their additivity in terms of identification. Concerning sample preparation, six extractants with different polarity were tested to evaluate their extraction performance and, in the particular case of GC–MS, two derivatization protocols were compared to check the influence of this step on the detection capability of the methods. The tested extractants were deionized water, MeOH, 1:1 (*v/v*) deionized water:MeOH, ethyl acetate, hexane and dichloromethane; and derivatization protocols with and without methoxymation were applied.

Combination of all the extractants and analytical platforms yielded a total number of 303 tentatively identified compounds, being the main families fatty acids, conjugates and derivatives, amino acids and analogues, carboxylic acids and derivatives, bile acids, bilirubins and derivatives, and carbohydrates and derivatives.

According to the results obtained in this research, the utilization of deionized water:MeOH as extractant for GC–MS analysis is recommended, while for LC–MS/MS analysis the extracts obtained with MeOH or deionized water:MeOH and ethyl acetate increased the number of identified compounds. Concerning the derivatization step, the implementation of methoximation previous to silylation provided the identification of three α-keto acids that were not detected with the other tested strategies. Thus, since these are important metabolites related to microbiome status, the implementation of a double derivatization strategy could be of interest for given studies. Concerning the complementarity of the two analytical platforms evaluated in this research (LC–MS/MS and GC–MS), their combined use allowed the identification of 303 metabolites, of which only 12 were common to both platforms. Thus, it is obvious that the use of the two platforms are complementary to obtain a comprehensive view of the pig feces metabolome.

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**NBO-P14**

## METABOLOMICS ANALYSIS OF HUMAN SWEAT BY GAS CHROMATOGRAPHY–TIME OF FLIGHT/MASS SPECTROMETRY IN HIGH RESOLUTION MODE

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Human sweat is a promising biofluid to be implemented in clinical metabolomics studies by virtue of its no invasive sampling and varied composition, which is modified by several pathologies. In fact, sweat has been recently used in metabolomics studies to discriminate between lung cancer patients and risk factor individuals (mainly smokers). Elucidation of sweat metabolome has so far been achieved using three analytical platforms (NMR, LC–MS and GC–MS). One hundred and three metabolites have been detected by combination of the three platforms, being GC–MS the approach providing the highest coverage, detecting more than 60% of them. Nevertheless, these previous studies did not focus attention on non-polar metabolites, presumably a minor fraction owing to the aqueous nature of sweat. The detection of non-polar compounds in sweat would increase the interest of sweat for clinical studies, especially in dealing with biomarkers search. With these premises, a method for metabolomics analysis of human sweat by gas chromatography–time of flight/mass spectrometry (GC–TOF/MS) in high resolution mode has been developed to expand the detection of compounds, with special emphasis on non-polar compounds. Different sample preparation strategies were tested to check their influence on the metabolomics profile of sweat. The performance of three extractants with different polarity (ethyl acetate, dichloromethane and hexane) and a deproteinization protocol were compared by using, in all cases, a dual approach: (1) application of a derivatization protocol prior to GC–MS and, (2) direct injection to detect volatile organic compounds. One hundred forty-three compounds were tentatively identified by high resolution MS. Mostly lipids, but also amino acids, benzenoids and other interesting metabolites, were identified. It is worthy to distinguishing the ability of the GC–MS protocol to detect lipids, family that represented around 32% of the compounds detected by the GC–MS platform. Among the compared protocols, methyoximation plus silylation after liquid–liquid extraction with dichloromethane was the most suited option to obtain a representative snapshot of sweat metabolome. Because of the involvement of most of the identified metabolites in key biochemical pathways, this study opens new possibilities to the use of human sweat in the search for lung cancer biomarkers.

**NBO-P15**

## COMBINACIÓN DE LA INMUNOHISTOQUÍMICA Y ABLACIÓN LASER-ICP-MS PARA LA BIOIMAGEN DE MMP-11 COMO NUEVO BIOMARCADOR DEL CÁNCER DE MAMA.

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Los procesos patológicos de la progresión del tumor mamario y las metástasis se asocian con la metaloproteinasas de matriz (MMPs), que son una familia de endopeptidasas dependientes de zinc estructuralmente relacionadas, y están implicadas con el crecimiento de tumores malignos.

En particular, la MMP-11 (Stromelysin-3) se expresa en in los fibroblastos del estroma que rodea a las células epiteliales tumorales y altos niveles de esta metaloproteinasa están asociados con un con la progresión del tumor y peor pronóstico del cáncer de mama (BC)[1]. Por ello, conocer los niveles de MMP11 y su distribución en el tejido mamario podrían ser un nuevo biomarcador para diagnóstico y pronóstico del cáncer de mama.

Las técnicas de bioimagen de metals en tejidos mamario ha surgido como una importante herramienta para aumentar el conocimiento del papel de los metales en el BC. Sin duda, la técnica normalmente utilizada para bioimagen de metales en tejidos es la ablación láser acoplada a la espectrometría de masas con fuente de plasma de acoplamiento inductivo (LA-ICP-MS). Además, la combinación ensayos de immunohistoquímica (IHC) con LA-ICP-MS ha sido aplicada con éxito para estudiar distribución de biomarcadores de proteicos[2] (MMPs, HER2, MUC-1) implicados en el BC con gran sensibilidad y resolución lateral.

Este trabajo se desarrolló un ensayo inmunohistoquímico basado en nanopartículas de oro en combinación con el LA-ICP-MS para estudiar la distribución de MMP-11 en tejidos de pacientes con cáncer de mama. La expresión de MMP-11 fue mayor y más heterogénea en muestras metastásicas en comparación con muestras de tumores no metastásicos. A modo de ejemplo, se presentan muestras de dos pacientes que fueron diagnosticadas con características y grados de cáncer de mama coincidentes. Aunque ambos pacientes tenían un pronóstico y tratamiento similares, solo uno desarrolló metástasis. Estos hallazgos demuestran que la obtención de imágenes de los tumores de mama por LA-ICP-MS puede ser una herramienta útil para ayudar al pronóstico y al tratamiento del cáncer de mama.

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**NBO-P16**

# GOLD NANOPARTICLES FORMATION AS AN INDICATOR OF ENZYMATIC METHODS: COLORIMETRIC L-PHENILALANINE DETERMINATION

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Quantitative determination of L-phenylalanine (L-Phe) in physiological fluids is crucial in diagnosis and therapy of disorders of phenylalanine catabolism, such as Phenylketonuria (PKU), a generic disorder characterised by a deficiency in the liver of the hepatic enzyme phenylalanine hydroxylase that catalyses the conversion of phenylalanine into tyrosine, leading to an excessive Phe accumulation in the serum. [1] This levels are most commonly monitored by chromatography methods, although there are also different enzymatic spectrometric methods not routinely applied. All of them are time consuming and require complex sample preparation and sophisticated instrumentation.

It is therefore that, in this work, an enzymatic-colorimetric method has been developed based on the reaction between L-Phe and the L-aminoacid oxidase (LAAO) in presence of Au (III), which lead to the formation of gold nanoparticles (Figure 1) that can be related to the concentration of L-Phe in the sample. These plasmonic nanoparticles show molecular absorption around 600 nm (Figure 2).

Since dissolved $O_2$ regenerate the enzyme and modify the mechanism of the kinetic reaction, we find two different types of nanoparticles formed with two concentration ranges: fluorescent nanoparticles for $[Phe]<[O_2]_{dissolved}$ (<200 µM) and the plasmonic nanoparticles for $[Phe]>[O_2]_{dissolved}$ (>200 µM). Thus, the amount of plasmonic nanoparticles can be related with Phe concentrations, which are associated with the PKU pathologies [2]. The Figures of merit of this methodology are being studied and applied to real samples.

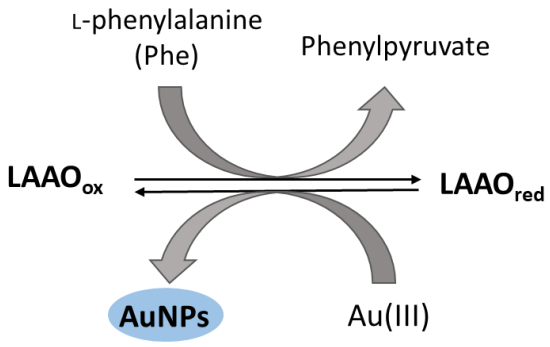


Figure 1: Scheme of the enzymatic reaction in presence of Au (III).

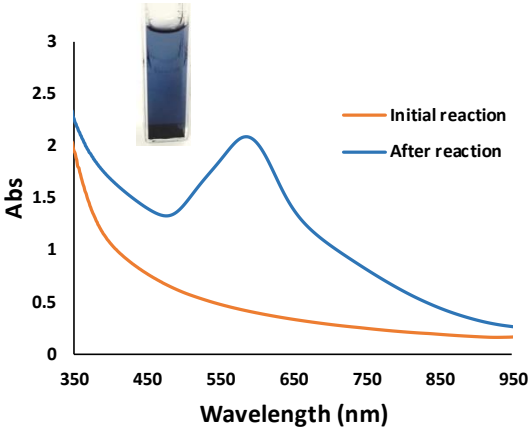


Figure 2: Absorption spectra before and after enzymatic reaction LAAO – L-Phe in presence of Au (III) ($[Phe]>[O_2]_{disolved}$)

Acknowledgements: This work has been supported by the MINECO of Spain (project CTQ2016-7684R) and by the founding to research group of the DGA-FEDER (E25_17R).

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**NBO-P17**

# "PLUG-AND-PLAY" POLYMERIC MONOLITHIC MICROCARTRIDGES WITH GOLD NANOPARTICLES FOR THE ANALYSIS OF PROTEIN BIOMARKERS BY ON-LINE SOLID-PHASE EXTRACTION CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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In this study, polymeric monolithic microcartridges with gold nanoparticles (AuNPs) were investigated for the analysis of protein biomarkers by on-line solid-phase extraction capillary electrophoresis-mass spectrometry (SPE-CE-MS) [1]. "Plug-and-play" microcartridges were prepared cutting into (7 mm) pieces a monolithic poly(glycidyl-co-ethylene dimethacrylate) (10 cm) capillary column modified with ammonia and subsequently functionalized with AuNPs [2]. To evaluate the performance of these novel microcartridges, human transthyretin (TTR), which is a protein related to different types of familial amyloidotic polyneuropathies (FAP) [3-4], was selected as a case study.

The different steps of the SPE-CE-MS method were optimized with standard solutions. Under the optimized conditions, TTR solutions were loaded during 5 minutes at 930 mbar in a 10 mM ammonium acetate solution at pH 5.0, which is a pH value close to the isoelectric point of the protein (pI~5.4). This solution was also used as separation electrolyte to prevent the elution of TTR while filling the capillary after sample loading, before the elution and the electrophoretic separation. For the elution, a small plug of 30 mM ammonium phosphate solution at pH 9.0 resulted in the best results. Limits of detection (LODs) for TTR by SPE-CE-IT-MS were 50 times lower than by CE-IT-MS (5 vs 250 $mg \cdot L^{-1}$). These LODs were slightly improved by SPE-CE-TOF-MS (1 $mg \cdot L^{-1}$), and were very similar to the values previously obtained using immunoaffinity microcartridges with intact antibodies against TTR (1 $mg \cdot L^{-1}$ by SPE-CE-TOF-MS) [5]. The SPE-CE-IT-MS method was linear between 5 and 25 $mg \cdot L^{-1}$ of TTR, repeatability in migration times and peak areas were similar to CE-IT-MS (2.3 and 6.0%, respectively) and the microcartridges could be reused more than 10 analyses. The optimized method was finally applied to analyze TTR in serum samples, after selecting an appropriate sample pretreatment to avoid the interference of the sample matrix, overcoming the limited selectivity of the polymeric monolithic sorbent with AuNPs.

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Acknowledgments:
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**NBO-P18**

## MASSIVE DETERMINATION OF POLAR LIPIDS IN PLASMA BY LC–MS/MS

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Lipids are biological molecules with a wide chemical diversity and varied functionality. Lipids are involved in numerous biological functions and have been widely associated to the development of several diseases. Polar lipid, especially glycerophospholipids, constitute the main components of cell membranes and have functions as precursors for signaling molecules in many cellular and physiological processes. For this reason, the development of methods with high capability for detection of lipids in biological samples is required. Discrimination of lipids in polar (mainly glycerophospholipids, sphingolipids and saccharolipids) and non-polar (glycerolipids, sterol lipids and fatty acyls) fractions is frequently used in clinical analysis since polar and non-polar lipids require different procedures for sample preparation, chromatographic separation and mass spectrometry detection. Several methods have been used for determination of polar lipids, with predominance of those based on LC–MS/MS due to their high ionization capability. The main problems in the determination of polar lipids are the chemical diversity, the isomeric character and the wide concentration range at which they can be detected.

The objective of this research was to develop a method for massive qualitative/quantitative determination of polar lipids in biological samples. The fragmentation of polar lipids in mass spectrometry has been extensively studied and the mechanisms involved in the activation reactions have been already described. With these premises, an LC–MS/MS strategy was designed by combination of data acquisition methods provided by a triple quadrupole mass analyzer. The strategy was carried out in two steps: a) A first step for detection of lipids by monitoring selective fragmentation patterns representative of each lipid family; and b) a second step for confirmation of lipid species by detection and identification of product ions associated to the fatty acids. Finally, the transitions list provided two MRM methods to ensure the detection of all transitions with enough instrumental sensitivity.

The combination of the two methods allowed the detection of 398 polar lipids in plasma in a total analysis time of 64 min (32 min per run) and with a very simple sample preparation protocol. Detected lipids were confirmed with the LipidMaps database. The studied polar lipids pertained to the following families: ceramides, sphingomyelins and sphingoid bases, lysoglycerophospholipids with different polar groups, glycerophosphatidylcholines, glycerophosphatidylethanolamines, glycerophosphatidic acids, glycerophosphatidylglycerols, glycerophosphatidylinositols, glycerophosphatidylserines and plasmalogens (O-alkyls and O-alkenyls families). The method has been applied to a cohort formed by 384 individuals to obtain a qualitative and quantitative distribution of polar lipids in plasma. The most concentrated lipid families in relative terms were lysoPLs, plasmalogens and PCs, with mean concentration of 58.0, 17.1 and 8.3%, respectively. Then, SMs and PEs reported a relative concentration of 2.0%, followed by PSs with 1.1%. The rest of families provided relative concentrations below 0.5%: PG (0.4%), PA (0.2%), CERs (0.2%), SG (0.1%) and PI (0.1%).

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**NBO-P19**

# CUANTIFICACIÓN DE BIOMARCADORES TUMORALES EN CÉLULAS INDIVIDUALES MEDIANTE ANTICUERPOS MARCADOS CON METALES Y SC-ICP-MS.

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El cáncer de mama es una de las principales causas de morbimortalidad en mujeres en el mundo, por lo que existe un gran campo de investigación en torno a la búsqueda de biomarcadores que permitan la detección temprana, el pronóstico y la predicción de la respuesta farmacológica de este tipo de tumores. En este sentido, la desregulación de la homeostasis del hierro está asociada a la progresión, la agresividad y la recurrencia del cáncer de mama [1]. La homeostasis del hierro involucra un buen número de proteínas, incluidas la transferrina y la ferritina, que están íntimamente relacionadas con la incorporación y el almacenamiento del hierro, respectivamente. En especial, la sobreexpresión de proteínas importadoras de hierro y la subexpresión de exportadores de este metal causan un incremento en el hierro intracelular, que es necesario para la supervivencia y rápida proliferación de las células tumorales. El receptor de transferrina (TfR) es una glicoproteína de membrana que media en la incorporación celular del hierro unido a la transferrina (Tf). Debido a las necesidades más altas de hierro que presentan las células tumorales para asegurar su proliferación, se espera que una mayor expresión de TfR en la membrana se relacione con una mayor malignidad del tumor mamario.

Existen varios métodos para la cuantificación de receptores de transferrina en cultivos celulares e, incluso, en tejidos. Sin embargo, al ser una proteína de membrana, su determinación no es sencilla y, además, no existe ningún método para la cuantificación de estos receptores a nivel de célula individual, lo que puede proporcionar información muy valiosa acerca de pequeñas poblaciones celulares que quedarían ocultas por la gran mayoría de células en análisis totales. Por ello, hemos desarrollado una metodología que permite la cuantificación de los receptores de transferrina en células individuales mediante *single-cell*-ICP-MS (SC-ICP-MS) en combinación con el uso de anticuerpos con marcas metálicas isotópicamente enriquecidas y un sistema de introducción de muestra específico. Los resultados obtenidos con esta técnica serán evaluados y comparados con los obtenidos mediante inmunoensayos tradicionales (ELISA) y mediante un inmunoensayo basado en marcas elementales desarrollado en el grupo de investigación, que permite la cuantificación de TfR mediante ICP-MS.

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**NBO-P20**

# TEJIDOS DE FIBRAS COAXIALES PARA EL DESARROLLO DE BIOSENSORES DESECHABLES Y SISTEMAS SENSORES MICROFLUÍDICOS

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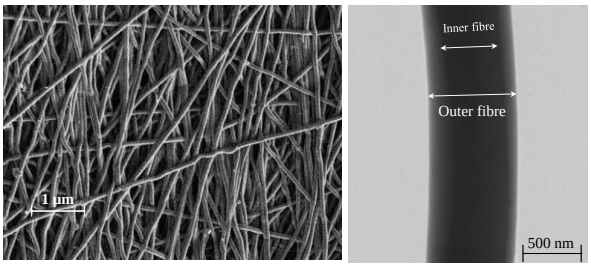
La transducción óptica de oxígeno es una herramienta potente y muy útil para el desarrollo de biosensores ópticos [1-4]. Pese a que en la bibliografía se pueden encontrar una gran variedad de biosensores con transducción óptica de $O_2$, la mayoría son solo dispositivos de laboratorio que no cumplen con las condiciones mínimas que son exigibles para su comercialización. Desde un punto de vista general se puede decir que los grandes inconvenientes que presentan estos biosensores son tres: 1) su elevado precio; 2) su limitada estabilidad; y 3) su difícil implementación en dispositivos que permitan medidas remotas.




En general, se puede decir que, en este tipo de biosensores, la fase sensora debe contener la(s) enzima(s) que catalizan la reacción y un compuesto que permita medir la concentración de $O_2$. Para ello es necesario disponer de un material con dos funcionalidades: 1) debe permitir la inmovilización de una biomolécula en su superficie para catalizar la oxidación del analito y 2) debe contener un luminóforo sensible a oxígeno para medir el consumo del $O_2$ durante la reacción de oxidación. Además, debido al carácter inherente de la biomolécula (hidrófila) y del indicador de $O_2$ (hidrófobo), estos materiales deben contener, al menos, dos entornos químicos diferentes: un entorno hidrofílico para la inmovilización de la enzima y un entorno hidrofóbico para la encapsulación del indicador de oxígeno, lo que dificulta y encarece la producción de estos materiales.

En esta comunicación se presenta el desarrollo y caracterización de un material coaxial multifuncional preparado a partir de dos soluciones poliméricas diferentes mediante co-electrospinning, que origina tejidos no tejidos con una gran superficie activa, excelentes propiedades mecánicas (resistencia a la abrasión, alta resistencia a la tracción, flexibilidad, fácil manejo), alta resistencia química y que simplifica el desarrollo de biosensores enzimáticos con transducción óptica de oxígeno, así como diferentes alternativas para la incorporación/inmovilización de biomoléculas (atrapamiento físico, inmovilización covalente dirigida o no, e inmovilización por afinidad).

Para demostrar la aplicabilidad de estos materiales en el desarrollo de biosensores y sistemas de microfluídica, se presentarán ejemplos de estos sistemas para la detección de glucosa, ácido úrico, colesterol y ácido láctico.

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**NBO-P21**

# DESARROLLO DE TEJIDOS DE NANOFIBRAS FUNCIONALIZADOS CON ESTREPTAVIDINA PARA MEJORAR LA INMOVILIZACIÓN DE URICASA EN EL DESARROLLO DE BIOSENSORES CON TRANSDUCCIÓN ÓPTICA DE OXÍGENO PARA LA DETERMINACIÓN DE ÁCIDO ÚRICO EN FLUIDOS BIOLÓGICOS

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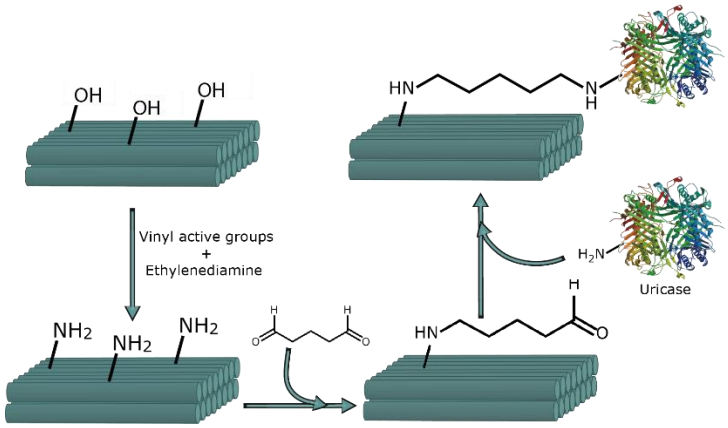
En este trabajo se ha modificado un tejido no tejido multifuncional producido mediante coelectrospinning para incluir en su superficie nuevos grupos funcionales que aumente sus prestaciones para el desarrollo de biosensores con transducción óptica de oxígeno.

Para ello, se partió de un material base desarrollado por la empresa NanoMyP® que estaba formado por fibras coaxiales, donde la fibra interna tenía carácter lipofílico y contenía in indicador de oxígeno (PdTFPP) y la fibra externa era de carácter hidrofílico y poseía grupos aminos primarios en su superficie. Para mejorar sus prestaciones se propone usar los grupos $NH_2$ para inmovilizar estreptavidina y usar el sistema Streptavidina-Biotina para inmovilizar la enzima en la superficie de la membrana; se seleccionó como enzima modelo la uricasa, por lo que fase sensora a desarrollar sería para la determinación de ácido úrico en fluidos biológicos.




Una vez optimizado el proceso de funcionalización se evaluaron dos estrategias para la inmovilización de la uricasa en el tejido; estrategias que diferían en la longitud del separador entre la estreptavidina y el grupo biotina, determinando para cada una de ellas tanto la cantidad de enzima inmovilizada como la actividad relativa de la enzima. Se determinó que las mejores prestaciones se conseguían usando Biotin-PEG como espaciador. La caracterización analítica del material para determinación de ácido úrico en suero determinó que el biosensor propuesto presenta un límite de detección de 4 µM y un intervalo dinámico lineal de 15 a 500 µM. Para validar el material, la misma muestra se analizó un método oficial de referencia, obteniendo un coeficiente de correlación de 0.996.

Agradecimientos. Este trabajo ha sido financiado por el programa People Programme (Marie Curie Actions, Multi-ITN) del 7 programa Marco de la Unión Europea (grant agreement n° 608104 (EUROMBR)) y el MINECO (proyecto CTQ2014-53442-P).

**NBO-P22**

# DEVELOPMENT OF A NOVEL THERAGNOSTIC PLATFORM BASED ON MESOPOUROUS HYBRID BIOFILMS

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In the last years, new complex nanostructures and multifunctional materials with new physicochemical properties have emerged in the field of biomedical nanotechnologies. These new materials have opened up not only to the development of diagnostic and therapeutic devices but also to “in vitro” regeneration. In this work we have integrated bioinspired silica mesomaterials, hybrid biofilms with 3D printed technologies for the construction of platforms that integrates the diagnostic, therapy and biomedical engineering.

As a “proof of concept” we have demonstrated the biocompatibility of new designed polymeric 3D material enabling the ordered deposition of heterofunctional polyelectrolytes multilayers, nanomaterials and immunochemistry for selective electrochemical sensing micro-devices. Complementary, hybrid "core-shell" nanoparticles based on biosilicates (core @ Si-shell) labelled with biomolecules and with controlled release capacity, have been synthesized and characterized. The possibility of controlling the surface area, the particle size (range: 200 nm - 1 μm) and most importantly, the shape, porosity as well as the surface charge properties have been demonstrated. Additionally, the use of these biosilicates on nanostructures organized with "layer by layer" technology have allowed to demonstrate; (i) the possibility of encapsulate biomolecules in a thermostable manner (ii) keeping its biological activity and (iii) their controlled release by a pH change, demonstrating its use as a possible element of theragnostic nanoplatforms.

Financial support from the Spanish Ministry for Economic Affairs and Competitiveness CTQ2015-73105-EXP and RTI2018-096278-B-C22 is acknowledged

**NBO-P23**

## OPTICAL MICROBIAL IMMUNOSENSOR BASED ON HYBRID BIOFILMS

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Bacterial infection is a common cause of morbidity and mortality worldwide. The identification and quantification of bacterial pathogens has become a key point in biodefense, diagnostics, and drug discovery research. In the last years, intensive research has been undertaken in diagnostic tools focused on point of care devices based on biosensing modules that allows a more effective management of the infectious diseases decreasing the mortality, hospitalization and, associated costs. In fact *Staphylococcus aureus* is the major cause of morbidity and mortality in patients suffering Cystic Fibrosis since they have a compromised immune system and suffer from both acute and chronic pulmonary infections by a multitude of opportunistic pathogens [1].

In this context, the present work shows results towards the design and characterization of heterofunctional polyelectrolytes multilayers integrating nanomaterials and immunochemistry for selective electrochemical sensing micro-devices for the detection of *S. aureus.* The rationale of our analytical approach relies on the completion of the affinity reaction within the assemblies using enzyme protein labelled immunoreagents. Detection is then achieved by the displacement of the immunoreagent by the presence of the sample rendering a concomitant signal decrease. The analysis only requires the addition of a sample drop to the immunochips and incubation for 20 minutes. The results obtained under optimal conditions shows a detection limit of $5x10^2$ CFU/mL, a EC50 of $2.0x10^6$ CFU/mL and LR=$3x10^4$ UFC/mL – $3.2x10^7$ UFC/mL. These values remain below the limits found with identical reagents with the traditional ELISA format, enabling a direct integration into microsystems.

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**NBO-P24**

## IN-DROP COLORIMETRIC PLASMONIC SENSING OF MERCURY INVOLVING GOLD NANORODS: APPLICATION TO THIOMERSAL DETERMINATION IN PHARMACEUTICALS

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The present work reports on the development of a miniaturized method for mercury sensing involving gold nanorods (GNRs). The basis of the method consists on the shift of the longitudinal plasmonic band of GNRs produced in the presence of elemental mercury ($Hg^0$). In this work, a three-phase microseparation approach has been employed with the aim of achieving improved sensitivity, selectivity and a nearly negligible consumption of GNRs. Thus, the method involved i) *in situ* reduction of Hg(II) into $Hg^0$, ii) trapping of the volatile into an aqueous microdrop containing GNRs exposed to the headspace above the sample, and iii) acquisition of the UV-vis spectra of enriched GNRs by a microvolume UV-vis spectrophotometric system. The effect of experimental parameters affecting the drop composition, namely GNRs aspect ratio, cetyltrimethylammonium bromide concentration and presence of water miscible organic solvents, on the analytical response was initially evaluated. Then, microextraction parameters were optimized following a 2-stage multivariate optimization, including Placket-Burmann and central composite designs for screening and optimization of microextraction parameters, respectively. The evaluated microextraction parameters included sample volume, extraction time, hydrochloric acid concentration, sodium borohydride concentration, sodium chloride concentration, stirring rate and temperature. In addition, the proposed sensing mechanism (amalgamation reaction) was assessed by microvolume UV-vis spectrophotometry, transmission electronic microscopy and scanning electron microscopy with energy-dispersive X-ray spectrometry. Under optimal conditions, a linear log-log calibration graph was obtained between the analytical response (shift of the longitudinal plasmonic band of GNRs) and Hg(II) concentration in the range 10-500 ng/mL. The limits of detection and quantification were found to be 0.5 and 1.5 ng/mL (as Hg), respectively, whereas the repeatability, expressed as relative standard deviation, was 8.4% (n=10). The method was finally applied to the determination of an organomercurial compound commonly used as antiseptic/preservative named as thiomersal (sodium ethylmercurithiosalicylate) in pharmaceuticals. A photochemical decomposition of thiomersal into Hg(II) was required prior to the analysis following the optimized procedure. Non-significant differences were found at a 95% level of confidence between the labeled concentration of thiomersal in the pharmaceutical samples analyzed and found concentrations. In addition, satisfactory average recoveries (92.8-103.9%) were obtained in all analyzed samples, thus demonstrating the applicability of the proposed method to the analysis of pharmaceutical samples.

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*
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## *COMUNICACIONES PÓSTER*

## *PREPARACIÓN DE MUESTRAS*

**PMU-P01**

# DEVELOPMENT AND VALIDATION OF A LC-MS METHOD FOR DETERMINATION OF FLUBENDIAMIDE IN BEE POLLEN USING AN ENHANCED MATRIX REMOVAL-LIPID SORBENT FOR EFFECTIVE CLEAN-UP

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Bee pollen is attracting particular attention as a food supplement for human consumption due to its high content of bioactive compounds such as essential amino acids, antioxidants, vitamins and lipids. However, insecticides residues, occurred in crop fields near the hive, can compromise the quality of apicultural products, such as bee pollen, and may further represent a potential risk for consumers. The insecticide flubendiamide belongs to a relatively recent class of chemicals called benzenedicarboxamides, and it is a compound which is extremely effective, especially against lepidopteron pests. At present, however, some countries are reviewing their registration of it because certain studies show that its use constitutes an environmental risk. Thus, the monitoring of flubendiamide residues in bee pollen could be an appropriate measure not only to ascertain its presence, but also because of the potential risk to the consumer's health, as well as the stringent maximum residue levels (MRLs) established by International legislation for this substance in honey and other apicultural products including bee pollen (50 µg/kg).

In this study, a new method has been proposed to determine the insecticide flubendiamide in bee pollen by means of liquid chromatography coupled to a single quadrupole mass detector. An efficient sample treatment, with average analyte recoveries between 90% and 102% and absence of the matrix effect, involved solvent extraction, centrifugation, freezing with dry ice, clean-up with an enhanced matrix removal-lipid sorbent, followed by evaporation. Chromatographic analysis (14 min) was performed on a $C_{18}$ based column. The mobile phase consisted of acetic acid (1 mM) in water and methanol, with a flow-rate of 0.5 mL/min in gradient elution mode. The method was fully validated and the data demonstrated that it is consistent, reliable and has a wide linear range of applicability. Low detection and quantification limits were obtained in all cases: 1 and 4 µg/kg, respectively. Finally, the proposed method was applied to flubendiamide analysis of commercial bee pollen samples from different Spanish regions; no residue of this insecticide was detected in any of the samples.

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**PMU-P02**

## SOLID PHASE EXTRACTION OF PESTICIDES FROM ENVIRONMENTAL WATERS USING A MSU-1 MESOPOROUS MATERIAL AND DETERMINATION BY UPLC-MS/MS

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To determine pesticides in complex matrices, such as ground and surface waters, sample treatment for pre-concentration and clean up is a crucial step, which has been defined as a bottleneck in the whole analytical process [1]. In the last decades, solid phase extraction (SPE) has been the most popular sample treatment technique and significant efforts have been carried out to develop new formats and advanced sorbent materials improving selectivity or specificity towards target analytes, sorptive capacity and physicochemical or mechanical stability [2]. Thus, research in new advanced materials has involved different disciplines such as materials science, nanotechnology, polymer synthesis and analytical chemistry [1].
This work describes the synthesis of a silica based MSU-1 mesoporous solid and its application as sorbent in solid-phase extraction to pre-concentrate 13 pesticides of low-high polarity (methomyl, cymoxanil, carbofuran, monolinuron, isoproturon, methidathion, methiocarb, malathion, phosalone, diazinon, penconazole, neburon and chlorotoluron) in ground and river water. The synthesis was based in an H-bonding interaction assembling ($I^0N^0$) between two non-ionic components (the inorganic silica surface, $I^0$ and the polyethylene oxide template, $N^0$) by adding tetraethoxysilane to the non-ionic surfactant Brij®100, this last previously dissolved in HCl 1M. 50 mL water samples adjusted at pH=3.5 were passed, at a flow rate of 5 mL/min, through a home-made cartridge containing 50 mg of MSU-1 sorbent, preconditioned with 5 mL of ultrapure water; then, the cartridge was washed with 5 mL of ultrapure water. Following elution with 5 mL of acetonitrile, the pesticides were determined by ultra performance liquid chromatography coupled to triple quadrupole-mass spectrometry. Two selected reaction monitoring transitions were monitored per compound; the most intense one was used for quantification and the second one for confirmation and 3 points were used for identification, as established in the Directive 96/23/EC for LC-MS/MS analysis, which deals with confirmatory methods for organic residues and contaminants listed in the Group B (veterinary drugs and contaminants). Medium matrix effect (|20%|>ME<|50%|) was found for methiocarb and malathion, whereas diazinon and phosalone showed strong matrix effect (ME>|50%|). Therefore, the standard addition methodology was applied by adding an adequate amount of the pesticide standard mix to the final sample extract (all pesticides were quantified using this approach for practical reasons, thus avoiding two different calibrations). The method quantification limit (MQL) of pesticides was 0.01 μg/L for all pesticides, except for diazinon (0.1 μg/L). Recoveries of the target pesticides at MQL and 0.25 μg/L concentration levels in blank river water were in the range 70.1-113.5% and 86.7-107.3%, respectively, with RSDs lower than 16.3% and 15.7 %. 4 ground water samples and 3 river water samples, all of them taken from Almería (Spain) were analyzed by the proposed method and only phosalone at a concentration level of 0.05 μg/L was found in one river water sample.

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**PMU-P03**

# APPLICATION OF SULFONIC-FUNCTIONALIZED MCM-41 AS SOLID PHASE SORBENT FOR THE PRECONCENTRATION OF UV FILTERS AND PARABENS IN WATERS AND DETERMINATION UPLC-MS/MS

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Water samples treatment techniques are in continuous development in order to reduce time and solvent consumption, as well as to improve versatility, allowing the simultaneous retention of analytes with very different nature and polarity. In this sense, the interest in the development of new materials is growing in recent years.
Silica based mesoporous materials have gained increasing interest in sample preparation because, in addition to their high pore volume and high surface area, they show the possibility of functionalization with hydrophilic, hydrophobic, polar or charged functional moieties, thus expanding the possibilities for the extraction of analytes with different nature and properties.
In this work, an silica based MCM-41 mesoporous material was modified with sulfonic groups (MCM-41-$SO_3H$) and it was used as a new sorbent for the solid phase extraction of three UV filters (4-hydroxybenzophenone, 2-4-dihydroxybenzophenone and 4,4´-dihydroxybenzophenone) and six parabens (methylparaben, ethylparaben, isopropylparaben, propylparaben, butylparaben, benzylparaben) at ultratrace levels in a municipal wastewater treatment plant (WWTP) effluent and swimming pool water. The functionalized material was obtained by co-condensation and was characterized by elemental analysis, low-angle X-ray diffraction, scanning electron microscopy, high resolution transmission electron microscopy and nitrogen adsorption-desorption isotherms. For pre-concentration, 50 mL water samples, containing 10% of sodium chloride and adjusted at pH=6.5, were passed through a home-made cartridge containing 100 mg of MCM-41-$SO_3H$ sorbent. After drying the sorbent, the retained compounds were eluted with 2 mL of ethyl acetate and the eluate was evaporated to dryness with a gentle $N_2$ flow. Finally, the residue was re-dissolved in 0.5 mL methanol:ultrapure water (20:80,v/v) and was injected in the ultraperformance liquid chromatography coupled to triple quadrupole-mass spectrometry system. Validation was carried out using a blank WWTP effluent from a municipal wastewater treatment plant. Low matrix effect (ME $\leq$|20%|) was found for most analytes in this water sample, the exception being isopropylparaben with ME = -27%. In order to overcome this drawback, the quantitation of the analytes in the WWTP effluent samples was performed using the standard addition methodology and the results were compared with those obtained by using solvent-based calibration curves. Recoveries at two concentration levels (0.05 and 0.2 $\mu$g/L in WWTP effluent) were in the range of 75.0-99.6% with RSDs <13% using the standard addition method and in the range 66.7-115.2% with RSDs < 9% using solvent-based calibration. Method quantitation limits, calculated according to the S/N $\geq$10 criterium, were between 0.5 ng/L and 5.0 ng/L in the WWTP effluent samples. The proposed methodology was applied to the analysis of two real samples, one t WWTP effluent and one swimming pool water. Methylparaben, propylparaben and benzylparaben were found in the WWTP effluent sample at 10.0, 10.0 and 6.0 ng/L, respectively, and butylparaben was also present but at a concentration lower than its method quantitation limit. The same four parabens were found in the swimming pool sample, methylparaben and propylparaben at concentrations slightly lower than in the WWTP effluent, whereas the levels for the others parabens were lower than their method quantitation limits. As for UV filters, 4-hydroxybenzophenone was detected in both samples and 4,4´-dihydroxybenzophenone was found in the swimming pool water but at concentration levels lower than their quantitation limits.

**PMU-P04**

# IN SYRINGE HYBRID MONOLITHS FUNCTIONALIZED WITH GOLD NANOPARTICLES FOR SELECTIVE EXTRACTION OF GLUTATHIONE IN BIOLOGICAL FLUIDS PRIOR TO ITS DETERMINATION BY HPLC

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Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is the most relevant low-molecular weight antioxidant synthesized in living cells. GSH is essential for the regulation of cell proliferation and keeps the thiol redox potential in the cells by preserving thiol groups of proteins in the reduced form. This amino thiol is also very significant for the cellular defense against free radicals, oxidative stress and diseases derived from those undesirable phenomena [1]. Thus, the accurate analysis of this compound in biological fluids is a relevant issue for the diagnosis of these serious diseases. Besides, due to the low-concentration of this analyte in biosamples (such as tissue, blood, serum, and urine), a pre-concentration step using solid-phase extraction (SPE) or other extraction techniques is indispensable to achieve a sensitive determination.

In last years, the use of nanomaterials such gold nanoparticles (AuNPs) has led to a wide variety of challenging possibilities in sample preparation. Their particular features such as large available surface area and multiform morphologies, which can used to increase analyte-sorbent interactions, speed up mass transfer and modify selectivity. However, most of works in extraction techniques are based on dispersive solid-phase extraction (SPE); producing this extraction mode analyte loss or desorption from the surface of NPs during washing or centrifugation steps. In this sense, the extraction and enrichment of GSH in complex mixtures could be improved by incorporating AuNPs onto a carrier or support to act as new kind of SPE sorbent. Thus, in recent years, the incorporation of AuNPs to monoliths have emerged as smart strategy to enhance selectivity and to improve the extraction efficiencies of polymer-based monoliths as well as NP-based materials [2, 3].

In this work, a hybrid material based on a polymer monolith modified with AuNPs was used as SPE sorbent for the selective extraction of GSH in biological fluid samples. For this purpose, glycidyl methacrylate-based monolith was firstly prepared within polypropylene syringes, which were previously modified (by photografting) with ethylene glycol dimethacrylate to assure a covalent attachment of monolith. Then, the polymer was treated with different ligands (ammonia, cysteamine and cystamine). The resulting materials (containing amine or thiol groups, respectively) were subsequently functionalized with AuNPs. The hybrid material that provided the largest AuNPs coverage was then selected as SPE sorbent and it was investigated in terms of extraction efficiency through parameters such as the sample pH, elution solvent, etc. Under the optimized conditions, the hybrid monolithic microdevices were applied to the extraction of GSH in saliva and urine samples followed its analysis by HPLC coupled with fluorescence detection.

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**PMU-P05**

# OPTIMIZACIÓN DE LOS PARÁMETROS OPERACIONALES DE UN SISTEMA DE MICROEXTRACCIÓN EN FASE LÍQUIDA CON FIBRA HUECA (HF-LPME) MEDIANTE DISEÑO EXPERIMENTAL (RCCD), APLICADO A $PCB_S$ EN LECHE MATERNA

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La leche materna se ha utilizado como marcador biológico de la contaminación ambiental ya que, por los procesos de bioacumulación en tejido graso, muchos compuestos químicos alcanzan concentraciones fácilmente medibles en la leche materna. El Comité de Lactancia Materna de la Asociación Española de Pediatría subraya la importancia de promover la lactancia materna como la opción más saludable, ya que sus beneficios superan claramente cualquier riesgo para la salud asociado a los contaminantes químicos presentes en la leche materna. Además, la leche materna contiene factores de protección que contrarrestan los efectos potenciales relacionados con la exposición prenatal a contaminantes ambientales.
A pesar de este hecho, cada vez surge una mayor preocupación social para reducir los niveles de contaminantes en la leche materna y se destaca la importancia de que se desarrollen programas para eliminar o reducir la contaminación química de los alimentos y el medio ambiente y prevenir de esta manera los efectos negativos para la salud infantil que se pueden derivar de la exposición a compuestos tóxicos.

Dentro de los compuestos orgánicos persistentes los bifenilos policlorados (PCBs) constituyen una familia de compuestos tóxicos de gran relevancia por su alto grado de toxicidad y persistencia. Son contaminantes orgánicos, que por sus propiedades físicas han facilitado su uso masivo en la industria. No son biodegradables y persisten en el ambiente, se transfieren dentro de la cadena alimenticia y tienden a bioacumularse. Numerosas investigaciones han demostrado que los PCBs afectan a la función de los sistemas endocrino, inmunológico y nervioso, defectos de nacimiento y cáncer entre otros [1].

La intranquilidad por los efectos de estos compuestos en la salud motiva la necesidad de desarrollar métodos analíticos nuevos y eficientes para su control y minimización. En la actualidad no existe método estándar para la cuantificación de PCBs en leche materna; la técnica más común empleada es la extracción líquido-líquido (LLE) y extracción en fase sólida (SPE) [2,3].

El presente trabajo muestra el desarrollo de un nuevo método de análisis en PCBs en leche materna empleando microextracción en fase líquida con fibra hueca mediante GC/MS. El método desarrollado consigue muy bajos límites de detección, buena repetibilidad, reproductibilidad y aporta ventajas como ausencia del efecto memoria y de contaminaciones cruzadas entre muestras, disminución de tiempos de análisis, bajo coste y una mayor sencillez, pudiendo ser aplicado en análisis de control de calidad rutinario. Para optimizar el método hemos aplicado diseño experimental multivariante (DOE), basándonos en el método Compuesto Central Rotacional (RCCD).

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**PMU-P06**

# DEVELOPMENT OF POLY(METHACRYLIC ACID-CO-ETHYLENE GLYCOL DIMETHACRYLATE) POLYMER AS EFFICIENT SOLID-PHASE SORBENT FOR HETEROCYCLIC AROMATIC AMINES EXTRACTION IN AQUEOUS SAMPLES

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Heterocyclic aromatic amines (HAAs) are a group of compounds known to be mutagenic and carcinogenic [1]. Thus, human contact with these compounds can occur by ingestion of foods and environmental exposure. Also, an increasing number of studies report a link with the combustion of various materials such as wood, biomass, and petroleum; among others [2]. Consequently, HAAs can be detected in cooking fumes, cigarette smoke, diesel exhaust, aquatic media and airborne particulate matter. Heterocyclic aromatic amines are able to migrate into the atmosphere causing pollution in remote areas. In this sense, HAAs have been identified in water from several areas around the world lately. Therefore, a proper control based on sensitive determination methodologies to ensure HAAs levels in compliance with the safety and water quality requirements are required.

In the last years, researchers have focused upon organic polymer monoliths in an effort to extend the range of materials available for solid-phase extraction purposes. These materials offer several potential advantages compared with packed particles. For instance, they do not require frits and their large permeability can increase the efficiency of extraction and decrease the backpressure. Since they can be prepared "*in situ*" within the confines of a mold (such as an SPE cartridge), their use as SPE sorbents has incredibly increased in the sample preparation [3]. Besides, due to the wide availability of monomers and cross-linkers, polymer monoliths can be tailored with different functional groups for specific purposes. Many of these materials are biocompatible and stable at extreme pH values (1-12).

In this work, a monolithic polymer based on poly(methacrylic acid-co-ethylene glycol dimethacrylate) (MAA-co-EDMA) was prepared as SPE sorbent for the efficient extraction of HAAs (quinoline and pyridine derivatives!!) in aqueous samples. The main advantages of this polymer underlies in its hydrophobic polymer bone structure jointly with the acidic pendant groups, which offer the possibility of electrostatic interaction, being an ideal sorbent for basic analytes like HAAs. Parameters affecting the extraction procedure were evaluated and the polymer was characterized in terms of breakthrough volume, reusability, and precision, among others. Under the optimized conditions, the developed sorbent was applied to the extraction and preconcentration of these compounds in environmental aqueous samples followed its analysis by HPLC-UV.

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**PMU-P07**

# SELECTIVE DETERMINATION OF GLYPHOSATE RESIDUES IN WINE BY SEQUENTIAL EXTRACTION-DERIVATIZATION AND LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Glyphosate [N-(phosphonomethyl) glycine] is used since 1975 as an effective, non-selective herbicide with, in principle, non-toxic effects on humans. Nevertheless, during recent years, concern about the possible carcinogenicity of this compound is rising abruptly, leading to controversial resolution in terms of regulation[1]. Glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) have been identified in different environmental samples, such as runoff water[2] and surface water[3], and in different food commodities elaborated from crops previously fumigated with this herbicide, some examples are beer[4] and soya drinks. There is also an increasing interest regarding indirect contamination of other vegetable origin foods due to aerial transport of glyphosate from nearby areas and/or compound uptake from treated soils. In this line, the existence of glyphosate residues in wines has been proposed using immunoassay techniques[5] however; to the best of our knowledge, these data have not been confirmed by mass spectrometry.

In this communication, we show a sensitive methodology for the determination of glyphosate and AMPA, with limits of quantification in the very low ng $mL^{-1}$ level. In a first step, both compounds are selectively extracted from the complex wine matrix using a molecularly imprinted polymer (MIP), employed in the SPE format. The obtained extract is derivatized by reaction with 9-fluorenylmethyl chloroformate (FMOC-Cl) before reversed-phase SPE cocentration and determination by Ultra-High-Performance Liquid Chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). This two-step approach avoids precipitation problems due to interaction of wine matrix components with FMOC-Cl, under basic pH conditions required for compounds derivatizatin, and achieves a better selectivity than anionic-exchange polymers for glyphosate extraction. The method is characterized in terms of analytical features and applied to red wine analysis. Obtained data confirmed the existence of measurable residues of glyphosate in some of the processed samples.

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**PMU-P08**

## EXTRACTION OF NITROIMIDAZOLES AND BENZIMIDAZOLES USING COMMERCIAL MIP CARTRIDGES FOR NITROIMIDAZOLES. APPLICATION TO ANALYSIS OF SAMPLES OF ANIMAL ORIGIN

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In this work, selectivity of commercial MIP cartridges for nitroimidazoles has been evaluated. To do it, a sample treatment based on molecularly imprinted solid phase extraction (MISPE) has been developed and applied to the simultaneous extraction of nitroimidazoles and benzimidazoles. These cartridges were compared with other sorbents commonly used for solid phase extraction of veterinary drugs, such as C18, HLB and MCX. Analysis of the extracts was carried out by HPLC-MS/MS. MIP cartridges provided successfully results, both in terms of recovery and precision and in the cleaning of the extracts, for all nitroimidazoles and for most of the benzimidazoles studied (except for the most apolar analytes). Thus, although MIPs have been designed to specifically binding a target molecule, the MIPs commercial cartridges for nitroimidazoles are able to extract other analytes such as benzimidazoles with high efficiency. The developed method was applied to the determination of nitroimidazoles and benzimidazoles samples of animal origin: urine and milk. Four nitroimidazoles and eight benzimidazoles have been successfully determined. Validation of the method afforded values in the range of 91-111% for recovery studies. Repeatability and reproducibility afforded relative standard deviation values lower than 11 % and 12, respectively. These results demonstrated that the proposed method is suitability for the determination of nitroimidazoles and benzimidazoles in samples of animal origin.

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**PMU-P09**

# ESTUDIO DE LA PRESENCIA DE MALONDIALDEHÍDO EN MUESTRAS DE ACEITES VEGETALES COMESTIBLES

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Debido a su composición química, los aceites comestibles vegetales son esenciales en la dieta humana ya que contienen macronutrientes, como ácidos grasos esenciales y glicéridos que no solo proporcionan energía metabólica, sino que también actúan como portadores de vitaminas liposolubles. [1]
Los aceites comestibles vegetales se pueden extraer de la semilla y la pulpa mediante diversos métodos térmicos, mecánicos o químicos. Para mejorar las propiedades organolépticas, estos aceites se refinan a altas temperaturas, lo que lleva a la formación de compuestos orgánicos indeseables que afectarán a su valor nutricional y a las propiedades generales de calidad [2-3](Custodio-Mendoza y otros., 2019; Nunes, 2014). Además, durante el almacenamiento, la peroxidación lipídica produce productos de oxidación primarios y secundarios, como el malondialdehído (MDA). La presencia de MDA en muestras de aceite puede disminuir su calidad y características nutricionales, pero también puede representar un riesgo potencial para la salud humana, debido a su alta reactividad con proteínas y ácidos nucleicos. [4-5]

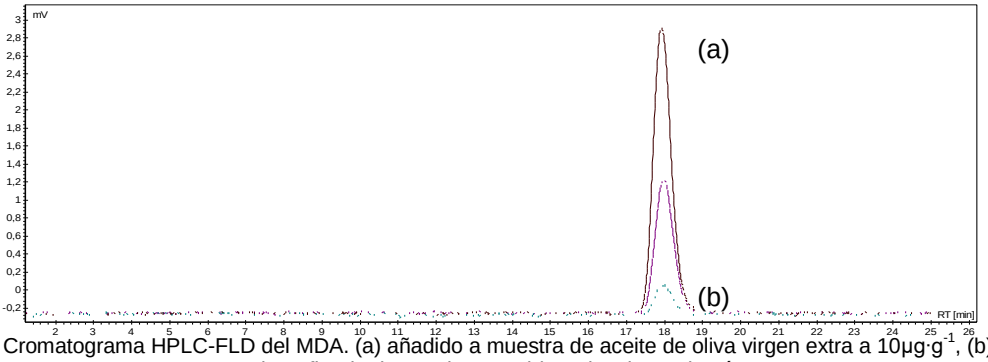


Figura 1. Cromatograma HPLC-FLD del MDA. (a) añadido a muestra de aceite de oliva virgen extra a 10µg·g$^{-1}$, (b) muestra de aceite refinado de cacahuete y (c) aceite virgen de sésamo.

En este estudio se ha desarrollado un procedimiento de extracción simple, barato y rápido basado en la microextracción por difusión de gas (GDME) con derivatización con ácido tiobarbitúrico (TBA). Esta técnica combina la difusión de analitos volátiles y semivolátiles en fase de vapor a través de una membrana porosa y microextracción. [6]
Los extractos se analizaron mediante HPLC-UV-FLD para determinar el contenido de MDA en cuarenta y cuatro muestras de aceite vegetal comestible que proporcionaron datos de evaluación de la presencia de MDA.

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**PMU-P10**

## MAGNETIC CELLULOSE NANOPARTICLES AND STIR BAR SORPTIVE DISPERSIVE LIQUID MICROEXTRACTION: A WINNER TANDEM IN SAMPLE PREPARATION

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Nanocellulose (NC) has emerged as one of the most interesting nanomaterials for sample preparation due to the combination of its excellent properties as a sorbent, i.e. large superficial area, chemical reactivity, lightness and surface tailoring by covalent and non-covalent interactions, together with its quick, cheap and easy production from renewable sources, and its biodegradability. The combination of NC with magnetic nanoparticles (MNPs) results in magnetic cellulose nanoparticles (MCNPs), which keep the inherent properties as sorbents and avoid tedious steps such as centrifugation in dispersive solid phase extraction modes.

MCNPs can be used in modern magnetic dispersive modes of solid phase extraction, such as stir bar sorptive-dispersive microextraction (SBSDME). This emerging mode consists of a magnetic stir bar coated with magnetic sorbents, typically MNPs, which is placed into the sample. At high stirring rate, MNPs are detached from the stir bar and dispersed into the sample so that extraction takes place. When the stirring stops, MNPs with the analytes come back to the stir bar surface. Then, the liquid is removed, and the back-extraction is carried out with the appropriate solvent [1]. SBSDME allows high preconcentration in a short time with minimal manual intervention.

This strategy has been used for the extraction and preconcentration of nine PCBs prior to gas chromatography coupled to mass spectrometry (GC-MS). The parameters that affect the extraction of the analytes by SBSDME which were studied were amount of MCNPs, salt concentration, pH and stirring time. The parameters studied in back-extraction were type of solvent and stirring time.

The limits of detection and quantification were in the range 2.1–54 ng $L^{-1}$ and 7.0–180 ng $L^{-1}$, respectively. Intra-day and inter-day precision were below 8.8% and 9.3%, respectively, in terms of relative standard deviation. The method was applied in commercial juice samples ($n$=5). The concentrations of all analytes were below the limit of detection in all samples. To evaluate the accuracy of the method, samples were spiked at 1 and 2 ng $mL^{-1}$ and submitted to analysis with quantitative recoveries.

The use of cellulose makes this approach environmentally friendly and the magnetism of the resulting sorbent in conjunction with SBSDME provides ease of handling and saving of time.

### Acknowledgments

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**PMU-P11**

# REVERSED-PHASE DISPERSIVE LIQUID-LIQUID MICROEXTRACTION PRIOR TO LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF ACRYLAMIDE IN COSMETIC PRODUCTS

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Among liquid-phase microextraction techniques currently developed, dispersive liquid-liquid microextraction (DLLME) [1] has become very popular due to its simplicity and the high enrichment factors achieved. However, its main drawback is the limited field of application in the extraction of polar compounds, due to the use of non-polar organic solvents as extraction solvents. This is a true problem when polar compounds have to be extracted, especially from fatty samples, such as cosmetic creams. A modification of the original DLLME termed reversed-phase DLLME (RP-DLLME) was subsequently proposed [2]. In this modified approach, a small volume of water, used as extraction solvent, is dispersed into a bulk organic solution containing the polar target analyte(s).

In this work, we have employed this methodology to the determination of acrylamide in cosmetic samples. Due to its mutagenic and potentially carcinogenic effects, the European Regulation on cosmetic products [3] forbids the use of acrylamide as an ingredient in such products. Acrylamide-based polymers, such as polyacrylamide, have been restricted in such a way the maximum residual acrylamide content is < 0.1 mg $kg^{-1}$ in cosmetic products without rinsing, and < 0.5 mg $kg^{-1}$ in other cosmetic products. In this sense, the determination of acrylamide in the presence of acrylamide-releasing ingredients is necessary for assuring the compliance with the legislation and the safety of users. Thus, the proposed method is based on vortex-assisted reversed-phase dispersive liquid-liquid microextraction (VA-RP-DLLME) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

This work shows the high potential of RP-DLLME, which opens a way to extract and concentrate highly polar compounds from cosmetic samples.

**Acknowledgements**
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**PMU-P12**

## COMPARACIÓN DE MÉTODOS PARA LA EXTRACCIÓN DE CAROTENOIDES EN HECES

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Introducción: los carotenoides son compuestos liposolubles presentes en la dieta humana potencialmente beneficiosos para la salud. La cantidad que el organismo absorbe y está disponible para ser utilizado (biodisponibilidad) es variable dependiendo de diversos factores, tanto dietéticos como otros ligados al sujeto. Un porcentaje importante de lo que se ingiere permanece en el tracto gastrointestinal, donde podrían ejercer acciones biológicas [1]. Por ello y también para el estudio de la biodisponibilidad, es de interés el análisis de los carotenoides en heces [2].

Objetivo: valoración de diferentes procesos de extracción de carotenoides en heces para seleccionar el que permita una mejor extracción de carotenoides mayoritarios en el organismo humano (luteína (L), zeaxantina (Z), licopeno (Lico) y a- y b-caroteno (a y b-Car).

Material: heces humanas liofilizadas y congeladas a -80ºC de sujetos aparentemente sanos y en un rango de edad de 45-65 años.

Métodos: 0,3 g de heces liofilizadas se homogeneizaron con mezclas de solventes diferentes: a) NaCl 10%, etanol y acetona; b) agua, etanol y acetona, y c) buffer salino -PBS 1x (p=6,7), etanol y acetona. A continuación, la extracción de los carotenoides se realizó con dietil éter: éter de petróleo (DE: EP) (1:1). Por otra parte, en tres muestras homogeneizadas con PBS 1x, se ensayaron cuatro solventes de extracción: a) DE:EP (1:1); b) hexano: diclorometano (Hex:DCM) (5:1); c) DE y d) dietil éter : metil-terbutil-éter (DE:tBME) (1:1).

Resultados: el mejor solvente para la homogeneización de las heces es el PBS, que permite una mejor extracción de la mayoría de los carotenoides en estudio (Tabla 1). El solvente DE:EP presenta especial eficacia para extraer L y Z (Tabla 2)

Tabla 1. Concentraciones de carotenoides (ug/g) en una muestra de heces liofilizada reconstituida con diferentes solventes y extraídos con DE:EP.

| | a-Caroteno | b-Caroteno | Licopeno | Luteína | Zeaxantina |
|---|---|---|---|---|---|
| PBS 1x | 41,54 | 160,72 | 42,62 | 20,76 | 4,59 |
| NaCl 10% | 24,94 | 100,35 | 26,69 | 14,25 | 4,05 |
| $H_2O$ | 33,02 | 135,64 | 38,52 | 17,53 | 5,18 |

Tabla 2. Concentraciones y porcentajes[1] de luteína y de zeaxantina [ug/g (%)] en heces liofilizadas, reconstituidas con diferentes solventes.

| | Hez 1 | | Hez 2 | | Hez 3 | |
|---|---|---|---|---|---|---|
| | Luteína | Zeaxantina | Luteína | Zeaxantina | Luteína | Zeaxantina |
| DE:EP | 12,36 (100) | 2,36 (100) | 89,81 (100) | 110,43 (100) | 59,54 (100) | 46,69 (100) |
| Hex:DCM | 11,32 (92) | 1,73 (73) | 43,32 (48) | 38,56 (35) | 12,95 (22) | 3,19 (7) |
| DE | 8,48 (69) | 1,54 (65) | 61,84 (69) | 33,52 (30) | 16,64 (28) | 5,86 (13) |
| DE:tBME· | 12,05 (98) | 1,93 (82) | 42,80 (48) | 23,80 (22) | 17,56 (29) | 1,19 (3) |

[1] se ha asignado el 100% al valor más alto obtenido.

Conclusiones: a) para una adecuada extracción de carotenoides de heces liofilizadas se deben homogeneizar usando PBS 1x; b) el mejor solvente de extracción de carotenoides en heces fue la mezcla de DE:EP (1:1).

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**PMU-P13**

# ANALYSIS OF 3MCPD MONOESTERS AND GLYCIDYL ESTERS IN EDIBLE OIL BY HPLC-APCI-MS/MS USING A NEW QUECHERS.

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3-monochloropropane-1,2-diol (3MCPD) is the most abundant and stable of a group of food contaminants induced by heat named chloropropanols. 3MCPD is formed during processing and has been reported in various food matrices including coffee, malt drinks, potatoes chips, vegetable oils, infant formula, etc. [1] In oils, 3MCPD could be present either free or esterified with fatty acids (3MCPDE), several toxicological studies have described 3MCPD carcinogenic activity, while the main interest in 3MCPDE is related to the fact that 3MCPD is released from them in human digestive track during enzyme hydrolysis. [1-3] For these reasons this compound has been classified as "possibly carcinogen to human" (Group 2B, IARC) [4] and a TDI has been set up at 0.8 and 4 $\mu g \cdot kg^{-1}$ of body weight per day respectively [5, 6]. Glycidol is consider a genotoxic carcinogen as well and has been classified as probably carcinogenic to humans (Group 2A, IARC) [7], in a similar way, glycidyl esters (GE) are capable of releasing glycidol in human body situation that will increase the risk of exposure.

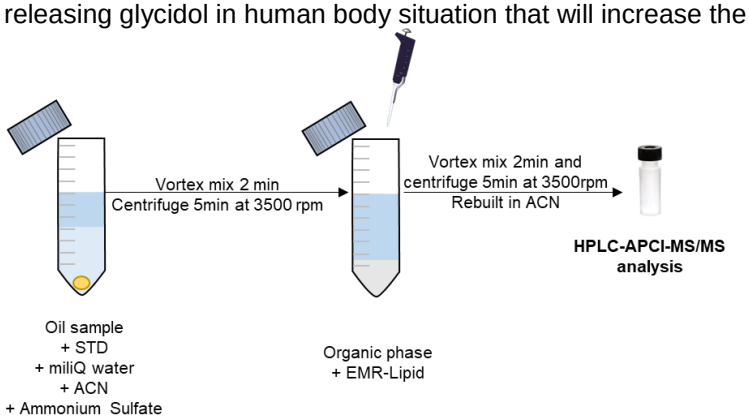


Figure 1. Scheme of QuEChERS method for analysis of 3MCPD monoesters and GE in oils.

Here, a direct method carried by HPLC-APCI-MS/MS using a new Quick, Easy, Cheap, Effective, Rugged y Safe (QuEChERS) procedure for the analysis of 3MCPD monoesters and GE in edible oils is presented. This method has been developed using isotopically labeled compounds as internal standards and validated following food and drug administration (FDA) guideline of analytical method validation.

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**PMU-P14**

## DEVELOPMENT OF DISPERSIVE LIQUID-SOLID MICROEXTRACTION: APPLICATION TO THE DETERMINATION OF CORTISONE AND CORTISOL IN HUMAN SALIVA

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Nowadays, sample preparation is one of the most hot-spot research trends in Analytical Chemistry, especially in trace analysis where it is usually necessary to perform both a preconcentration of the analytes and a cleaning-up step to eliminate potentially interfering compounds.

In this work, a new hybrid microextraction technique that combines dispersive liquid-liquid microextraction (DLLME) and dispersive solid-phase extraction (DSPE). In this new approach, termed dispersive liquid-solid microextraction (DLSME), a magnetic composite is dispersed into the liquid employing a disperser solvent. Later, employing an external magnetic field, the sorbent is kept whereas the sample is retired with a syringe. Finally, the analytes are desorbed in a small amount of organic solvent.

To show its utility, the extraction of cortisone and cortisol from human saliva has been chosen as model analytical application. Measurement of levels of cortisol in serum samples has been used to study the malfunction of the adrenal grand, the pituitary and the hypothalamus, also can be an indicator of Cushing disease, hypertension etc . However, measurements of salivary cortisol are usually performed because it is a relatively non-invasive method for sample acquisition. Moreover, salivary cortisol shows a good correlation with serum cortisol. Nevertheless, the action of enzyme 11-β hydroxysteroid dehydrogenase (11-β HSD2) present in the parotid gland turns part of free cortisol into cortisone. Thus, the concentration of salivary cortisone will be bigger than salivary cortisol. For that reason, measurement of salivary cortisone has gained interest in recent years as a marker of the amount cortisol in blood [1]. In addition, the cortisol to cortisone ratio can be used as indicator of a deficiency in the activity of 11-β HSD2 [2].

In order to determine salivary cortisone and cortisol, the proposed method is based on DLSME employing a composite made of $CoFe_2O_4$ magnetic nanoparticles embedded into a reversed-phase polymer as sorbent, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) as measurement technique.

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**PMU-P15**

## USE OF MICROEXTRACTION BY PACKED SORBENTS COUPLED TO GC-MS FOR THE DETERMINATION OF POLAR COMPOUNDS IN BIOLOGICAL SAMPLES

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Sample preparation is one of the most critical steps of any bioanalytical method development. Classical extraction procedures mainly include liquid-liquid extraction (LLE) or solid-phase extraction (SPE). However, in recent years, attention has been drawn to the development of microextraction methods with lower sample and solvent consumption. Among techniques, microextraction by packed sorbents (MEPS) has gained broad attention.

MEPS is a miniaturized version of SPE, where around of 4 mg of sorbent is packed inside a syringe as a plug or placed between the barrel and the needle as a cartridge [1,2]. Sample extraction and enrichment can be accomplished on the packed sorbent. MEPS devices can be connected on-line to gas chromatography and liquid chromatography without any further modification in the analytical instrument.

Here we propose, for the first time, the use of MEPS for the determination of polar endogenous compounds (putrescine, cadaverine, spermidine, L-ornithine and GABA) in biological samples obtained using non-invasive methods of collection (saliva samples). Due to the polarity of the compounds, a derivatization reaction is mandatory to perform the analysis. Ethyl chloroformate is proposed as derivatization reagent. Chloroformates have been widely used for treating amino groups, as well as esterification reagents, and present important advantages, such as a very rapid reaction with no need for heat or water exclusion, negligible reagent cost and simple after reaction workup [3]. Saliva is a clear, slightly acidic mucoserous exocrine fluid secretion formed by approximately 99 % water, with a variety of electrolytes and proteins, along with glucose and nitrogenous metabolic products [4]. Saliva samples have been successfully used for the determination of several kind of compounds, such as drugs or organic contaminants, among others [4].

After optimizing the reaction conditions, the microextraction step (MEPS) and the analysis procedure using a PTV coupled to GC-MS, we have been able to reach limits of detection (LODs) in the range of µg/L. Matrix effect has been checked and quantitation of samples performed using the one-point standard addition method and normalization to IS. Accuracy (89-131 %) and precision values in terms of repeatability (2.9-15.2 %) and reproducibility (1.5-26.5 %) have also been successfully proven to be within acceptable values. Finally, the method has been applied in the diagnosis of real saliva samples, finding results in accordance to those described in literature. Samples from diagnosed subject or heavy smoker presented higher concentrations than those corresponding to non-diagnosed ones.

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**PMU-P16**

# OPTIMIZATION USING TAGUCHI EXPERIMENTAL DESIGNS OF A MICROWAVE ASSISTED SAMPLE DISSOLUTION PROCEDURE FOR THE DETERMINATION OF TOTAL CONTENTS OF TRACE ELEMENTS IN SOILS.

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Trace elements, especially heavy metals, are categorized as the most hazardous anthropogenic environmental pollutants due to their persistent nature and high toxicity. The determination of total concentrations of toxic trace elements in sediments and soils is commonly done to evaluate the degree of contamination of aquatic and terrestrial environments.

ICP-MS is widely used for the determination of trace elements as it combines high sensitivity and wide dynamic range with multi-elemental capability. Prior to determination, soil and rock samples must be dissolved. Acidic digestion by microwave, MWD; is to date the most appropriate method for the dissolution of complex materials such as soil and sediment [1]. The partial digestion methods using acids are considered appropriate for pseudo-total analysis, but they do not completely dissolve silicates. Only the microwave-assisted US EPA 3052 method in presence of hydrofluoric acid allows a total sample digestion [2]. In spite of the large number of publications on rock sample digestion there are different and sometimes contradicting conclusions on the efficiency of the MWD technique [1,3], thus pointing to the necessity of MWD optimization.

In this work, the optimization of MWD procedure to dissolve soil samples for the determination of total trace elements by ICP-MS has been carried out using a Taguchi experimental design. The control factors assayed were: volumes of concentrated nitric acid (N), hydrofluoric acid (F) and hydrochloric acid (C), digestion time (t), temperature (T) and method used for excess HF elimination (E). The six factors were assayed at three levels to detect quadratic effects and assigned to six columns of a Taguchi's $L_{27}(3^{13})$ orthogonal design, with 26 degrees of freedom. The remaining columns and degrees of freedom of the experimental design were used to investigate factor interactions F×T, F×E and T×E. This experimental design was applied to the determination, by ICP-MS, of 17 trace elements in a soil sample with certified contents of the elements (CRM GBW07402 GSS-2, Institute of Geophysical and Geochemical Exploration, Langfang, China). The elements were selected to represent groups of different characteristics or reactivity: toxic elements occurring in agricultural soils, chloride-forming elements, hardly soluble elements, light elements, volatile elements and rare earths (As, Be, Co, Cr, Cs, Cu, Dy, Ga, Ni, Pb, Sb, Sn, Th, Tl, U, Y, Zn). The purpose of the experiment was to minimize bias in the determination of trace elements by maximizing the amounts of elements determined. Aliquots of 0.25 g of the soil sample were digested in the different conditions fixed by the experimental design and the digestates were diluted to volume with ultrapure water. Calibration standards and digested samples were measured by ICP-MS in comparison with the adequate internal standards to improve accuracy.

ANOVA of the results of the experiment showed the factors with significant effect on the amounts of elements: E > F > C. The effects of t, N and T were in general non-significant. However, the optimal MWD conditions were not identical for the 17 elements measured, The optimal levels of the factors yielding maximum recovery of most assayed elements were: N3 (6 mL), F2 (2 mL), C3 (2 mL), t2 (15 min), T1 (180 ºC) and E2 (excess HF removed by evaporation) for Be, Cr, Cu, Ni, Sb, Tl, Zn, or E3 (addition of 1.4 g of boric acid and 5 min MW heating at 180 ºC) for As, Cs, Dy, Ga, Sn, Th, Tl, U, Y.

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**PMU-P17**

# ADVANCES IN THE DEVELOPMENT OF A SIMPLE AND EFFECTIVE METHODOLOGY FOR THE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM VEGETABLE OILS

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Polycyclic aromatic hydrocarbons (PAHs) are a group of molecules formed by several fused aromatic rings that can be found as food contaminants, especially in oils and fats, due to their lipophilic character. Their proven mutagenicity has promoted that regulatory organisms (EPA and EU boards) have fixed their permitted levels in edible oils. Thus, the development of analytical methodologies that allow a robust and effective determination of PAHs in vegetable oils is essential. Most of the already existing analytical strategies include long and tedious steps at the sample treatment stage and normally require high amounts of solvents too.

In this contribution, our ongoing experiments to develop a fast, simple and reliable method to extract PAHs from vegetable oils will be discussed. In a first stage of the project, a compound-stability study was conducted, finding that preconcentration techniques, such as vacuum evaporation or nitrogen stream may conduct to substantial losses of the analytes. Afterwards, the fluorimetric characterization of the 16 PAHs that have been recognized by EPA as hazardous compounds was achieved, finding its optimum excitation and emission wavelengths in acetonitrile and water.

Chrysene (Chr) was selected as a proper representative of the 16 EPA PAHs, since it is one of the most recurrent PAHs in contaminated vegetable oils and is also part of the PAH8 -a group of eight PAHs that has been advised by EU to be monitored in vegetable oils-.

The extraction process that we propose is based on the use of a novel material that consists on a multifunctional core-shell material where the core is magnetic and the shell is a molecularly imprinted polymer (MIP). Therefore, the MIP provides the selectivity to extract the PAHs and the magnetic core highly simplifies the collection of the extractant particles, giving the possibility of collecting them with magnets. The efficiency of the MIP has been verified in several matrices so far (for instance, water and water/ACN 9:1 samples (spiked with Chr)), obtaining satisfying results in terms of recovery and repeatability. The optimization of the protocol to extract Chr from oil samples is currently been conducted in our laboratory. Our preliminary studies suggest that oil samples need to be diluted in an adequate solvent that modifies medium polarity and viscosity, forcing Chr to leave the oily matrix in favor of the MIP. After that, the MIP (containing the retained Chr) is collected with a magnet and washed with acetone in order to elute the analyte of interest. Approximately 70% of the total Chr content is recovered during a first elution, whereas second and third elutions both contribute in lower percentages. Acetone is evaporated and the residue is redissolved in ACN to be measured by fluorescence spectrometry; measurements cannot be carry out directly in acetone as it may lead to a quenching phenomenon of the fluorimetric signal.

Thus, the present contribution will discuss the effect of the solvent in which contaminated oils are dissolved (prior to the extraction) and our latest results regarding the optimization of the extraction methodology.

**PMU-P18**

# ANÁLISIS DE ÉSTERES DE 3- MONOCLOROPROPANO-1,2-DIOL EN ACEITES DE CONSERVAS MEDIANTE HIDROLISIS ENZIMATICA- DLLME Y GC-MS

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Durante el refinado de los aceites vegetales, además de alcanzar las propiedades organolépticas adecuadas que pueden tener una influencia positiva en la aceptación de los consumidores, tiene lugar la formación de compuestos orgánicos no deseados, principalmente en las etapas llevadas a cabo a altas temperaturas como la deodorización [1,2]. Ejemplo de ello son los cloropropanoles, un grupo de contaminantes inducidos por el calor. El 3-monocloropropano-1,2-diol (3-MCPD) es el compuesto más destacado, para el cual diversos estudios toxicológicos han descrito actividad cancerígena y genotóxica, haciendo que sea considerado como posible carcinógeno para humanos (categoría 2B, IARC) [3]. Recientemente, ha despertado interés del análisis de sus ésteres con ácidos grasos (3-MCPDE) debido al hecho de que el 3-MCPD se libera a partir de sus ésteres durante la hidrolisis enzimática en el tracto digestivo humano, incrementando con ello el riesgo de exposición. Por ello, en 2016 se han establecido niveles de ingesta diaria tolerable de 0.8 $\mu g \cdot kg^{-1}$ (EFSA) y 4 $\mu g \cdot kg^{-1}$ (JECFA) de peso corporal al día [4-5].
Los métodos indirectos permiten determinan 3-MCPDE aunque para ello se requiere la hidrólisis de los ésteres y una etapa adicional de derivatización. La ventaja es que se cuantifica el 3-MCPD libre liberado por los ésteres, compuesto sobre el que hay límites legislados. El objetivo de este trabajo es el desarrollo y aplicación de una nueva metodología analítica que requiera un menor volumen de disolventes y menor tiempo de preparación de muestra, basada en una hidrólisis enzimática, usando una amano lipasa de *Burkholderia cepacia* (BCL), combinada con una microextracción líquido-liquido dispersiva que incluye la derivatización con n-Heptafluorobutirilimidazol del 3-MCPD generado y análisis mediante GC-MS. Los resultados obtenidos al analizar 42 muestras de aceite de conservas de pescado, se encuentran en el rango 1,1-22.0 µg/g para aceite de girasol y 0,17-5,5 µg/g para aceite de oliva.

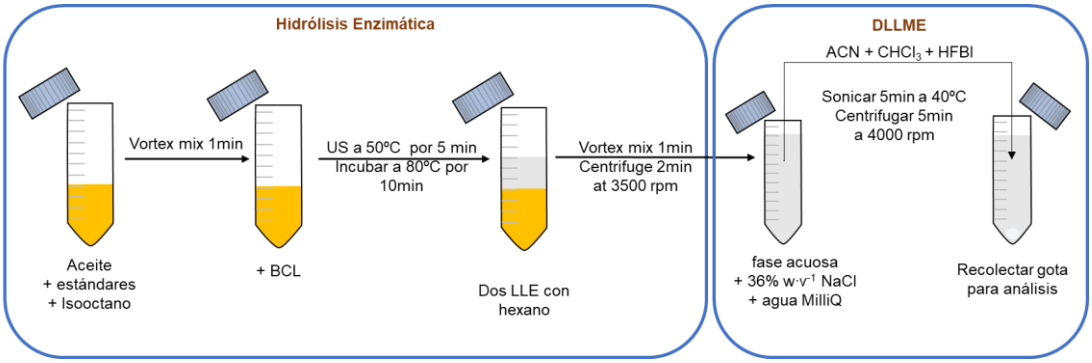


Figura 1. Esquema de la hidrolisis enzimática acoplada a DLLME para el análisis del 3-MCPD total por GC-MS

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *TÉCNICAS DE ANÁLISIS*

**TAN-P01**

## ALTERACIONES HOMEOSTÁTICAS A NIVEL ELEMENTAL E ISOTÓPICO OBSERVADAS EN SUERO SANGUÍNEO DE PACIENTES CON DEGENERACIÓN MACULAR ASOCIADA A LA EDAD

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La degeneración macular asociada a la edad (DMAE), principal causa de ceguera irreversible en personas mayores de 60 años, es una enfermedad ocular que evoluciona con pérdida de la visión central y aguda. El principal inconveniente de esta patología ocular es que en muchos casos su diagnóstico se produce cuando la enfermedad se encuentra en un estado tardío debido a que, en sus inicios, los pacientes asocian la pérdida de visión con vista cansada y con desórdenes propios de la edad. La DMAE se caracteriza por la acumulación de depósitos celulares (drusas) en la región macular de la retina y que se producen debido a una degeneración de las células del epitelio pigmentario retiniano. En este contexto, estudios a nivel básico supondrían una mejor comprensión de los procesos de modulación que dan lugar a la formación de estos depósitos y así facilitar la posibilidad de encontrar (bio)marcadores tempranos de esta patología.

Si bien las alteraciones de la DMAE se limitan al sistema ocular, teniendo en cuenta que idealmente la toma de muestras biológicas debería ser lo menos invasiva posible, se planteó utilizar suero sanguíneo como muestra de estudio. Así, el objetivo del presente trabajo ha sido estudiar, por un lado, la existencia de posibles alteraciones homeostáticas a nivel elemental que se derivan de esta enfermedad. En particular, nos hemos centrado tanto en elementos que se encuentran a nivel mayoritario (Na, Mg, P y K) como elementos esenciales (Fe, Cu y Zn) del suero sanguíneo. Por otro lado, y dado el potencial que ha demostrado la medida de relaciones isotópicas de elementos traza en clínica en la actualidad [1,2], se evaluará si existen alteraciones a este nivel para el Cu, elemento directamente involucrado en el ciclo visual. Tanto para los estudios a nivel elemental como isotópico, hemos empleado técnicas basadas en espectrometría de masas, con plasma de acoplamiento inductivo (ICP-MS e ICP-MS de tipo multicolector), y se han empleado un total de 40 muestras (20 controles sanos y 20 pacientes de DMAE seca).

Agradecimientos:

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**TAN-P02**

# ELECTROCHEMICAL BEHAVIOUR OF TIN IN THE DEEP EUTECTIC SOLVENT CHOLINE CHLORIDE – ETHYLENE GLYCOL (1:2)

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The electrochemical behaviour of anhydrous $SnCl_2$ dissolved in the deep eutectic solvent ChCl-EG (1:2) has been studied, under argon atmosphere, for temperatures ranging from 323-373 K. Transient electrochemical techniques, such as cyclic voltammetry, chronopotentiometry and chronoamperometry were used in order to study the reaction mechanism and the transport parameters of electroactive species at a glassy carbon electrode.

The deep eutectic solvent was prepared by weighing and mixing choline chloride ($HOC_2H_4N(CH_3)_3Cl$, Sigma-Aldrich >98%) and ethylene glycol ($HOCH_2CH_2OH$, Sigma-Aldrich 99.8%) in a 1:2 molar ratio. This mixture was kept in an oven at $70^0$C until a colourless homogeneous liquid was formed. Karl Fischer titrations of the as-prepared solvent revealed a water content of approximately 1400 ppm. This level can be reduced to 150 ppm by careful preparation and drying procedure using activated molecular sieves.

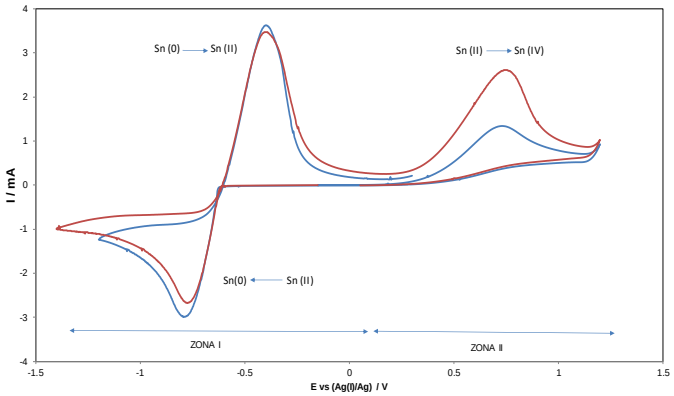


Fig.1.- CVs obtained after dissolution of anhydrous $SnCl_2$ in ChCl-EG (1:2) at a GC electrode

Examples of the good quality voltammograms recorded with a Sn(II) solution at the stationary GC electrode are shown in Figure 1. In zone I, the waves arising from the bulk deposition and stripping of tin can be observed, and in zone II the voltammogram exhibits a broad oxidation wave with a peak potential of approximately 0.75 V ascribed to the oxidation of Sn(II) to Sn(IV), which exhibit limited stability (at 323 K the tin(IV) is quickly lost from the melt by volatilization).

Electrocrystallization of tin seems to be the controlling electrochemical step. Chronoamperometric studies indicated instantaneous nucleation of tin with three dimensional growth of the nuclei.

Mass transport towards the electrode is a simple diffusion process, and the diffusion coefficient of the electroactive specie Sn(II) has been calculated. The validity of the Arrhenius law was also verified by plotting the variation of the logarithm of the diffusion coefficient versus 1/*T*.

**Acknowledgements**

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**TAN-P03**

## DEEP EUTECTIC SOLVENT ROUTE FOR Se AND $CuGaInSe_2$ ELECTROSYNTHESIS

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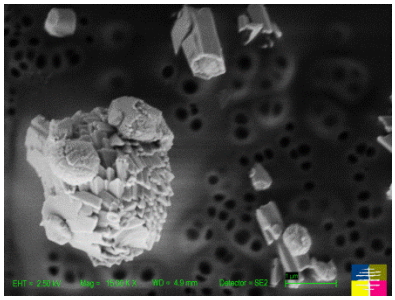
Electrosynthesis appears as one of the best methods to prepare advanced materials under the form of coatings or thin layers, due to its many advantages: i) economics (e.g. low investment is required for an easy composition control, with large coverage areas and high deposition rates), and ii) ecologic (no product loss, and efficient use of raw materials). Nevertheless, aqueous electrolysis presents two important shortcomings; the low crystallinity of the electrodeposited material (due to the low working temperature) and the hydrogen discharge that hinders the electrodeposition of very electronegative elements such as Ga and In, reduces the plating efficiency, and has destructive impacts on the thin film quality causing the appearance of pinholes and dendritic morphologies. In order to prevent these drawbacks, the use of deep eutectic solvents as electrolytes could be a promising way to elaborate materials with tailored structures and properties. In this communication, the method has been successfully applied to the electrodeposition of Se and the semiconductor $CuGa_xIn_{1-x}Se_2$ which is a good candidate for window layers realization in thin film solar cells.

Electrochemical investigations performed in the eutectic mixture ChCl-EG (1:2) at 343, 373 and 383 K are reported. DRXs and SEM analysis of the samples obtained under potentiostatic electrolysis indicated that: i) red and black Se were electrodeposited on Mo and glass sheets covered with $SnO_2$, and ii) $CuGa_xIn_{1-x}Se_2$ (with x ranging from 0.4 to 0.6) were electrodeposited on glass sheets covered with $SnO_2$.



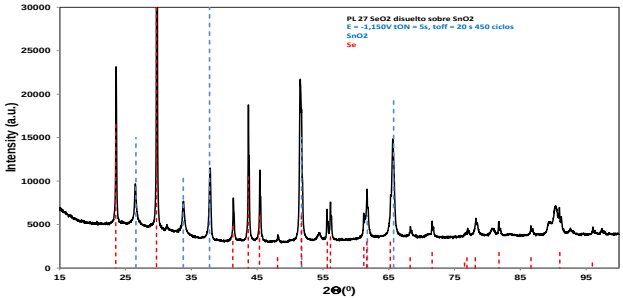
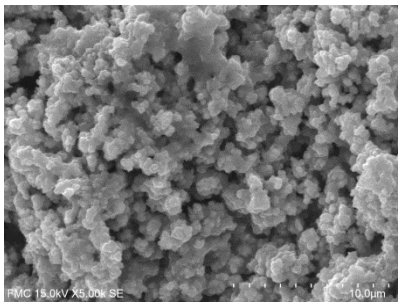



Figure 1.- SEM and DRXs of black Selenium obtained at -1.15 V in the eutectic ChCl-Eg(1:2) at 343 K



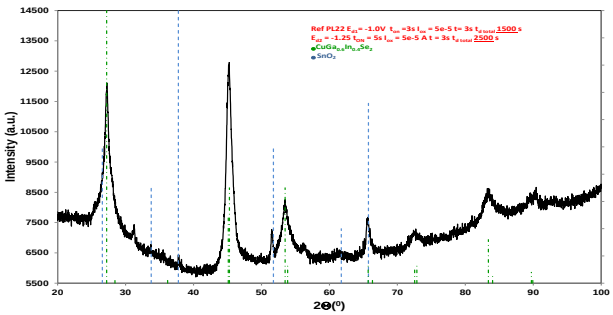



Figure 2.- SEM and DRXs of $CuGa_{0.6}In_{0.4}Se_2$ obtained at -1.00 V in the eutectic ChCl-Eg(1:2) at 343 K

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**TAN-P04**

## TARGETED ANALYSIS OF TROPANE ALKALOIDS AND POST-TARGETED SCREENING OF CONTAMINANTS IN HONEY SAMPLES APPLING LC-HRMS

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Honey is a natural foodstuff produced by honey bees (*Apis* spp.) from nectar and it has several health benefits due to its antioxidant, antibacterial, antidiabetic, anti-inflammatory, antitumoral and antimicrobial activity [1]. However, it can be contaminated by anthropogenic contaminants [2], drug residues [3] or natural toxic compounds, as tropane alkaloids (TAs) [4,5], and there is a growing concern regarding honey safety.

TAs are a group of more than 200 secondary metabolites that naturally occur in many plant families and some of them, such as atropine and scopolamine, which are the main TAs, are toxic. Taking into account that several TAs have been found in different food and feed [4,5] but to our knowledge, only atropine and scopolamine have been analysed in honey sample. Therefore, a multi-analite method for the analysis of 11 TAs (anisodamine, apoatropine, atropine, homatropine, littorine, physoperuvine, pseudotropine, scopolamine, scopoline, tropine and tropinone) in honey was performed.

For that purpose, a simple and fast an extraction procedure based on a solid liquid extraction, using methanol/water/formic acid (75/25/0.4, *v*/*v*/*v*) as extraction solvent, and clean-up step with magnesium sulphate and graphitized black carbon was optimized. After that, a liquid chromatography, equipped with an ACE HILIC-A column, and coupled to high resolution mass spectrometry (LC-HRMS-Orbitrap) was carried out using 5 mM of ammonium formate and 0.1% of formic acid in water (eluent A) and methanol (eluent B) as mobile phases. The total running time was 18 min.

The method has been fully validated, providing adequate linearity (>0.99), trueness (recoveries between 71-120%) and precision (relative standard deviation ≤20.1%), with LOQ at 20 µg/kg for all the compounds, except for anisodamine and scopoline, with LOQ at 40 µg/kg.

Finally, a total of 19 honey samples were analysed. Ten out of 19 samples were positive in relation to the occurrence of TAs, but only a sample presented scopolamine levels above the LOQ.

On the other hand, a post-targeted screening of about 1500 contaminants (pesticides, veterinary drugs, mycotoxins and metabolites) was performed, showing that 47% of honey samples were contaminated. Two veterinary drugs and one metabolite of them were putatively identified in 2 samples, while 6 herbicides and one metabolite were tentatively identified in 8 samples. Moreover, insecticides were detected in 4 samples

Although contamination of honey commercialized in Spain with TAs seems to be rare or is at trace levels, this method is a powerful tool for fast determination of TAs in honey as well as suitable screening analysis of contaminants.

Acknowledgement

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**TAN-P05**

## DEVELOPMENT AND VALIDATION OF A QUANTITATIVE METHOD FOR TARGET SCREENING OF 200 PESTICIDE RESIDUES IN FRUITS USING GC-QORBITRAP-MS

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High-resolution mass spectrometry (HRMS) is a powerful tool for semi-targeted or non-targeted screening, HRMS strongly competes with classical tandem mass spectrometry in the field of quantitative multiresidue methods (e.g., pesticides residues or veterinary drugs in foodstuffs). It is one of the most promising tools when moving towards non-targeted approaches, which is gaining greater acceptance every year.

The purpose of this study has been to develop a high throughput screening and simultaneously determination of 200 pesticides in various types of seasonal fruits samples (banana, watermelon, pear and strawberry) combining an effective modified QuEChERS method with gas chromatography coupled to high resolution accurate mass spectrometry (GC-Q-Orbitrap-MS). Samples were extracted with acetonitrile, and then magnesium sulphate, sodium chloride, trisodium citrate dehydrate and of sodium hydrogencitrate sesquihydrate were added, stirred and centrifuged, after cleanup with magnesium sulphate and PSA. Internal standard (propoxur-d7) for recovery and injection control was applied. The method was properly validated in all matrices according to SANTE document [1]. Nine compounds showed extraction difficulties (dimethomorph, ethoxyquin, fenamiphos, fenamiphos sulphoxide, fenamiphos sulphone, methamidophos, oxyfluorfen, sulprofos and tetrachlorvinphos). The other 191 compounds were evaluated in the validation tests.

Linearity was obtained from the analysis of spiked blank samples at six concentration levels ranging 1 to 100 µg/kg. All pesticides (191) were detected in three extracts (banana, watermelon and pear) at 1 µg/kg level, however, in the case of strawberry was detected at 5 µg/kg. Determination coefficients ($R^2$) were 0.99 and the relative standard deviation of response factor (RSD) for each calibration curve was less than ±20%. Finally, the limit of quantification (LOQ) was 5 µg/kg for all the analytes. Recovery (70-120%) and precision (≤ 20%) were studied at 5 and 10 µg/kg concentration levels, pesticides showed results as it were expected. The validated method was applied to the analysis of 31 field samples of banana (6), watermelon (5), pear (12) and strawberry (8). Thirteen pesticide residues were identified and quantified below European MRL. Concerning samples, residues were present in a 20% of watermelon samples, 33% of banana, 37% of strawberry and the 67% of pear samples.

In conclusion, the developed and validated method has demonstrated to be robust enough and appropriate for quantification purposes working in full scan mode, which has additional advantages such as its applicability to the analysis of non-target compounds and retrospective evaluation.

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**TAN-P06**

# MULTIPLE HEADSPACE SAMPLING COUPLED TO A PROGRAMMED TEMPERATURE VAPORIZER FOR THE DETERMINATION OF PAHs IN SALIVA

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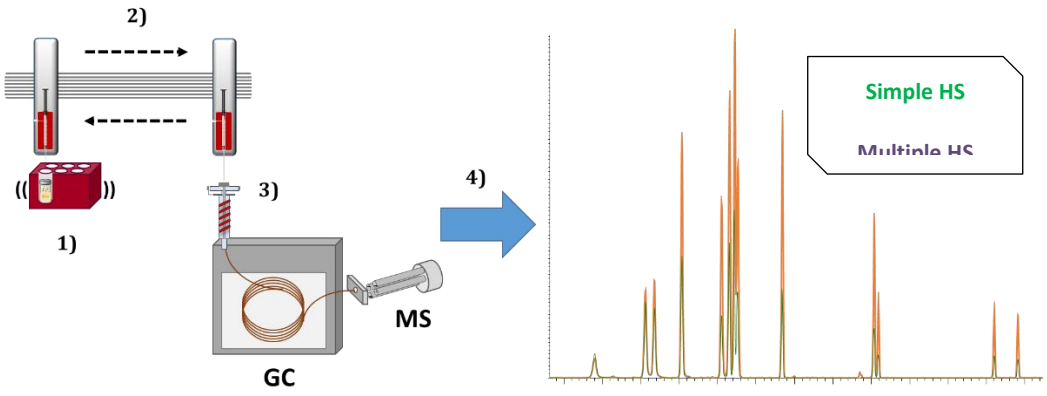
Here we propose for the first time the use of a multiple headspace methodology for the determination of 14 different polycyclic aromatic hydrocarbons (PAHs) in saliva samples.

PAHs are organic compounds formed by at least 2 fused aromatic rings with carcinogenic characteristics as stated by the International Agency for Research on Cancer (IARC). They are the result of incomplete combustion of organic matter and can be present ubiquitously in solid, aqueous and air samples entering the human body mainly by ingestion. These compounds have been studied from different types of human samples, using a plethora of extraction, separation and detection techniques [1]. In case of saliva samples, air-assisted dispersive micro-solid phase extraction (AA-dμ-SPE), liquid-liquid extraction (LLE), and atmospheric pressure solid analysis probe (ASAP) have been the procedures used for sample preparation, coupled to GC-FID, PTV-GC-qMS, Q-TOF-MS or HPLC-MS/MS. However, to the best of our knowledge, headspace (HS) has never been used to determine this class of compounds in saliva, probably due to their low volatility and scarce presence in this type of biological samples.

In this work, after a very simple sample treatment (just dilute 1mL of saliva to a final 5mL volume with UHQ water and add 2.5g of NaCl in a 10mL HS vial), this method utilizes a headspace autosampler coupled to a programmed temperature vaporizer (PTV) interfaced to a GC-MS for separation and detection. After only 5 minutes of headspace generation at 90 °C, PAHs are stepwise extracted (4 times) from the same vial and introduced into the PTV (Tenax liner) where they are focused below their boiling point (115 °C) using solvent vent mode to purge gas solvent. With a fast heating (12 °C/s), analytes are then introduced into the GC column for their separation in only 6 minutes of runtime. The use of this methodology allows to increase sensitivities up to 7 times for the least volatile compounds (up to 14 times in case of Chrysene). Furthermore, with this much simpler technique, we can obtain similar limits of detection (within the low ng·L$^{-1}$ range) to those reported using the methods aforementioned, minimizing sample manipulation.

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**TAN-P07**

# DETERMINATION OF GLYPHOSATE IN SOIL SAMPLES USING CDTE/CDS QUANTUM DOTS IN CAPILLARY ELECTOPHORESIS

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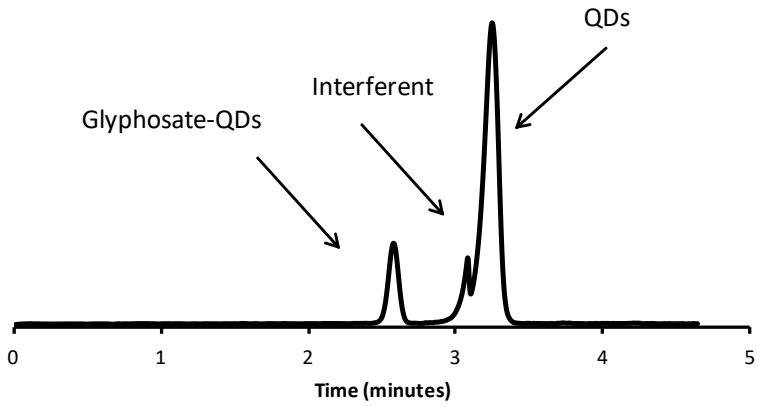
In this work, CdTe/CdS quantum dots were incorporated in a capillary electrophoresis system for the determination of glyphosate in soil samples. Fluorescence spectroscopic evaluation indicates there is an interaction between CdTe/CdS quantum dots and glyphosate, which produces an enhancement of the emission signal as a result of the formation of quantum dots-glyphosate complex. A capillary electrophoresis system for the separation of the complex from the remaining CdTe/CdS quantum dots is design. The variables involved in the separation were evaluated and optimized using a central composite design. Signals are elucidated under optimal conditions by calculating the difference in their electrophoretic mobility. The methodology selectivity allows the determination of glyphosate in complex matrices such as soil samples. The proposed method exhibits a linear range from 78 to 700 mg $kg^{-1}$ with a limit of detection of 26 mg $kg^{-1}$ and adequate repeatability and reproducibility (%RSD < 5.0%). The described methodology was applied to 7 soil samples collected from crop fields in Hidalgo, Mexico, found concentrations varied from 320 to 607 mg $kg^{-1}$.

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**TAN-P08**

## DEVELOPMENT AND VALIDATION OF AN LC MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF SHORT-CHAIN FATTY ACIDS IN PLASMA OF MULTIPLE SCLEROSIS PATIENTS

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In recent years, interest in the intestinal microbiota and its potential relationship with different diseases is gaining prominence [1-3]. Among the metabolites generated by the gut microbiota that could be acting as modulators of the evolution of different neuro and immune disorders, short-chain fatty acids (SCFAs) are the ones that could be more relevant [4]. There is therefore an urgent need for an analytical method that is sufficiently sensitive to enable SCFAs to be determined satisfactorily in biological samples.

In the present article, a novel method based on LC-QqQ-MS working on MRM mode has been optimized for the determination of the three main SCFAs found in plasma: acetate, propionate and butyrate. The proposed method has achieved significant improvements in analytical features, especially in the case of the sensitivity, where lower detection and quantification limits than those obtained in previous approaches have been obtained. Finally, the optimized method has been successfully applied for the determination of SCFAs in plasma samples collected from multiple sclerosis patients. The results have shown an existing correlation between the levels of SCFAs in plasma and the disease, thus demonstrating the relevance of the proposed approach as a rapid, simple and reliable clinical tool for better understating the role of the gut microbiota with the evolution of certain diseases.

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**TAN-P09**

# ELECTROCHEMISTRY OF INDIUM AND ELECTROCHEMICAL FORMATION OF Cu-In INTERMETALLIC COMPOUNDS IN CHOLINE CHLORIDE-ETHYLENE GLYCOL (1:2)

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Indium and its alloys are suitable materials for producing semiconductor compounds (e.g. InSb, InAs, GaInAs, $CuInSe_2$ and $Cu(In,Ga)Se_2$) which are widely used in electronic and optoelectronic technology. Polycrystalline thin films of the chalcopyrite materials $CuInSe_2$ and $Cu(In,Ga)Se_2$ are proposed as absorbent materials for photovoltaic solar cells, due to their high optical absorption coefficient and p-i-n-type electrical conductivity.

The processes to prepare chalcopyrite absorber layers involve electrodeposition of precursor films of Cu-In alloy, $CuInSe_2$, $Cu(In,Ga)Se_2$ followed by re-crystallization by thermal annealing at high temperature in selenium atmosphere. An alternative to aqueous electroplating solutions is to use as electrolytes low cost room temperature ionic liquids, as the deep eutectic solvents (DES) due to their important properties (i.e high conductivity, relatively wide potential range of electrochemical stability, low vapor pressure and the ability to solvate many metal salts).

As a part of a project to look into the possibilities offered by DES in the formation of semiconductor compounds, the present work is concerned with the electrochemical behaviour of indium in the eutectic mixture ChCl-EG (1:2). The study has been carried out using different substrates as working electrodes: i) W as an inert material and ii) Cu as a reactive electrode.

$InCl_3$, dissolved in the rich chloride media as $InCl_p^{3-p}$, is reduced on a tungsten electrode to indium metal via only one electrochemical step. Conversely, InCl undergoes the following disproportionation reaction when dissolved in the eutectic ChCl:2EG.

$$3InCl(s) + (p-3)Cl^- \rightleftharpoons 2In(0) + InCl_p^{3-p}$$

Transient electrochemical techniques were used in order to study the reaction mechanism and the transport parameters of the electroactive species at a tungsten electrode. The results showed that electrocrystalization of In plays an important role in the electrodeposition process. Experimental current-time transients have been compared with the theoretical models based on instantaneous and progressive nucleation.

Mass transport towards the electrode is a diffusion process, and the diffusion coefficient of In(III) and the activation energy for diffusion have been calculated.

The electro-reduction of In(III) solutions was also investigated at a copper substrate. The resulting cyclic voltammograms evidenced the formation of In-Cu intermetallic compounds. Hence, In-Cu alloy films were obtained by continuous potentiostatic electrolysis and intensiostatic pulse electrolysis. The obtained samples, characterized by XRD and SEM, revealed the formation of the metastable CuIn phase that could be transformed into $Cu_{11}In_9$ by thermal annealing.

**Acknowledgements**

Authors thank the Junta de Castilla y León (RIS3 de Castilla y León 2014-2020), Project CLU-2017-09 for financial support. S. García, also thanks the youth employment operative programme (European Social Fund, ESF, and youth employment initiative, YEI).

**TAN-P10**

## SCREENING OF SEMI-VOLATILE COMPOUNDS IN INDOOR DUST USING GAS CHROMATOGRAPHY-ACCURATE MASS SPECTROMETRY

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Indoor dust contains a complex mixture of anthropogenic and synthetic compounds closely related to dermal and respiratory diseases, usually grouped under the so-called sick building syndrome [1]. Target methods have been developed for the quantification of different groups of substances in dust samples, based on the combination of well-tuned sample preparation with selective analytical techniques, such as liquid and gas chromatography tandem mass spectrometry (MS/MS). These procedures permit the sensitive determination of pre-selected compounds [2,3]; however, they are totally blind to any compound not included in the list of MS/MS transitions. For that reason, the comprehensive characterization of the different species existing in dust remains as a challenging issue.

Herein, a new screening strategy is presented. Pressurized Liquid Extraction (PLE) and gas chromatography coupled to quadrupole time-of-flight mass spectrometry, using electron ionization (GC-EI-TOF-MS), was applied as sample preparation and determination technique, respectively.
A data mining workflow is proposed for the non-target identification of species present in dust. First, the Unknown Analysis algorithm was applied for spectral deconvolution of compounds in the GC-MS chromatograms. For tentative/confirmed identification of the detected compounds, a preliminary comparison was developed with nominal resolution EI-MS spectra in the NIST17 library. In addition to experimental m/z values compiled in this library, the accurate ratios calculated for fragment ions with known structures were also considered, thereby the identities of more than 75 compounds were confirmed against authentic standards, and compiled in a customized library. Thus, some species, such as indigo, phthalic anhydride, 2,4-toluene di-isocyanate, phthalimide, certain UV absorbers and octyl isothiazolinone were reported (with a confidence level 1 in the categorization scale set for non-target screening in accurate MS) in indoor dust for the first time. A semi-quantitative determination of semi-volatile compounds was developed and a range of concentrations for a group of 44 pollutants were estimated in a set of 27 dust samples.

Acknowledgments
This study was supported by Xunta de Galicia (grant GRC-ED431C 2017/36), and the Spanish Government (grant CTQ2015-68660-P).

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**TAN-P11**

# ASSESMENT OF SUPERCRITICAL FLUID CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY FOR THE DETERMINATION OF NEONICOTINOID RESIDUES IN WINE SAMPLES

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Neonicotinoid pesticides have been introduced two decades ago as a new generation of safer insecticides. Despite their improved selectivity and lower toxicity versus other groups of insecticides; nowadays, the use of this family of compounds is controversial due to their side effects in non-target organisms. In particular, the spread of neonicotinoids has been correlated with the collapse of hives[1,2,3]. At medium term, a reduction in the population of pollinators might have a dramatic, negative effect in the productivity of agriculture. In order to face this situation, EU has recently forbidden the open applications of three neonicotinoid compounds: Imidacloprid[4], Thiamethoxam[5] and Chothianidin[6]. However, other compounds from the same group (Acetamiprid and Thiacloprid) are still permitted in Europe. In other countries, additional compounds belonging to the same family, such as Nitenpyram and Dinotefuran, are also authorized.

Production of vinification grapes employs not only fungicides, but also a relevant amount of insecticides to treat different pests, such as *Lobesia Botrana*, spiders and other diseases affecting vineyards. In some production regions, i.e. Bordeaux (France), treatment of vineyards with insecticides is not an option, but a compulsory practice to control pests. So far, our group has reported the presence of Imidacloprid in wines elaborated by year 2018, using liquid chromatography (LC) tandem mass spectrometry (MS/MS). Herein, we investigate the performance of supercritical fluid chromatography (SFC), combined with electrospray (ESI) MS, as an alternative technique for the determination of the currently known neonicotinoid insecticides in samples of red and white wines. Performance of compounds separation is evaluated using different polar SFC columns. Analytical features of SFC-MS are investigated in detail and compared to those achieved by reversed-phase UHPLC-ESI-MS, using the same MS platform (quadrupole time-of-flight mass spectrometry, QTOF) as determination technique. The developed SFC-QTOF-MS method is applied to study the presence of neonicotinoid residues in samples of commercial wines.

Acknowledgements: This study has been supported by the Spanish Government, Xunta de Galicia, E.U. FEDER funds and EU Interreg SUDOE program (projects CTQ2015-68660-P, GRC-ED431C and SOE1/P2/F0246). L.P.M acknowledges a FPU contract to the Spanish Ministry of Education (FPU16/03942).

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**TAN-P12**

# CARACTERIZACION DE RESIDUOS DE DE DISPARO MEDIANTE LA TÉCNICA ABLACIÓN LASER DE BARRIDO ACOPLADA A ESPECTROMETRÍA DE MASAS CON FUENTE DE PLASMA DE ACOPLAMIENTO INDUCTIVO (SLA-ICP-MS)

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El creciente uso de las armas de fuego, ha suscitado una alta demanda en el ámbito de la ciencia forense, para desarrollar nuevas metodologías analíticas que permitan la identificación de residuos de disparo (GSR). Los productos de descarga de las armas de fuego están formados por una colección de material particulado y vapores que se depositan en los alrededores del disparo. Hoy en día, la detección e identificación de estas evidencias físicas puede proporcionar una valiosa información a la investigación forense y ser considerada una evidencia en procesos judiciales [1]

De acuerdo con lo establecido por la *European Network of Forensic Science Institutes* (ENFSI) y la *American Society for testing and materials* (ASTM), las partículas características de GSR de munición convencional deben de estar compuestas por $^{121}$Sb, $^{137}$Ba y $^{208}$Pb [2,3]. El análisis forense convencional de esta triada utiliza la técnica SEM-EDX que implica, entre otros inconvenientes, un tiempo elevado de análisis. La ablación laser de barrido acoplada a espectrometría de masas con fuente de plasma de acoplamiento inductivo (SLA-ICPMS) constituye una alternativa para la detección e identificación de GSR. El modelo de ablación y asi como las condiciones del ICPMS fueron optimizadas para la detección de partículas características ($^{121}$Sb, $^{137}$Ba, y $^{208}$Pb). Además, se han monitorizado otros isotopos ($^{27}$Al, $^{29}$Si, $^{31}$P, $^{33}$S, $^{35}$Cl, $^{39}$K, $^{44}$Ca, $^{57}$Fe, $^{60}$Ni, $^{63}$Cu, $^{66Zn}$ y $^{118}$Sn) que permiten obtener información adicional y clasificar las partículas de GSR en características o consistentes. En el caso de la munición exenta de plomo (*lead-free*) otras triadas de elementos han sido también determinadas por esta técnica para identificar los GSR característicos ($^{157}$Gd, $^{49}$Ti, $^{66}$Zn y $^{71}$Ga, $^{63}$Cu, $^{118}$Sn).

La metodología desarrollada ha mostrado su efectividad en muestreos realizados en la mano del tirador o en las fosas nasales, empleando para ello diferentes dispositivos (*tape lifting y swabbing)* que se han diseñado, modificado y evaluado [4, 5].

Se han realizado numerosos análisis reales tras la descarga de armas de fuego de diferente calibre y utilizando municiones con y sin plomo. En todas las muestras analizadas, se han encontrado partículas características o consistentes con el método analítico propuesto.

En vista a los resultados obtenidos, se puede concluir que con el método analítico desarrollado basado en SLA-ICPMS, se permite identificar y caracterizar partículas de GSR en muestras tomadas tras el disparo de armas de fuego, en un tiempo de análisis inferior a 60 minutos. Es de esperar que esta técnica pueda considerarse próximamente como técnica *bona fide* en el ámbito judicial.

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**TAN-P13**

## MONITORING THE PRESENCE OF MYCOTOXINS IN EDIBLE OILS AND NUTS

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Mycotoxins are secondary metabolites produced by various species of fungi and they can appear in food under certain conditions of humidity and temperature. Among them, the presence of aflatoxins (produced by the species Aspergillus) in food is a main concern [1]. All of them are toxic to both human and animal cells, and according to Food and Agriculture Organization (FAO), which estimates that more than 25% of all agricultural products are contaminated with mycotoxins, the upper limit of them in a food product should not be greater than 15 µg/kg. The processed vegetable oils and nuts can be contaminated easily with mycotoxins due to certain weather conditions, such as high temperatures, relative humidity and rainfall, as well as storage conditions, so there is an increase in the interest of validated analytical methods for their determination. However, to our knowledge, there are scarce studies that evaluate the presence of aflatoxins in edible oils and nuts [2].
In the present study, an analytical method based on a QuEChERS approach (quick, easy, cheap, effective, rugged and safe) with a clean-up step by dispersive solid phase extraction (d-SPE) has been performed for the extraction of mycotoxins (zearalenone (ZEA) and α-zearalenol (α-ZOL), and aflatoxins B1, B2, G1 and G2) from edible oils and nuts. The analysis of the extract was performed by ultra high performance liquid chromatography coupled to a triple quadrupole mass analyzer (UHPLC-QqQ). The method was fully validated and the detection limit is 0.5 µg/kg, for all the mycotoxins in edible oils and nuts, except 1 µg/kg for ZEA and α-ZOL in nuts. Suitable recoveries were obtained at low concentration levels, ranging from 80 to 110%. Intra and inter-day precision were also evaluated and relative standard deviation was lower than 20%. The validated method has been applied to more than 200 samples of different types of edible oils (olive oil, lampante olive oil, refined olive oil, olive pomace oil, crude olive pomace oil, sunflower oil, crude sunflower oil, soy oil and corn oil) and nuts (almonds, hazelnuts, peanuts pistachios and walnuts). AFB2 and AFG1 have been detected at very low concentrations, while AFG2 and ZEA were detected at higher concentrations; up to 7 and 25 µg/kg respectively ZEA has been detected in 20% of the studied edible oils. In addition, almost the 40% of the nuts analysed were contaminated with AFG2 or AFG1, detecting AFG2 in all the samples of almonds and pistachios studied at a concentration up to 6 µg/kg.

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**TAN-P14**

# DEVELOPING A METHOD FOR THE ANALYSIS OF MINERAL OIL SATURATED HYDROCARBONS (MOSH) AND MINERAL OIL AROMATIC HYDROCARBONS (MOAH) IN EDIBLE OILS

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Mineral oils are products obtained from the distillation of petroleum and are mainly composed of hydrocarbons, but they are also synthetically produced from coal, natural gas and biomass. Lubricating oils for food use are a complex mixture of these aliphatic saturated hydrocarbons (MOH), linear or branched (paraffins), ranging from C20 to C54.
Mineral Oils Saturated Hydrocarbons (MOSH) accumulate in tissues, lymph nodes, spleen and liver and can cause microgranulomas, and Mineral Oils Aromatic Hydrocarbons (MOAH), are considered as possible carcinogenic and mutagenic substances. Thus, the contamination of edible oils by MOHs is becoming of great importance. These MOHs usually come from different sources, either extracting the oil from its fruit, by contact with materials that have mineral oils such as paperboard or inks, mineral oils used in machinery used during the oil manufacturing process, or even food additives [1].
The European Commission has stablished a legal limit of the group of MOH, an unresolved chromatographic hump below de natural hydrocarbons of the sample ranging from C10 to C56, of 50 mg/kg in sunflower oils [2]. In the present study an analytical method based on an offline solid phase extraction (SPE) has been performed a silver nitrated silica gel for the extraction and separation prior to the analysis of MOSH and MOAH from edible oils.
The analysis of the extracts was performed using a large volume injection method (LVI) by gas chromatography with flame ionization detector (GC-FID). The method was fully validated and the detection limit was 10 mg/kg. Suitable recoveries were obtained ranging from 80 to 110%. Intra and inter-day precision were also evaluated and relative standard deviation was lower than 20%. The validated method has been applied to more than 20 samples of different types of edible oils (olive oil, sunflower oil, etc…) detecting MOSH and MOAH in the majority of them.

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**Acknowledgements**

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**TAN-P15**

# PORTABLE COLORIMETRIC SENSOR SUPPORTED IN NYLON FOR SILVER ION DETERMINATION AS CATALYST

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This paper reports the fabrication and the utility of a new solid sensor for silver ion (Ag (I)) detection at µM level. The optical sensor was prepared by incorporating of both reagents, pyrogallol red (PGR) and 1,10-phenantrhroline (Phen), in a nylon membrane. The sensor is based on the catalysis of Ag (I) for the oxidation of PGR by potassium persulphate in presence of Phen as activator[1]. The decrease in sensor absorbance at 485 nm and change of color from red to grey were proportional to Ag(I) concentration. Visual inspection of colored sensor has been evaluated for semiquantitative analysis. Quantitative analysis was carried out by its diffuse reflectance (DR) measurement or by a digital image-processing tool (GIMP) using a smartphone. The sensor exhibited a linear relationship toward Ag (I) concentrations ranging from 0.4 to 10 µM. The limit of detection was found to be 0.12 µM. The relative standard deviation achieved for several batches of sensors was lower than 2 %. Finally, water samples from refrigerate circuits containing solid biocides (in which sold biocides leaching Ag (I) were employed), have been analysed to demonstrate the performance of the proposed device.[2]

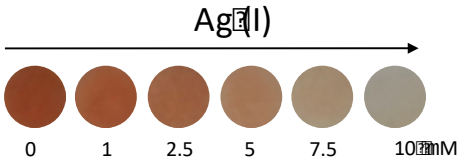


**Figure 1**: Colorimetric response of the sensor as function of the concentration of Ag(I) at µM level

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**TAN-P16**

# ON-SITE DETECTION AND QUANTIFICATION METHOD FOR KETAMINE IN ILLICIT DRUG STREET SAMPLES BASED OF ITS REACTION WITH GOLD BROMIDE

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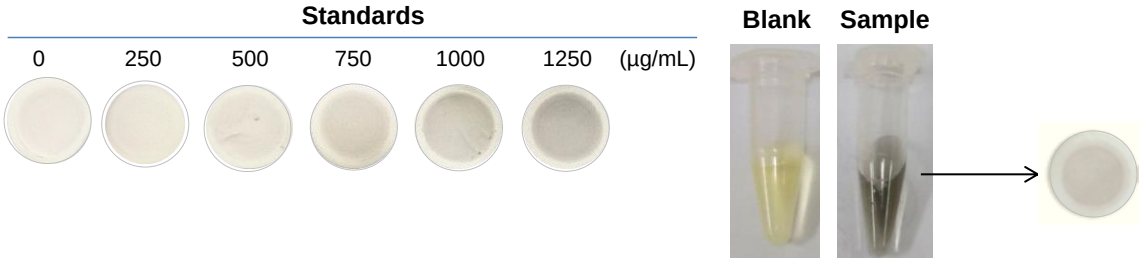
Because of its psychoactive properties the prescription drug ketamine is widely used in illicit street samples, alone or in combination with other drugs. Thus, it is frequently found in seizures all over the world, and several studies have proved its incidence in criminal activities [1]. Consequently, there is an increasing demand of reliable, simple, fast and cost-effective methods for the presumptive detection of ketamine, not only for prosecuting drug trafficking but also in the context of point-of-care services. Ideally, these methods should also provide quantitative or semiquantitaive information, as it may be essential to take informed decisions. In this respect, classical colorimetric spot tests are of renewed interest because they can be easily used in combination with digital colour analysis [2].

We describe here a new approach for the on-site detection and quantification of ketamine based on its reaction with alkaline gold bromide and subsequent collection of the product of reaction (colloid gold) onto nylon filters. The obtained filters are then photographed and processed by digital colour analysis in order to estimate the amount of ketamine in the samples. Different reaction and measurement conditions have been optimized. The selectivity towards other drugs and diluents commonly found in drug illicit drug street samples has been also studied. For quantitative purposes, both the RGB and CYMK colour systems have been tested. The black coordinate of the CYMK colour system was selected as it provided the best linearity, as well as suitable sensitivity and reproducibility (RDS ≤ 13 %).

The quantitative performance of the proposed approach has been evaluated and compared with the results obtained through the measurements of the absorbance of ketamine in solution, as well as the absorbance of the filters in diffuse reflectance mode. The method has been applied to different illicit drug street samples. An example of positive result is shown in the next figure, which also shows the filters obtained for a set of calibration.

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**TAN-P17**

# EVALUATION OF CALIBRATION STRATEGIES FOR LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS): DETERMINATION OF Al E Pb IN ELECTRONIC WASTE

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The LIBS allows the direct analysis of solids, complex and refractory samples that present's difficult acid decomposition, such as electronic waste (waste electrical and electronic equipment, WEEE). This material is reach in oxides of Mg, Si and Ti, flame retardants, base (Cu, Ni) and noble (Ag, Au) metals and polymers. The generation of WEEE has been growing annually, and international legislation for correct disposal procedures and maximum permissible values of potential toxic elements. The LIBS presents itself as an interesting analytical technique for the monitoring of Al and Pb in WEEE, as it allows the direct analysis of this sample. However, for the quantitative analysis, some difficulties are found, since analyte and matrix are analyzed integrally, and thus matrix effects can compromise the accuracy and precision of the measurements. Thus, some calibration strategies can be used to minimize matrix effects, and to determine analytes with satisfactory figures of merit. In this study, we evaluated four calibration strategies: i-Matrix-matching calibration (MMC), ii-Calibration free (CF), iii-One-point and multi-line calibration (OP MLC); and iv- Single-sample Calibration (SSC) for the direct determination of Al and Pb in printed circuit boards – PCBs - of computers by LIBS. Six PCBs samples were ground in a knife mill for analysis. Using a full factorial design $2^3$, with center and axial points the instrumental conditions were optimized: delay time of 2 µs, gate width 3 µs and laser energy of 42.5 mJ. For all calibration strategies evaluated, 200 mg of sample was used to obtain pellets (pressed at $10x10^4$ N for 2 min), and 100 spectra were obtained for each sample (n=3). For MMC a calibration curve in the range of 3.1 to 55 $gkg^{-1}$ Al (r= 0.9025, Al I 396.15nm) and 0.72 to 11.6 $gkg^{-1}$ of Pb (r= 0.9179, Pb I 405.76 nm) were obtained using four samples of PCBs as standards, and recoveries between 99 and 116% were obtained for Al and Pb. For CF only emission intensity and physic-chemical parameters of the obtained plasma are necessary for the quantification, not requiring a calibration standard.[1] Recoveries between 78 and 113% were obtained for Al and Pb using CF LIBS. For the OP MLC,[2] only one sample is used as calibration standard and several emission lines to obtain linear calibration models for Al and Pb. Recoveries ranging from 78 to 109% for Al (except sample S3, 57%) and 83 to 103% for Pb were obtained. In the SSC method, only one sample is used as standard and different emission lines of the analyte and elements present in the sample and standard.[3] For this strategy the emission lines for Al I 396.15 nm, Pb I 405.78 nm and Mg II 279.55 nm were used. Recoveries between 82 to 116% for Al (except for sample S1, 220%) and 71 to 116% for Pb were obtained using SSC. Thus, MMC, CF, OP MLC and SSC were calibration strategies that allowed analyzes of solids to determine Al and Pb in WEEE by LIBS with satisfactory accuracy, considering the complexity of the sample evaluated.

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**TAN-P18**

# DETERMINATION OF BISPHENOL A IN THERMAL PRINTING PAPER USING DIRECT ANALYSIS IN-REAL TIME (DART) ACCURATE MASS SPECTROMETRY

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Bisphenol A (BPA) is a high-production-volume chemical commonly used as a developer in thermal paper products, such as cash register receipt paper. This compound is a well-known endocrine disrupter, involved in a wide range of health outcomes in animals and humans [1]. Direct contact with thermal paper tickets is recognized as a relevant source of exposure to this compound [2]. In fact, increased urinary levels of BPA have been reported for cashiers and operators of thermal paper manufacturing companies [3]. In order to reduce this exposure route, the EU has limited the concentration of BPA to a maximum of 0.02 % (equivalent to 0.2 mg $g^{-1}$) for thermal printing paper commercialized after 2020 [4].

The main objective of this presentation is to demonstrate the suitability of direct ionization in real-time (DART), combined with accurate mass spectrometry, as a faster alternative to chromatographic-based methods for the quantitative determination of BPA, and three of its analogues species, in common cash receipts and tickets from different establishments. The efficiency of compounds ionization is evaluated under different conditions, and the effect of instrumental parameters of the DART source in the observed responses is discussed.
Under optimized conditions, the new proposed methodology provided recoveries in the range from 90 to 110 % and limits of quantification below the maximum concentration established for 2020. Accuracy of BPA levels found in non-spiked samples was confirmed using GC-EI-MS as reference technique. Obtained data reveals that BPA was systematically noticed in all the processed samples with concentrations ranging from 0.005% to more than 6%, and that only 6 out 17 studied samples fulfil the future legislation regarding maximum BPA levels in thermal printing paper.

## Acknowledgements

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**TAN-P19**

## DETERMINATION OF CHLORPYRIFOS BY A MULTICOMMUTATED PHOTOCHEMICALLY-INDUCED FLUORESCENCE OPTOSENSOR

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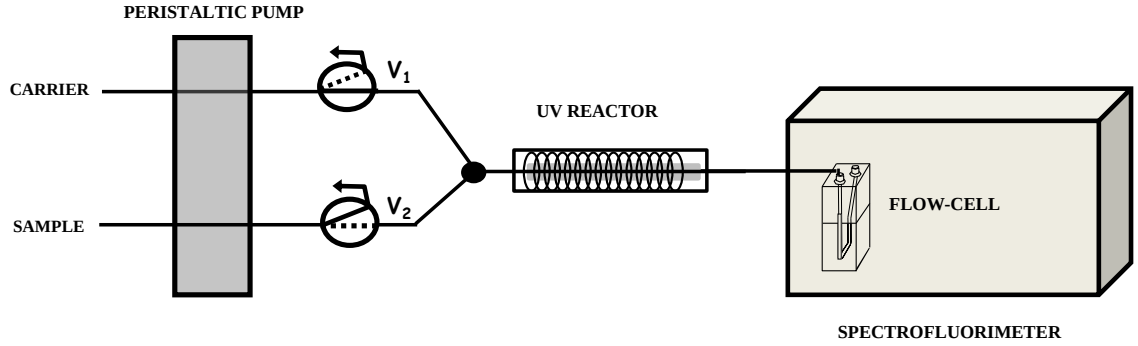
We propose a multicommutated flow-through optosensor (see Figure) for the quantitation of the organophosphate pesticide chlorpyrifos in dried chili peppers. We used photochemically-induced fluorescence detection to overcome the negligible native fluorescence of the target pesticide. Firstly, the photodegradation of chlorpyrifos was performed online with a UV lamp. Then, the fluorescent photoproduct was retained on an anion-exchange solid support (Sephadex QAE A-25) placed inside the flow-cell, where the analytical signal was recorded. The online photodegradation and preconcentration of the photoproduct on the solid support were critical for the automation, rapidity, and high sensitivity obtained. A QuEChERS procedure was selected for sample treatment, obtaining recovery yields close to 100%. The method proposed presents a quantitation limit of 18 mg $kg^{-1}$ in real samples, hence fulfilling the maximum residue limit specified in the Codex Alimentarius for dried peppers: 20 mg $kg^{-1}$. Therefore, it could be used as a novel method for screening purposes in the agri-food sector.



**TAN-P20**

## MULTICOMMUTATED PHOTOCHEMICALLY-INDUCED OPTOSENSOR FOR THE QUANTITATION OF THE NEONICOTINOID THIACLOPRID

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Neonicotinoid pesticides are the most widely used class of insecticides worldwide, representing a 25% share of the insecticides market in 2014 [1]. They present a wide range of applications: plant protection (crops, vegetables, fruits), veterinary products, and biocides to invertebrate pest control in fish farming. Therefore, they can be present in a wide variety of samples, hence the need to have reliable methods of analysis for their control in food samples. We report a novel analytical method for the quantitation of the neonicotinoid thiacloprid in different kinds of lettuce samples. This analyte does not present native fluorescence. It is thus required to develop different strategies for its luminescence detection. In the proposed method, the analyte is UV-photoirradiated on-line, generating a fluorescence photoproduct. This photoproduct is retained on a solid support, Sephadex SPC-25, placed in the flow-cell, where it develops its analytical signal. The whole system was automated by means of a multicommutated manifold, using 3-way solenoid valves.
The analytical method presents a detection limit of 15 µg L-1 and a linear dynamic range of 50-500 µg L-1. For the analysis of thiacloprid in lettuce samples, a QuEChERS method was carried out and, after the proper dilution, the method detection limit was low enough to comply with the Maximum Residue Limit for thiacloprid. Hence, although chromatography is still the benchmark methodology for the analysis of pesticides in food samples, new easy-to-handle and non-expensive methods may represent an alternative as screening methods.

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**TAN-P21**

## PORTABLE INSTRUMENTATION: VALIDATION FOR IN-SITU MONITORING

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Today, the increment of the demand of devices that allow the monitoring of environment, leads to the development of in situ analysis methods [1,2]. One option is the use of photometric probes, which are portable devices designed to perform both, in situ and even continuous analysis, showing some advantages (e. g. higher data frequency, real-time information, greennes) over the classic laboratory intrumentation.

This work aims to evaluate the analytical quality parameters offered by an optical fiber probe for the control of the presente amount of the biocide 2,2-dibromo-3-nitrilopropionamide (DBNPA) in refrigerate circuits. Two methods have been tested: native absorbance and colorimetric derivative formation. Direct measurement of the absorbance at 230 nm and the measurement of the formation of $I_3^-$ from DBNPA have been tested. Moreover, the behavior against turbidity of both methods has been studied for several waters, parameter that can be relevant in the mentioned circuits (see Fig 1). This study has allowed to conclude that the DBNPA determination with the colorimetric method provides higher analytical quality results, specially for selectivity, sensibility and detection and quantification limits (LOD and LOQ), which are markedly superior for controlling the amount of the DBNP in the circuit at the required levels. For example, LOQ for the direct method was 1.3 mg/L instead for the colorimetric method was 0.1 mg/L. On the other hand, there are not significant differences between the figures of merit of the optical fiber probe and a laboratory spectrophotometer.

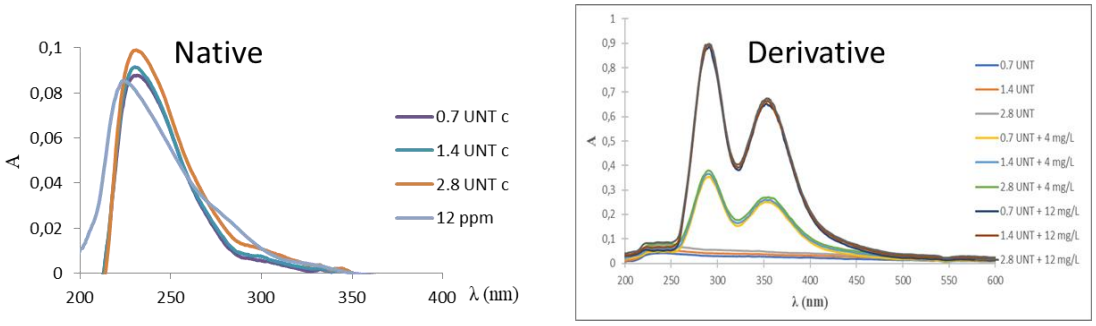


Figure 1. Influence of the level of turbidity at several amounts of DBNPA for the two methods: native absorbance and colorimetric derivative formation

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**TAN-P22**

# APPLICATIONS OF LOW FLOW SECONDARY ELECTROSPRAY IONIZATION FOR VOLATILE CHARACTERIZATION OF BIOLOGICAL AND INDUSTRIAL PROCESESSES

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Secondary ElectroSpray Ionization (SESI) in tandem with Atmospheric Pressure ionization Mass Spectrometry (API MS) has achieved sensitivities in the sub-ppt range for polar vapors. Low-Flow SESI (LF-SESI) was developed in order to overcome classic SESI disadvantages, such as dilution by MS counterflow gas and space charge scattering. In this work we present some examples of real-time applications of our Low-Flow SESI, which can be coupled to a pre-existing MS, such as breath analysis, volatilome of yeast and plants or analysis of electronic cigarettes. The results show that the LFSESI-MS architecture is a powerful method to discover new analytes and analyze complex systems kinetics.

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**TAN-P23**

## SENSITIVE FLUOROMETRIC SCREENING OF QUINCLORAC RESIDUES IN RICE

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Rice is considered one of the most important foods in the world due to its high nutritional value. Quinclorac, a selective herbicide, is one of the most detected pesticide residues in rice crops according to pesticide monitoring studies around the world. Usually, the most common methods for the determination of quinclorac in rice are very time-consuming and labor-intensive, so it is important to develop alternative sensitive and simple analytical methods able to detect quinclorac in food samples. Here we propose a fluorometric method for the screening of this herbicide (monitoring its fluorecence signal at excitation/emission wavelengths of 238/358 nm/nm). A modified QuEChERS method was selected for sample treatment due to its simplicity and high recovery yields. The proposed method presents a detection limit of 0.75 ng $mL^{-1}$ and satisfactory precision. Recovery experiments were performed in different kinds of rice (white and brown) at or below the Maximum Residue Limit established in European Union. In all cases, recoveries close to 100% were achieved. The simplicity, high sensibility and good selectivity of the method proposed fulfill the requirements for its application in quality control.

**TAN-P24**

## DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICALS AND BIOLOGICAL FLUIDS BY QUENCHING ON EUROPIUM LUMINESCENCE

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In this work, we present a novel strategy for the quantification of ascorbic acid (vitamin C). The method developed is based on the quenching effect produced by ascorbic acid on the time-resolved luminescence signal of europium ions (excitation/emission wavelengths of 388 nm/612 nm). The use of europium instead of terbium at the same concentration resulted in approximately a 50% enhancement in the analytical signal. On the other hand, the addition of surfactants did not provide an enhancement of the quenched signal.

The method presents a linear dynamic range of 0.2-2 μg $mL^{-1}$, with a detection limit of 0.06 μg $mL^{-1}$ and relative standard deviation lower than 6%. It was applied to the determination of ascorbic acid in pharmaceutical preparations of Spanish Pharmacopoeia. The results were compared to those obtained by the AOAC method [1] (*t*-test and *F*-criterion; 5% significance level) and no statistically significant differences were found in all cases. We also performed recovery experiments in the same pharmaceuticals, human serum and human urine. Recovery yields were close to 100%, demonstrating the suitability of this proposed method for the analysis of ascorbic acid in pharmaceuticals and biological samples, requiring no sample treatment except a dilution step.

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**TAN-P25**

# EVALUATION OF INTERFERING COMPOUNDS IN THE NON-SEPARATIVE DETERMINATION OF POLYAMINES AND RELATED COMPOUNDS IN URINE BY FIA-MS-QqQ

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The use of non-separative techniques based on mass spectrometry detection presents a great interest for biomarker discovery due to the rapid output of results and the wide range of compounds that can be determined. Moreover, currently there is a great interest in the determination of compounds of clinical significance in biological matrices involving non-invasive methods of sample collection.

In this work, we have developed a high performance method for the determination, by means of a screening-confirmation approach, of polyamines (putrescine, cadaverine, spermidine, spermine, N-acetylputrescine and N-acetylspermine) and related compounds (L-ornithine and γ-aminobutyric acid) in urine samples. In order to perform the analysis, the screening step was carried out using a non-separative method by flow injection analysis coupled to a triple quadrupole mass spectrometer (FIA-MS-QqQ), while the confirmation step was carried out using a separative method by means of a liquid chromatographic system coupled to the aforementioned MS system (HPLC-MS-QqQ).

One of the main drawbacks when using non-separative techniques is that there may be interfering compounds i.e., isobaric molecules, in the evaluated matrix that could interfere in the analysis. These interfering compounds can yield parent ions of the same mass-to-charge ratio, and if they exhibit similar gas-phase ion chemistry, they can also produce fragment ions of the same mass-to-charge ratio, causing quantification interferences when multiple reaction monitoring (MRM) is used.

In order to evaluate this problem, in this work an exhaustive evaluation was carried out of those compounds present in urine that could have a fragmentation pattern similar to the target analytes. Within this purpose, first, a bibliographic search (in articles and different databases, such as Human Metabolome Database or METLIN) was performed of those compounds present in urine previously described that share the same precursor ion and a fragmentation pattern similar to the analytes of interest. From this bibliographic search, 20 compounds were selected. Then, aqueous standard solutions of these compounds were prepared and working diluted samples were injected directly in the mass spectrometer. Precursor ions and fragmentor values were optimized for each of them. Following this, product ion spectra were obtained at different collision energy values in order to evaluate if they share similar fragmentation patterns that the analytes of interest. Next, these compounds were injected in the separative mode (HPLC-MS-QqQ) to identify their retention times. Finally, four different urines were analysed using the separative mode (HPLC-MS-QqQ) in order to evaluate which one interfered with the target analytes.

It was observed that only 3 of the 20 compounds really interfere with the analytes of interest. The other 17 were discarded since they did not present similar MRM transitions or they were not detected in urine samples.

The methodology was applied to the analysis of urine samples from non-diagnosed subjects. Firstly, the non-separative method (FIA- MS-QqQ) was used and subsequently the results obtained by this modality were confirmed with the HPLC-MS-QqQ method.

**TAN-P26**

# FAST BLUE B FUNCTIONALIZED SILICA-POLYMER COMPOSITE TO EVALUATE DIETARY TRANSGRESSION IN PATIENTS WITH CELIAC DISEASE

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Celiac disease is an immune-mediated systemic disorder elicited by gluten and related prolamines present in genetically susceptible individuals. The actual treatment is a strict and lifelong gluten-free diet. However, compliance with the gluten-free diet is not always adequate and many food products contain low concentrations of gluten. As a consequence, the determination of dietary transgressions is a challenge for patients, physicians and dietitians. Alkylresorcinols (AR) have been proposed as sensitive and specific biomarkers of gluten consumption [1]. Herein, the objective of the present work was to evaluate silica –polymer composites doped with fast blue B (FBB) colorimetric reagent to estimate alkylresorcinols in biological samples [2]. The proposed colorimetric device was synthetized by immobilizing FBB into Polydimethylsiloxane-Tetraethylortosilicate composite. The assay was based on the spontaneous release of FBB to the solution containing AR (5-pentylresorcinol as target analyte) and the formation of the azo complexe that can be measured at 520 nm (Fig. 1).

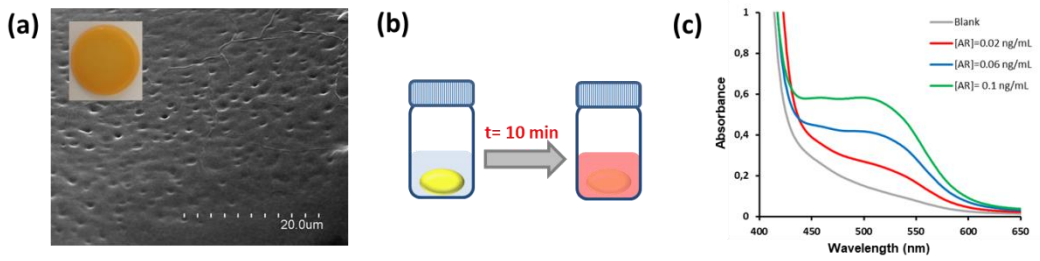


**Fig. 1.** (a) SEM micrograph of the FBB functionalized-PDMS-TEOS composite.(b) Schematic diagram of the experimental set-up (c) UV-vis spectra as a function of alkylresorcinol concentration.

Under the optimum experimental conditions, LOD was 10 ng/mL (estimated as [AR] total concentration) by adding a SPE step (C18 cartridges) prior to the analysis. Precision was also tested, providing adequate results (RSD<7%). Preliminary studies in urine samples have shown successful results. The main advantages of the proposed device are the simplicity of the whole procedure and portability. It is also a cost-effective and energy efficient device.

**Acknowledgements**
The authors thank to the VLC-BIOMED 2017 program (University of Valencia). EU FEDER and the Gobierno de España MCIU-AEI (CTQ2017-90082-P) and to the Generalitat Valenciana (PROMETEO Program 2016/109.

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**TAN-P27**

# FROM VISUAL OBSERVATION TO COMPLEX SPECTROMETERS: PORTABLE INSTRUMENTS FOR IN SITU ANALYSIS OR SMARTPHONE AS COLORIMETRIC DEVICES

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Nowadays the society demands new devices for monitoring the environment [(1)] and the health [(2)] (including security and environmental protection) in the point of the sample (in situ), thus are call point of care (POC) or point of need (PON) devices [(3)]. These devices are also characterized by low-cost, versatile and easy to handle by non-qualified people. Concerning to this topic many different methodologies have been developed, being the employment of colorimetric devices one of the most used. In this work, the operability of portable instrumentation for colorimetric analysis has been displayed. Portable instruments such as naked eye, smartphone spectrometers (imagen and spectra data) and portable reflectance spectrometer have been used as a colorimetric device (Figure 1). To perform the instrument validation a comparative study has been performed using a conventional laboratory reflectance spectrometer. In order to obtain broad information, these instruments have been compared from 1) the analytical point of view, considering the quantitative parameters such as precision (intra e inter day precision), robustness, size, components, costs…), and from 2) environmental point of view, based on the footprint as kg of $CO_2$ of the employed instrumentation. No significant differences in the precisions of the different instruments were obtained with RSD (%) values lower than 5% for all the instruments. However, other aspects such as portability, cost or footprint of $CO_2$ were better for portable instrumentation, especially for those colorimetric devices that used smartphones. As a case of study, these instruments have been employed to measure the color developed on three different solid sensors (supports based on PDMS, paper and nylon used to determine $NH_4^+$, $H_2S$ (for ppm concentrations) and $H_2S$ (for ppb concentrations). The results acquired indicated that the use of a smartphone (as color reader or as spectrometer) is a good alternative to obtain appropiate analytical signals which can be processed in order to obtain the analyte concentration. These results represent a great potential of the portable instrumentation used in this work, being excellent analytical tools for POC colorimetric analysis and broad accessibility in resource limited settings.

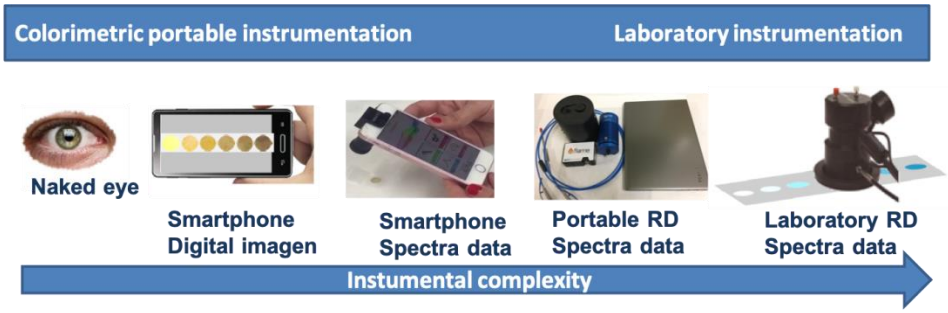


**Figure 1.** Colorimetric instruments classified as portable for in-situ analysis and non-portable for laboratory analysis.

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**TAN-P28**

## CARBON QUANTUM DOTS-TERBIUM IONS AS NOVEL, SENSITIVE AND SELECTIVE LUMINESCENT PROBES FOR THE DETERMINATION OF IMIDACLOPRID IN FOOD

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Pesticides are widely used to guarantee large-scale food production and to support the demand worldwide. Considering their high toxicity for humans, it is fundamental to perform exhaustive quality controls within the agri-food field. Neonicotinoids are systemic pesticides used to control harmful insects and one of the most used families of pesticides worldwide. These pesticides, which are nerve agents, cause many harmful effects to insects, especially to individual bees, such as damaging memory and reducing queen numbers. One of the most important neonicotinoids is imidacloprid, due to its direct relationship with the issue known as "colony collapse disorder". In fact, the use of imidacloprid has been banned in the European Union in recent years.

Pesticides analyses are usually performed with chromatographic methods. We propose an alternative approach for imidacloprid determination, based on terbium-sensitized luminescence (TSL). We report for the first time the implementation of carbon quantum dot (CQDs) nanoparticles in TSL detection to improve its sensitivity and selectivity. CQDs can react with terbium ions through the carboxylic groups present in their structure. These Tb(III)–CQDs complexes, formed *in situ* in aqueous solution, can be used as time-resolved luminescent probes.

The proposed method presents a limit detection of 15 ng $mL^{-1}$ and recovery experiments in cane fruits demonstrated that our method can be useful with screening purposes in quality control laboratories. Based on the results obtained, the implementation of CQDs in TSL can lead to the development of novel time-resolved luminescent probes with high analytical potential in the agri-food sector.

**TAN-P29**

# NOVEL SELECTIVE AND SENSITIVE LUMINESCENT STRATEGIES FOR THE DETERMINATION OF CONTAMINANTS IN AGRI-FOOD FIELD MAKING USE OF CARBON QUANTUM DOTS-EUROPIUM IONS SYSTEM

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Lanthanide-sensitized luminescence (LSL) have been used in Analytical Chemistry for decades due to its interesting features that make it a good detection technique. LSL detection is based on the formation of chelates between lanthanide ions and organic analytes. The detection process is as follows: in these complexes, the energy absorbed by the organic chromophore (usually the analyte) at its characteristic excitation wavelength is transferred to a triplet state of the molecule and then transferred to a resonance level of the lanthanide ion (such as europium), which finally emits luminescence at its particular emission wavelength. With the additional use of luminescent nanoparticles (carbon quantum dots, CQDs), a high enhancement in sensitivity and selectivity is achieved, allowing the determination of residues of contaminants in the agri-food field, in which low levels of analytes have to be quantified. CQDs can react with europium ions through the carboxylic groups present in their structure. In this way, these systems can be used as time-resolved luminescent probes. Several fungicides (carbamates) have been selected as target analytes for the application of the analytical method proposed, obtaining detection limits around 25-50 ng $mL^{-1}$. These preliminary results show that the implementation of nanoparticles in LSL detection provide novel, simple, sensitive and selective analytical methods with applications in the agri-food sector for the screening of contaminant residues.

**TAN-P30**

## DIFFERENT ANALYTICAL METHODOLOGIES FOR CLEANING IN PLACE (CIP) PROCESS CONTROL

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Cleaning procedures are important in the alimentary industry to ensure hygiene in food processing lines by eliminating organic or inorganic materials and bacteria from surfaces. This work focuses on the application of 2 analytical methods with different physicochemical principles in the control of cleaning in place (CIP) wash waters that contains egg remains. The first method is based on the use of a solid sensor that responds to ovoalbumin. In this sensing technology, the derivatization reagent 1,2-naphtoquininone-4-sulphonate (NQS) is embedded into a polydimethylsiloxane-tetraethylortosilicate-$SiO_2$ nanoparticles composite (PDMS-TEOS-$SIO_2$NPs) [1]. Upon contacting the sample with sensor, the ovoalbumin is extracted from the solution and derivatized inside the PDMS matrix after 10 minutes at 100 °C. The device changes its color from yellow to brown dependent the ovoalbumin concentration and quantitative analysis was carried out by measuring diffuse reflectance at 590nm. The second method is the direct measurement of tryptophan-type compounds by native fluorescence (exitation at 280, emission at 340). From sample 2 (S2) to S23 (Figure 1) decreasing tendency of signals was observed for both indicated analytical methodologies, which shows that they are suitable for a CIP control.

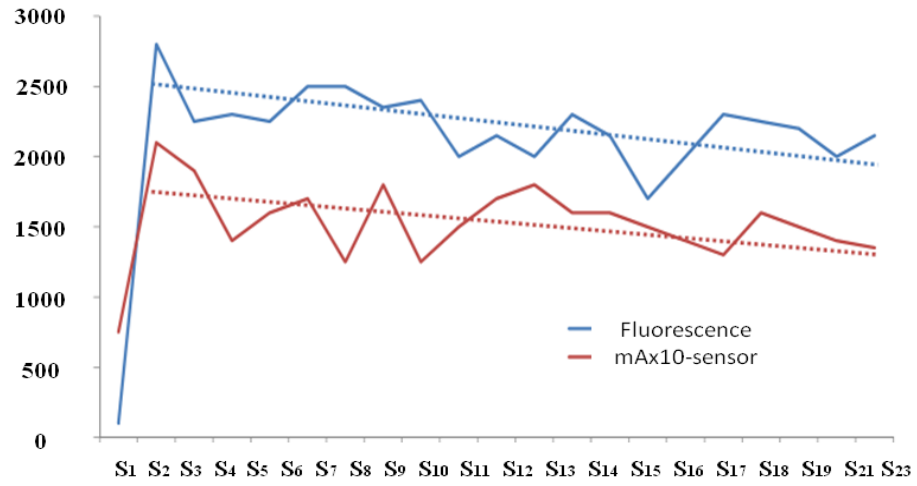


**Figure 1.** Signals obtained for the measurement of the sensor for ovoalbumin and the native fluorescence for tryptophan-type compounds. Fluorescence intensity at 340 nm and miliabsorbance x10 of the solid sensor.

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**TAN-P31**

# DETERMINATION OF $H_2S$ EMITTED BY CARDIOMIOCITES USING A PLASMONIC COLORIMETRIC SENSOR BASED ON SILVER NANOPARTICLES ON SOLID SUPPORTS

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## ABSTRACT

Heart attack is one of the leading causes of death in Western countries. Currently, the only treatment that minimizes damage is reperfusion of the occluded artery. However, restoration of blood flow to the heart after a significant period of ischemia causes myocardial damage, a process known as reperfusion injury. Several studies have shown that hydrogen sulfide ($H_2S$) has protective effects on myocardial ischemia induced by cellular apoptosis [1, 2]. The reduction of $H_2S$ concentrations in a model of cardiac injury increases the size of myocardial infarction, suggesting a role for the production of endogenous $H_2S$ in the prevention of myocardial ischemia injury [3]. In contrast, the therapeutic administration of $H_2S$ in a model of myocardial ischemia reduces the size of the lesion, decreases the mortality rate, improves cardiac function, suppresses inflammation and attenuates fibrosis [4]. In this context and due to the interest of this issue, we have developed a method to determine $H_2S$ emitted by cardiac cells in different clinical conditions using a plasmonic solid colorimetric sensor. The sensor is based on the aggregation of AgNPs retained on nylon membranes [5]. When these sensors are exposed to different $H_2S$ concentrations (ppb level) we observed that they change from yellow to brown and the plasmon band shifts to higher wavelengths. In this application, the sensor will detect $H_2S$ emitted by cardiomyocytes and the signal measured (absorbance) can be correlated with the $H_2S$ concentration. The solid sensor has been adapted to requirements of this application (eg. higher sensitivity, experimental conditions necessary for cell grow (37 °C to 95% humidity, 8 hours), and multiwell analysis). Simulated conditions in culture cells and in cardiomyocytes were tested. Stability against a wide range of temperatures, humidity and solar radiation was also evaluated. In order to perform a multiwell assay a multisensory sheet (96 spots) was developed. The figures of merits of standards ($H_2S$) in culture cell were obtained. The LODs was 0.128 µM. Precision was also evaluated and the RSD(%) values were < 10 %. This method has been satisfactory applied to determine the amount of $H_2S$ emitted by cardiomyocytes exposed to different clinical conditions (ischemia, reperfusion and pharmacological administration). According to these results, it can be concluded that the developed approach is adequate for in-situ multi analysis monitoring of $H_2S$ in cardiomyocytes, is.energy – efficient, it does not require pre-treatment or external instrumentation, rapid and easily handled by non-specialized personal.

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**TAN-P32**

# REUSABLE SOLID BIOSENSOR FOR IN SITU CHEMILUMINISCENT DETERMINATION OF HYDROGEN PEROXIDE: APPLICATION TO REAL SAMPLES

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In-situ monitoring technologies appeared as an advance for complementing the classic methods in the laboratory. Traditional chemistry analysis sometimes means a process so long that it impedes a quick actuation in case of not expected outcomes. In this context, biosensors appear as a reliable alternative which can be applied to numerous analytical processes in several fields like healthcare, food and drink industry including environmental and security monitoring [1-2]. Most of the sensors are for just one use, due to the limitation of the reagents employed or its format. The capability of reusability and the use of in situ portable instruments for transduction are a challenger of the biosensors. In this communication we reported an chemiluminescent biosensor based on the covalent immobilization of the horseradish peroxidase enzyme (HRP) on a polydimethylsiloxane (PDMS) support to quantify in-situ hydrogen peroxide ($H_2O_2$) and also compounds which liberates $H_2O_2$ like sodium percarbonate ($Na_2CO_3 \cdot 2H_2O_2$) (Fig.1). The chemiluminescent reaction, based on the use of luminol as oxidizable substrate with HRP as catalyst, has been used in order to quantify $H_2O_2$ as oxidant agent. The performance of the proposed biosensor has been demonstrated to be able to determine $H_2O_2$ liberated by cells in a culture medium and for evaluating the delivery of $H_2O_2$ from denture cleaner tablets as examples of application. For both analysis, the results indicated that the biosensor is cost-effective, sensitive and selective with detection limits of 0.02 µM and a good linearity over the range 0.06 to 10 µM. Precision was also satisfactory (relative standard deviation, %RSD<6). The strength of this biosensing system is the simplicity, portability and reusability of the devices; it can be applied at least 60 times by keeping its activity at 90%.

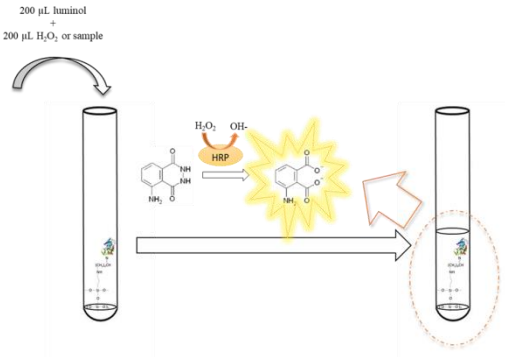


**Figure 1.** Graphical of the developed biosensor.

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**TAN-P33**

## EXCITATION-EMISSION FLUORESCENCE (EEMs) MULTIVARIATE ANALYSIS FOR THE DISCRIMINATION AND QUANTIFICATION OF GRAPEVINE LEAVES

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Precision agriculture is an increasingly adopted practice for viticulture providing greater control over all stages of wine production. It is known that factors like plant productivity can be highly variable within blocks containing the same grapevine variety [1] and that the same variety planted in different soils will grow differently, and thus, the grapevine leaves will reflect the soil where the vines are located [2]. As far as it is known, there are no studies regarding the discrimination of leaves of the same grapevine variety collected at different vineyards located in two geographical regions using fluorescence spectroscopy.

In this sense, in this work, fluorescence spectroscopy in combination with multivariate analysis was explored as a potential tool for classification and quantitative analysis of grapevine leaves. The leaf samples belonging to the same variety “Touriga Nacional”, were collected in two wine regions in Portugal and in four different dates during the ripening period of the grape (from June to September). Excitation-emission matrices (EEMs) were obtained directly on the lyophilized leaf samples using an optical fiber. For each sample, EEMs were recorded in three spectral regions (emission spectra in the range 384-496 nm in the excitation range of 282-369 nm for the first region, 504-566 nm and 420-492 nm for the second one and 654-756 nm and 390-600 nm for the third region), corresponding to polyphenols, carotenoids and chlorophylls and derivatives, respectively.

The classification was performed according to the geographical origin and also the sampling date at each geographical origin, exploring different spectral regions. The EEMs were processed with parallel factor analysis (PARAFAC) and PARAFAC supervised by linear discriminant analysis (LDA). The proposed models allowed the discrimination between samples from the two geographical origins in the three spectral regions assayed. According to the date of sampling, good discrimination were found in all the regions between samples collected during June and July, with respect to those collected in August and September.

On the other hand, models using unfolded-partial least squared (UPLS) were built for the quantification of total chlorophyll and total polyphenol contents. Good correlations were found between the content obtained by proposed and by the spectrophotometric reference methods.

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**TAN-P34**

# DESCARGAS LUMINISCENTES A PRESIÓN ATMOSFÉRICA GENERADAS SOBRE MUESTRAS LÍQUIDAS PARA EL ANÁLISIS DE METALES PESADOS Y ANÁLISIS ISOTÓPICO MEDIANTE SCGD-OES.

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Las descargas luminiscentes (GD) a presión atmosférica han demostrado un relevante potencial analítico para el análisis rápido de líquidos mediante espectroscopia de emisión óptica (OES). En particular, la descarga luminiscente con una solución como cátodo (SCGD), también conocida como descarga luminiscente de cátodo electrolítico (ELCAD), es una técnica alternativa a otras técnicas de referencia para el análisis elemental rápido de líquidos [1,2]. En esencia, SCGD es un plasma de pequeñas dimensiones generado a presión atmosférica entre una solución electrolítica (cátodo) y una varilla metálica de tungsteno (ánodo). La luz emitida (emisión atómica y molecular) se utiliza para identificar y cuantificar la composición de la solución. Debido a su simplicidad, el sistema puede modificarse fácilmente dependiendo de la aplicación y de las necesidades analíticas.

SCGD-OES ha demostrado ser una herramienta competitiva debido a su tamaño compacto, bajo coste, bajo consumo y buena sensibilidad práctica [3]. Además, su uso como herramienta para el análisis de elementos relevantes desde el punto de vista ambiental/industrial, incluyendo por ejemplo Cd, Cu, Pb, o Zn, ha mostrado bajos límites de detección, en el orden de las ppb [4]. SCGD muestra algunos beneficios en comparación con el plasma de acoplamiento inductivo (ICP), tales como no necesitar un nebulizador, gas de transporte o sistema de enfriamiento. Por todo ello, SCGD-OES está generado interés en la comunidad científica e industrial con varios prototipos desarrollados y, además, se ha comenzado la comercialización de varios instrumentos.

En este trabajo, se presenta un prototipo SCGD-OES desarrollado en nuestro laboratorio; se investiga la influencia de los distintos parámetros de la descarga en el potencial analítico de la técnica para el análisis de aguas. Por otro lado, se evalúa la capacidad del equipo como herramienta de espectroscopía para análisis isotópico. La posibilidad de llevar a cabo análisis isotópico se basa en la presencia de moléculas, formadas por el analito de interés y oxígeno o nitrógeno, en el plasma. Los espectros de emisión generados muestran emisiones moleculares con desplazamientos isotópicos relativamente grandes cuando se modifica la abundancia isotópica del elemento de interés [5]. En concreto, se estudian los cambios en los espectros producidos en muestras que contienen B con distintas abundancias isotópicas.

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**TAN-P35**

# CUANTIFICACIÓN ESPECTROELECTROQUÍMICA SIMULTÁNEA DE DOPAMINA, ÁCIDO ASCÓRBICO Y ÁCIDO ÚRICO

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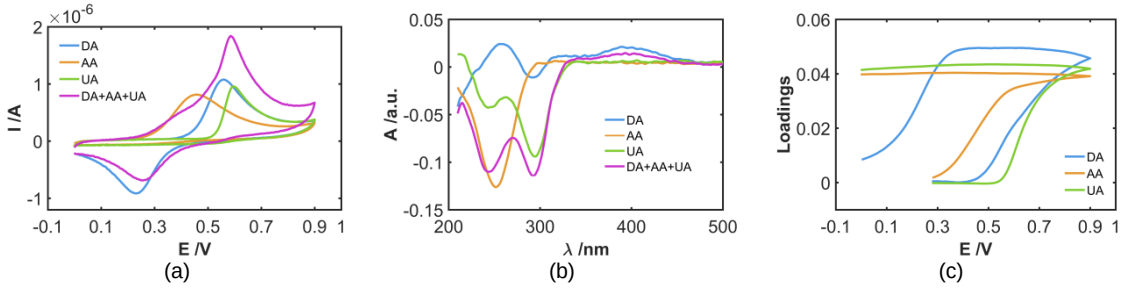
Los medios biológicos reales son muy complejos en cuanto a su composición, siendo un gran reto la determinación fiable de varios analitos de forma simultánea. Por ejemplo, las determinaciones de dopamina en medios reales se suelen ver afectadas en gran medida por la presencia de ácido ascórbico y ácido úrico. Dos de las técnicas más utilizadas para estudiar estos sistemas son la electroquímica y la espectroscopia de absorción molecular. Pero esta mezcla de compuestos supone un gran reto para estas dos técnicas ya que electroquímicamente se oxidan a potenciales similares (Figura 1.a), y sus espectros de absorción presentan bandas que se solapan (Figura 1.b), complicando en gran medida su cuantificación.

La espectroelectroquímica (SEC) es una técnica de análisis que permite estudiar en un único experimento una reacción electroquímica mientras se registran de forma simultánea espectros relacionados con el consumo de los reactivos presentes, la formación de los productos de reacción, y/o la generación de intermedios de reacción. De entre todas las técnicas espectroelectroquímicas, la de absorción en el UV/Visible (UV/Vis-SEC) es una de las más útiles en la identificación y cuantificación de multitud de moléculas. La alta resolución temporal de esta técnica permite determinar diferentes especies simultáneamente correlacionando los cambios detectados en los espectros de absorción UV/Vis característicos de cada especie con los procesos de oxidación o reducción de las mismas en la superficie del electrodo de trabajo.

Gracias a un dispositivo de SEC de absorción UV/Vis de largo camino óptico y capa fina basado en fibras ópticas [1] se ha podido abordar con éxito el estudio de una mezcla de gran complejidad que contiene dopamina, ácido ascórbico y ácido úrico. El análisis de los datos con una herramienta quimiométrica muy potente como es PARAFAC, el carácter trilineal de los datos, y la alta resolución temporal conseguida en estos experimentos ha permitido resolver satisfactoriamente esta mezcla (Figura 1.c) y determinar simultáneamente estos tres analitos, alcanzándose límites de detección en el orden micromolar.




(a) (b) (c)

Figura 1. (a) Voltamperogramas cíclicos y (b) espectros de absorción a +0.80 V obtenidos simultáneamente durante la oxidación de disoluciones puras de ácido úrico, dopamina y ácido ascórbico, y de una disolución mezcla de las tres moléculas con UV/Vis-SEC de largo camino óptico y capa fina. (c) Representación de los loadings frente al potencial obtenidos tras realizar el análisis por PARAFAC de los datos espectroelectroquímicos durante una serie de experimentos donde las concentraciones de estos analitos se han variado de forma aleatoria.

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**TAN-P36**

# Determinación de sulfato de gentamicina en solución inyectable mediante voltamperometría cíclica utilizando electrodo carbón vidrio

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La gentamicina es ampliamente usada como un antibiótico potente en el espectro de aminoglucósido, siendo utilizado en el tratamiento de infecciones de gram-negativo. El uso indiscriminado de este antibiótico aumenta la resistencia a bacterias requiriendo una dosis mayor a la recomendada. Y concentraciones superiores a 12mg $L^{-1}$ puede causar ototoxicidad o nefrotoxicidad [1], entre otros efectos secundarios como insuficiencia renal.
Por lo que es necesario realizar una vigilancia adecuada de este compuesto, en este sentido se han desarrollado varias técnicas de análisis como: la determinación de gentamicina en plasma por HPLC [2], espectrometría de masas [3] y recientemente se determinó la gentamicina mediante una técnica electroquímica [4]. Destacando su simplicidad y robustez.
En este sentido el presenta trabajo plantea la cuantificación de gentamicina mediante voltamperometría cíclica, utilizando como electrodo de carbón vitrificado como electrodo de trabajo, donde se evalúa el pH de análisis (buffer Britton Robinson 0.1 M) en un intervalo de 4-10, obteniendo mejor respuesta a pH 10 y posteriormente se realiza línea de calibrado en un intervalo de concentración de 10-70 mg $L^{-1}$ (Figura 1b) obtenidos de los voltamperogramas cíclicos (Figura 1a), para posteriormente obtener los parámetros analíticos destacando el límite de detección de 5.3 mg $L^{-1}$.

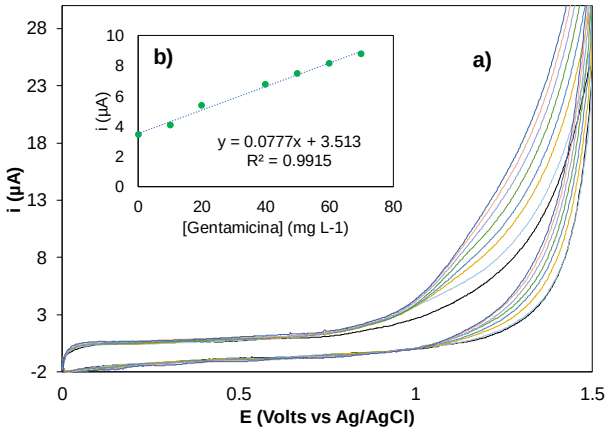


Figura 1. a)Voltamperogramas cíclicos de la gentamicina a pH 10 en un intervalo de concentración de 10-70 mg $L^{-1}$. b) línea de calibrado obtenida de la gentamicina

La técnica propuesta es una alternativa viable para la cuantificación de gentamicina debido a que esta técnica permite disminuir el potencial de análisis de 2.0 Volts [4] a un potencial de 1.1 Volts lo que disminuye significativamente los posibles interferentes en una muestra. Adicionalmente el intervalo de concentración es adecuado para prevenir efectos nocivos en la salud.

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**TAN-P37**

## DETERMINATION OF METHYLONE IN ORAL FLUIDS BY MICROEXTRACTION BY PACKED SORBENT AND ION MOBILITY SPECTROMETRY

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New psychoactive substances (NPS) are defined by the United Nations Office on Drugs and Crime (UNODC) as substances of abuse which have similar effects to drugs under international control conventions, but not controlled yet. The NPS market is characterized to be very active and dynamic with the presence of novel compounds in most countries around the world, being reported more than 800 substances to the UNODC early warning advisory up to 2017. These substances are continuously introduced in the market with slight structural modifications to conventional drugs, being amphetamine-type stimulants, synthetic cannabinoids, synthetic cathinones, and phenethylamines the largest categories found in the market. Methylone (3,4-methylenedioxy-N-methylcathinone) is a synthetic cathinone with euphoric and stimulant effects, but other adverse effects like hyperthermia, seizures, and kidney damage may also appear [1]. Abuse drug consumption analysis is typically carried out in plasma, serum and urine. Nevertheless, the use of oral fluid as alternative fluid is a new trend in the last years because of its simplicity, non-invasive collection, and most drugs remain unmetabolized. Common analytical methods for the analysis of abuse drugs in biological fluids are based on liquid-liquid and solid-phase extraction, followed by gas or liquid chromatography–mass spectrometry determination. In the present communication is proposed a new procedure for the analysis of methylone in oral fluids by microextraction by packed sorbent (MEPS) followed by ion mobility spectrometry (IMS) determination. MEPS is a simple, fast, and on-line sample-preparation technique, with several advantages such as low sample and solvent consumption and high automation potential [2]. While IMS is a gas-phase separation technique in which ions are separated under a weak and homogenous electric field and atmospheric pressure, providing simple and fast determinations in few second with a high sensitivity in the µg $L^{-1}$ scale [3]. The combination of MEPS with IMS determination provides promising advantages regarding automation and portability widely required in field analysis. Octadecyl silica ($C_{18}$) MEPS sorbents were employed and experimental working conditions were studied, such as the effect of sample pH and ionic strength, the number of loading steps, and the number of elution steps using methanol, chloroform, acetonitrile and 2-propanol as elution solvents. The proposed procedure was validated in terms of linearity, sensitivity, selectivity, trueness and precision. The obtained limit of detection was set in 20 µg $L^{-1}$ and precision, calculated as the relative standard deviation of five determinations, was lower than 20%. Recovery studies were carried out using synthetic saliva spiked with methylone at 50, 100, 250 and 400 µg $L^{-1}$ with quantitative results ranging from 78 to 94 %. Additionally, field saliva samples, collected from healthy individuals, were blind spiked at different concentration levels of methylone and analyzed by the proposed procedure and by a reference methodology. Thus, the developed methodology based on the MEPS and IMS combination can be proposed as a promising alternative to conventional analytical methods for the in-field abuse drug consumption analysis due to its high degree of automation and portability.

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**TAN-P38**

# DETERMINATION OF CHLOROPHYLL IN LEAVES BY SMARTPHONE

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The aim of this work is the development of a direct and green methodology for the determination of chlorophyll in leaves by employing a smartphone, avoiding the consumption of pollutant reagents and reducing the waste generation. Mathematical models were created for the prediction of the chlorophyll concentration critic leaves from the color of them. Seventy-eight leaves were employed for built the calibration set and thirty-four samples were used in the validation set. Each leaf was photographed and by using Matlab software the RGB values and color CIELAB descriptors were obtained for each image. Chlorophyll content was quantified by UV-Vis spectrophotometer and by a direct technique as CCM (Chlorophyll Content Meter). Concentrations predicted by the models were correlated with the data obtained by CCM and the concentration obtained by UV-Vis after solvent extraction.

For chlorophyll extraction, different volumes and quality grade of ethanol and acetone were probed as less contaminant extractans. Additionally, the stability of the extracted chlorophyll taking into account the incident light, the time and the temperature was checked. Once the extraction parameters were selected, the extract was measured by UV-Vis and the concentration was obtained interpolating the signal in the corresponding equations. The direct measurement by CCM was done in circles of each leave.

Each leaf circle was photographed and the values of R, G, B, L, h, C, a and b were obtained. Calibration models provided values of determination coefficients from 0.87 till 0.97. There is a good correlation between concentrations predicted by the models employing RGB, Lab and LhC and the concentrations quantified by reference methods obtaining determination coefficients from 0.86 till 0.98.

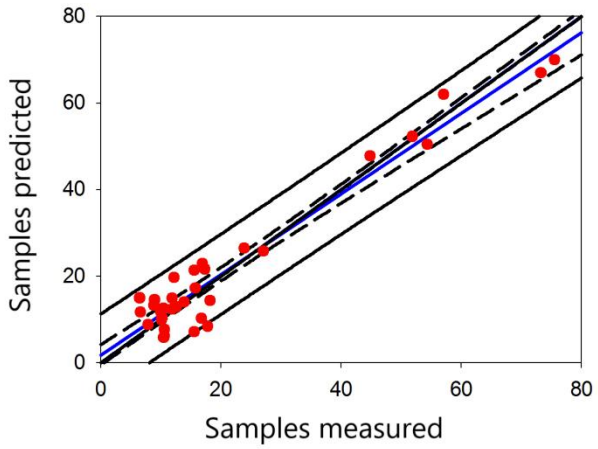


Figure 1. Passing-Bablock of the samples predicted by the RGB model in front of the concentration quantified by CCM.

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**TAN-P39**

## VALORIZATION OF THE MULTIPLE PHARMACOLOGICAL PROPERTIES OF *HARUNGANA MADAGASCARIENSIS* LAM. EX POIR. - A UNIQUE AFRICAN TRADITIONAL MEDICINAL PLANT

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This study attempts to valorize the multiple pharmacological properties of *Harungana madagascariensis* Lam. ex Poir, also known as dragon's blood tree, which is a unique species of the *Harungana* genus with wide applications in African traditional medicine. The antioxidant and inhibitory activity of *H. madagascariensis* leaf and stem bark extracts (ethyl acetate, methanol, and aqueous extracts) against enzymes related to diabetes ($\alpha$-amylase, $\alpha$-glucosidase), Alzheimer's disease (acetyl and butyryl cholinesterase), and epidermal hyperpigmentation problems (tyrosinase) were evaluated. The phytochemical profiles of the extracts were studied by HPLC-DAD-MS, observing the presence of procyanidins and flavonoids, particularly in leaf extracts. TPC and TFC assays demonstrated that aqueous and methanol extracts of leaves of *H. madagascariensis* contained the highest amounts of phenolics (195.72 mg GAE/g) and flavonoids (40.61 mg rutin equivalent/g), respectively. The radical scavenging and reducing power of *H. madagascariensis* leaf extracts were higher than the stem bark extracts. The methanol extracts of leaves (4.61 mg galantamine equivalent/g extract) and stem barks (4.68 mg galantamine/g extract) of *H. madagascariensis* inhibited acetyl cholinesterase. The methanol extract of *H. madagascariensis* stem bark (5.01 mg galantamine equivalent/g extract) showed the highest butyryl cholinesterase inhibitory activity. Methanol extracts (153.55 and 147.07 mg KAE/g extract, for leaf and stem bark extracts, respectively) of *H. madagascariensis* showed high tyrosinase inhibition. The observed pharmacological effects of *H. madagascariensis* support that this plant could be a promising candidate for the development of novel pharmacophores for the management of diabetes, Alzheimer's disease, epidermal hyperpigmentation problems, and other oxidative-stress-related complications.

**TAN-P40**

## CHARACTERIZATION OF MICROMETRIC-SIZE PARTICULATED MATTER BY LASER IONZATION MASS SPECTROMETRY

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Nowadays there is an increasing interest in aerosol characterization. Particles with aerodynamic diameters in the micrometers range can be transported by suspension for long distances. But not only in the environmental field are this kind of material important. Particulate matter with diameters under 100 μm represent an important risk for humans' health since they can be easily inhaled, especially particles smaller than 10 μm, which can penetrate deeper in the lungs. In addition, the riskiness of this material not only depends on its size, but also on its chemical composition. For this reason, stablishing a connection between particles sizes and its components is fundamental in order to know and prevent human and ecological impact of this pollutants.

Mass spectrometry techniques have been extensively used for this purpose. In this sense, Laser Ionization Time-of-Flight Mass Spectrometry (LI-TOF-MS) allows to know aerosol composition without any sample pretreatment, which avoid the loss of any specie. The use of lasers as ionization probes bring the opportunity of localized analysis and make possible obtaining different mass spectra in function of the laser energy regime. Low energies processes mean that compounds are soft desorbed making possible the appearance of organic signals, while ionization regime is required for more energetic species observation.

Direct determination of particulated matter is challenging due to the inherent lack of reproducibility derived from the size distribution and non-regular geometries of the sizes. In conditions where the probe size is larger than that of the particle, the heating of the particle may be assumed complete and the vaporization/excitation yield approaches 100%. If the particle size is larger, such value decreases. On the other hand, even under a successful hitting of the particle, the sample composition may affect the results as the amount of refractory oxides alter the heating dynamic of the particle. In this sense, the conventional analytical strategy demands a controlled optimization of the energy per pulse required to overcome the enthalpy barrier. Another strategy focuses on the taking advantage of the better energy coupling of ultrashort lasers to increase the excitation yield.

In this work, an off-line LI-TOF-MS analysis of urban soil particles with different sizes was carried out. A relation between particles sizes and chemical composition for this sample was elucidated. Besides, the knowledge of the particles compounds allowed to trace their origin. On the other hand, the information dependence on the laser energy and pulse width were examined. For that, a comparative study with femtosecond and nanosecond lasers at several energies was performed.

The present communication compares data obtained with the same Time-of-Flight Mass Spectrometry where two optical lines have been implemented to allow the excitation with a nanosecond laser (5 ns pulse width) and a femtosecond laser (35 fs pulse width).

**TAN-P41**

# DETERMINACIÓN DE CALCIO Y NITRATO EN DIFERENTES MUESTRAS POR ANÁLISIS DE IMAGEN DIGITAL

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Un fertilizante o abono es cualquier tipo de sustancia orgánica o inorgánica que contiene nutrientes asimilables por las plantas y sirven para mantener o incrementar el contenido de esos elementos en el suelo, estimular el crecimiento de las plantas, etc. Se conocen como nutrientes de plantas, los elementos químicos que las plantas necesitan para poder crecer, mantenerse y producir frutos y semillas y poder así reproducirse. Estos nutrientes se clasifican en dos categorías; macronutrientes y micronutrientes. Además, los macronutrientes pueden diferenciarse entre nutrientes primarios (N, P, y K) y secundarios (Ca, Mg y S). El objetivo del trabajo es desarrollar métodos rápidos y sencillos basados en análisis de imagen digital para la determinación de macronutrientes como son el calcio y los nitratos en muestras de fertilizantes y en aguas de consumo.

Para ello se empleará la técnica de análisis de imagen digital, basada en la medida del color de los productos con la ventaja de que se trata de una técnica no destructiva. Para obtener estas imágenes se han empleado equipos tan sencillos y tan extendidos como un escáner de mesa y un *smartphone*. Para la reproducibilidad de las imágenes se ha utilizado un patrón de color y para confirmar su reproducibilidad los gráficos de control. La ventaja de utilizar estos métodos es la posibilidad de analizar varias muestras a la vez empleando pequeños volúmenes, inferiores a 400 μL. Mediante estas imágenes se han obtenido modelos de calibración univariantes para cada analito y se han estudiado figuras de mérito como la reproducibilidad y límites de detección. Posteriormente se ha validado con métodos de análisis de referencia como son la espectrometría UV-Vis y de absorción atómica y cromatografía iónica.

Para el calcio se ha seguido el procedimiento descrito por Damasceno *et al*,[1] basado en el uso del negro de eriocromo negro T a pH básico dando lugar a una coloración azul-morado. Se han obtenido unos coeficientes de correlación superiores a 0,98, valores de RSD inferiores al 15% y errores relativos menores del 14%.

Para el nitrato, se ha basado en la reacción con ácido cromotrópico[2], el cual genera un color amarillo en función de la cantidad de analito en la muestra. Los coeficientes de correlación obtenidos son superiores a 0,97, valores de RSD inferiores al 15% y errores relativos inferiores al 9%.

Estos métodos se han aplicado satisfactoriamente a muestras reales obteniendo en la mayoría de las veces, buenos resultados.

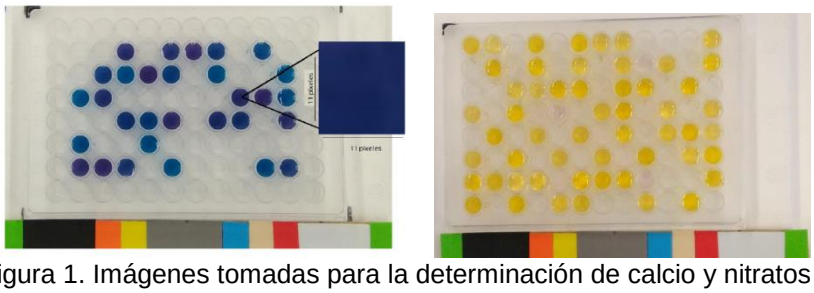

Figura 1. Imágenes tomadas para la determinación de calcio y nitratos

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**TAN-P42**

# ELECTROCHEMICAL BEHAVIOUR OF COBALT IN THE EUTECTIC MIXTURE CHOLINE CHLORIDE-UREA (1:2)

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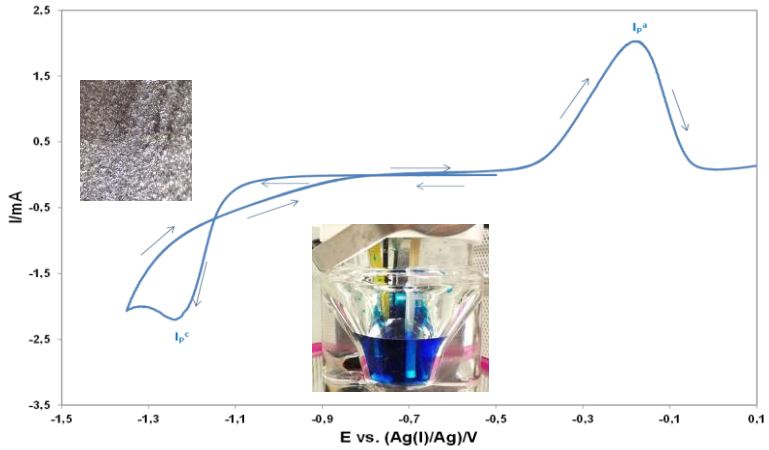
Cobalt is a metal with attractive properties (e.g. high temperature and corrosion resistance) and is one of the few metals that can maintain its magnetic properties after magnetization). These qualities, combined with the ability to be electro-deposited, make Co an ideal metal for coatings. However, the electrodeposition of cobalt in aqueous solution is generally difficult due to the narrow electrochemical window of water, and Co electrodeposition occurs together with hydrogen evolution. Therefore, ionic liquids (ILs) with larger electrochemical windows are required as electrolytes for electrodeposition of cobalt or its alloys.

As a part of a project to look into the possibilities offered by deep eutectic solvents (DES) in the electrodepositon of metals and alloys, the present work is concerned with the electrochemical behaviour of Co(II) solutions in the eutectic mixture ChCl-Urea (1:2) at W and GC working electrodes.

Anhydrous $CoCl_2$, dissolved in the rich chloride media, probably as $CoCl_4^{2-}$, giving a blue solution, which is reduced on tungsten and glassy carbon electrodes via only one electrochemical step.

$$CoCl_4^{2-} + 2e^- \rightarrow Co + 4Cl^-$$




Cyclic voltammetry and chronoamperometry were used in order to study the reaction mechanism and the transport parameters of the electroactive species at the W and GC electrodes. The results showed that the electrochemical reaction is irreversible and that electrocrystalization of Co plays an important role in the electrodeposition process. Experimental current-time transients have been compared with the theoretical models based on instantaneous and progressive nucleation.

Mass transport towards the electrode is a diffusion process, and the diffusion coefficient of Co(II) and the activation energies for diffusion have been calculated.

**Acknowledgements**

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**TAN-P43**

# DETERMINACIÓN DE ARSÉNICO EN MUESTRAS DE AGUA Y ORINA UTILIZANDO ELECTRODOS SERIGRAFIADOS DE CARBONO MODIFICADO CON NANOPARTÍCULAS DE ORO

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El arsénico es un elemento ubicuo encontrado en la atmósfera, suelos, rocas, aguas naturales y organismos vivos. En la zona Norte, principalmente las 7 fundiciones de cobre activas generan un impacto regional muy preocupante debido a las elevadas concentraciones de As aerotransportado dispersado en pequeñas partículas. La producción de cobre a partir de minerales sulfurados como la enargita y tenantita utilizan un proceso pirometalúrgico en el que ≈ el 63 % del arsénico se recoge en forma de vapor y polvos eliminados junto a los gases de fundición, afectando principalmente a los trabajadores y comunidades aledañas por la inhalación de trióxido de arsénico.

Para determinar arsénico, las técnicas electroanalíticas, son una buena alternativa debido a su rapidez, selectividad, sensibilidad, bajos límites de detección, relativa simplicidad y equipos de bajo costo comparado con otras técnicas; y por otra parte, son irreemplazables al momento de necesitar portabilidad. La concentración de arsénico en orina es considerada una buena medida con respecto a la exposición de este elemento. Generalmente se utiliza para estudios ambientales (ingestión de alimentos y agua) y ocupacionales (inhalación) en personas que están expuestas crónicamente al arsénico debido a que la orina es la principal vía de excreción.

En esta determinación se utilizó electrodos serigrafiados de carbono modificados con nanopartículas de oro (DRP-110GNP, Dropsens). Con el objetivo de obtener una metodología sensible y selectiva se optimizaron los parámetros más importantes realizando medidas en presencia y en ausencia de orina sintética Bio-Rad. En la celda electroanalítica se adicionó As(III) 0,50 $mgL^{-1}$ en presencia de HCl 0,20 mol $L^{-1}$ y se agregó alícuotas desde 0 a 500 µL llevados siempre a un volumen total de 10,0 mL. Se aplicó un potencial de acumulación ($E_{ac}$) de -0,60 V durante 30 y luego se realizó el barrido desde -0,20 a 0,40 V mediante SWV. En presencia de orina, la señal de As se desplaza hacia potenciales más positivos y la corriente baja en un 40 % al adicionar 500 µL de orina. Posteriormente se realizó el estudio en función de la concentración de HCl, $E_{ac}$, $t_{ac}$, frecuencia de la onda cuadrada y velocidad de agitación en la etapa de acumulación. En las condiciones óptimas, se construyó las respectivas curvas de calibrado y se determinó el rango lineal, límite de detección (LD) y sensibilidad de la metodología. El rango lineal fue de 0,05 a 1,00 mg $L^{-1}$, mientras que LD fue de 42,0 µg L-1 y la sensibilidad de 18,56 µA/mg $L^{-1}$. Para evaluar la repetibilidad se realizaron 15 mediciones consecutivas con el mismo electrodo obteniendo una desviación estándar relativa de 3,52 y 2,71 5 en ausencia y presencia de orina respectivamente. Para validar la metodología se utilizó agua potable del laboratorio y orina humana correspondiente a personas con bajo nivel de exposición a arsénico. El agua potable se dopó con 100,0 µgL-1 obteniendo 103,9 µg $L^{-1}$, mientras que la orina fue dopada con 50,0 µg $L^{-1}$ obteniendo 53,6 µg $L_{-1}$. Para realizar el estudio de interferencias se analizó, en términos de selectividad y competitividad, el efecto de Cu(II), Zn(II), Fe(III), Bi(III), Sb(III) y As(V). El efecto se evaluó en cuatro niveles de concentración de interferentes: 1,0; 10,0; 100,0 y 1000,0 mg $L^{-1}$. La competitividad se estudió en presencia de 5,0 mg $L^{-1}$ de As(III). Las principales interferencias son presentadas por Sb(III) y Bi(III) cuando está sobre 100,0 mg $L^{-1}$ y Cu(II) cuando está sobre 1000,0 mg $L^{-1}$. Posteriormente, se aplicó el método en la determinación de As(III) y $As_{total}$ en un residuo industrial líquido de una planta de abatimiento de As obteniendo 4,1 y 12,0 g $L^{-1}$, mientras que los valores obtenidos por ICP-MS fueron 3,93 y 13,06 g $L^{-1}$ respectivamente. El LD obtenido con las muestras de orina es relativamente alto (≈ 50 µg $L^{-1}$), sin embargo hay que considerar que en esta metodología se agrega directamente una alícuota de orina sin previo tratamiento.

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**TAN-P44**

# RESULTADOS DE PARTICIPACIÓN EN EJERCICIOS DE INTERCOMPARACIÓN PARA LA VALIDACIÓN DEL MÉTODO DE MEDIDA DE URANIO (235 Y 238) MEDIANTE ICP-SFMS EN MUESTRAS DE ORINA.

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El Laboratorio de Espectrometría de Masas del Servicio de Dosimetría Personal Interna del CIEMAT realiza, entre otros ensayos, la determinación de concentraciones de isótopos de Uranio (235U y 238U) en muestras de orina para la vigilancia de las dosis de radiación recibidas por los trabajadores expuestos a radiaciones ionizantes con riesgo de incorporación de radionucleidos. El laboratorio trabaja en el marco de un sistema de calidad basado en la norma ISO/IEC 17025 y está en proceso de validación del método de ensayo para completar la implantación de los requisitos de la citada norma y estar en condiciones de solicitar su acreditación.
Además de los métodos internos de verificación anual efectuados por el Laboratorio (revisión periódica de las calibraciones, controles de equipo, medidas de los fondos), la participación en ejercicios de intercomparación entre laboratorios es necesaria para verificar que los métodos de ensayo, y las técnicas de medida son adecuados.
El plan definido para la validación del método, incluye, entre otra metodología, la participación en ejercicios de intercomparación como herramienta fundamental para la demostración de la competencia técnica del personal y para la comprobación de la exactitud y nivel de confianza de los resultados que proporciona el laboratorio.
Por todo ello, la participación en los ejercicios internacionales de intercomparación organizados por PROCORAD aportan una garantía de calidad a los métodos analíticos desarrollados y utilizados en el Laboratorio de Espectrometría de Masas.

El laboratorio de Espectrometría de Masas ha participado de forma programada y periódica, desde el año 2012, en ejercicios de intercomparación organizados por PROCORAD, organización sin ánimo de lucro que organiza intercomparaciones en radiotoxicología.
La participación en el ejercicio consiste en analizar 3 muestras de orina, A, B y C. Dos de ellas (A y B) están trazadas con diferentes cantidades de uranio natural. La muestra “C” puede ser un blanco de orina o bien una orina “sorpresa”.

Para la determinación de concentraciones de los isótopos de Uranio ($^{235}U$ y $^{238}U$) en orina, el Laboratorio emplea la espectrometría de masas de alta resolución con fuente de plasma de acoplamiento inductivo (ICP_SFMS), que es una técnica instrumental universal y específica, altamente sensible y que permite la identificación y cuantificación inequívoca de una elemento.
Las muestras de orina recibidas se miden previa dilución 1:20 con $HNO_3$ al 4% de manera directa, tal y como se expone en los procedimientos de calidad del laboratorio.

Se han comparado los resultados obtenidos a lo largo de los años en estos intercomparativos, así como los resultados obtenidos por los distintos analistas del laboratorio, y se han realizado diferentes pruebas estadísticas de comparación de resultados (test de Student, ANOVA, etc.) concluyendo que no hay diferencias significativas entre los resultados obtenidos y los valores de referencia de las muestras analizadas, así como entre los resultados obtenidos por distintos los operarios del laboratorio.

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**TAN-P45**

# DETERMINACIÓN DE Ra-228 SOPORTADO EN AGAR-AGAR MEDIANTE ESPECTROMETRÍA GAMMA EN AGUAS DE CONSUMO Y CONTINENTALES

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En la actualidad para el cumplimiento del RD 140 de aguas potables [1] es necesario en determinados casos la medida de Ra-228. Existen diferentes metodologías normalizadas para su medida pero el procedimiento más extendido consiste en evaporar a 80 ºC, hasta sequedad, de grandes volúmenes de muestra sobre un film, plegarlo, recogerlo en una placa Petri y medirlo en espectrometría gamma. En este trabajo se ha validado esta metodología con las siguientes modificaciones: las muestras se han evaporado a pH < 2 en vaso hasta un volumen de 20 ml. Una vez reducido el volumen se ha gelificado con agar-agar en una placa Petri. En esta forma la muestra queda homogéneamente repartida y con una geometría similar a la del detector.

En primer lugar se realizaron una serie de experimentos de prevalidación para establecer algunas condiciones experimentales del método de ensayo a validar puesto que, a veces, no están suficientemente detalladas en el método analítico de partida. Estos ensayos fueron pH óptimo de trabajo y posterior neutralización, cantidad de trazador a emplear (Ba-133), control de blancos, influencia del volumen final y establecimiento de la eficiencia o linealidad.

Se dispuso de un cóctel de calibración, compuesto por distintos radionucleidos emisores en un amplio espectro gamma, y se construyeron curvas de eficiencia específicas para cada radionucleido gamma del cóctel. Durante el diseño de experimentos necesario para la validación se prepararon muestras de control empleando un MRC-ICP de Th con alto contenido en Ra-228 previamente caracterizada su actividad radioquímica, y varias muestras provenientes de un ejercicio de intercomparación organizado por el Organismo Internacional de Energía Atómica, IAEA.

Para estudiar el rendimiento del proceso se prepararon distintas muestras de agua con cantidades crecientes de Ra-228 empleando Ba-133 como trazador. A partir de las curvas de eficiencia se comprobó que la medida de Ba-133 dio un rendimiento del proceso del 98%.

La reproducibilidad se calculó como la desviación entre las medidas de una misma muestra preparada con el MRC de Th y el sesgo se evaluó a partir de las diferencias entre los valores medidos en las muestras suministradas por IAEA y los valores asignados a dichos materiales. Los límites de decisión y de detección se estimaron de acuerdo a la norma UNE EN ISO 10703. La incertidumbre se evaluó mediante el modelo de propagación de errores descrito en el documento *Guide to the Expression of Uncertainty in Measurement* [2] y mediante un modelo *in house* a partir de los resultados obtenidos durante la validación.

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**TAN-P46**

# E-NOSE "AZOTIC 1.0" APLICADA A LA DETERMINACIÓN DE LA ESTABILIDAD DE LAS PÓLVORAS

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La nitrocelulosa (NC) es un éster nítrico polimérico, componente principal de las *pólvoras sin humo*. La nitrocelulosa es inestable, incluso a temperatura ambiente, presentando un envejecimiento natural basado en una desnitración fuertemente exotérmica y autocatalítica [1]. La seguridad en el almacenamiento de una pólvora suele requerir de las *pruebas clásicas de estabilidad,* numerosas [2] pero, la mayor parte de ellas, agrupables en dos familias de métodos: las primeras utilizan un papel indicador para determinar el tiempo de viraje provocado por los vapores emitidos al calentar una pólvora; el segundo grupo recoge la evolución de los anteriores métodos cuando cuantifican gases o propiedades de la pólvora en condiciones isotermas. La determinación del estado de utilidad de una pólvora, en base a estos métodos, es arriesgada por la escasa información que estos métodos proporcionan.

La relación entre la constante de descomposición y la temperatura sigue un comportamiento basado en la ecuación de Arrhenius, manteniéndose la velocidad de descomposición constante en una reacción de primer orden hasta alcanzar la autocatálisis [3] o, posteriormente, una desnitración tan severa que la pólvora ya no siga siendo un propulsante.

Los estudios originales de Koehler y Marqueyrol [4] identificaron, en la descomposición de la NC, como principales gases emitidos al $NO_2$, $CO_2$ y CO; por su parte, los estudios de Will [5], demostraron una relación entre la emisión de nitrógeno con el tiempo ($\Delta n/\Delta t$) y la estabilidad de la pólvora.

Siguiendo estos estudios, se instrumentaliza un ensayo de estabilidad a temperaturas entre 100ºC y 135ºC para diferentes muestras de pólvora desarrollando un sistema de detección electrónico con semiconductores quimioresistivos, que pudiera permitir un control monitorizado a distancia de los posibles procesos de degradación, sin necesidad de un acceso presencial a las zonas de depósito. De los resultados obtenidos, se concluye una buena correlación entre los tiempos del viraje del procedimiento de referencia y la detección de emisión de $NO_x$ mediante el sistema desarrollado. Sin embargo, la técnica sigue presentando interferencias cruzadas con otras sustancias volátiles presentes en los vapores emitidos por las pólvoras calentadas. Aunque los resultados son prometedores, es necesario un estudio más amplio para alcanzar una validación del sistema propuesto, que consideramos de gran interés a la hora de garantizar la seguridad en el almacenamiento de pólvoras de base nitrocelulósica.

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**TAN-P47**

# MODIFICACIÓN DE ELECTRODOS CON NANOTUBOS DE CARBONO A PARTIR DE DISPERSIONES EN POLÍMEROS: EFECTO DEL DISPERSANTE EN SU APLICACIÓN AL ELECTROANÁLISIS DE POLIFENOLES EN PRODUCTOS DE LA INDUSTRIA VITIVINÍCOLA

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Las técnicas electroanalíticas son una alternativa interesante a las espectrofotométricas para el análisis de polifenoles en muestras complejas, como pueden ser los alimentos. Esto se debe a que la mayoría de estos compuestos pueden ser oxidados electroquímicamente a potenciales moderados, por lo que pueden ser detectados de forma bastante selectiva y sencilla. La integración de nanomateriales de carbono, como nanotubos o grafeno, en la superficie de transducción de dispositivos electroanalíticos generalmente supone una mejora ostensible en la respuesta de los polifenoles, especialmente al emplear voltamperometría o amperometría, ya que su señal de oxidación puede obtenerse aplicando menores potenciales con gran estabilidad y reproducibilidad.

En esta comunicación se describe la preparación de electrodos modificados con una capa de nanotubos de carbono de pared múltiple (NTC), obtenidas a partir de dispersiones acuosas del nanomaterial, que presentan mejoras en la respuesta electroquímica de polifenoles. Para la preparación de estas dispersiones se evaluaron varios polímeros como agentes dispersantes de los nanotubos de carbono mediante tratamiento de ultrasonidos. Entre ellos se emplearon polietilenimina (PEI), polivinilpirrolidona (PVP), cloruro de polidialildimetilamonio (PDDA) y cloruro de hexadeciltrimetil amonio (CTAC). Para ello se evaluó la concentración de NTC y agente dispersante, la presencia de disolvente orgánico, la energía de ultrasonidos aplicada y la centrifugación y aislamiento del nanomaterial no disperso. Se pudo comprobar que, en las condiciones óptimas de preparación para cada agente dispersante, es posible obtener dispersiones estables de forma reproducible, las cuales son capaces de proporcionar películas de nanotubos que, en general, proporcionan respuestas voltamperométricas para polifenoles de referencia, como ácido gálico, ácido cafeico o catequina, con mayor sensibilidad, mejor relación entre la señal faradaica y la capacitiva así como un aumento en la reproducibilidad en las señales respecto a las obtenidas con el electrodo de base (carbono vítreo) sin modificar.

La aplicación de estos electrodos al análisis de polifenoles en diversas muestras procedentes de la industria vitivinícola (vino, mosto, vinagre) mediante técnicas voltamperométricas o acoplados a detectores amperométricos en sistemas de análisis por inyección en flujo (FIA) o electroforesis capilar (EC) indican que pueden aportar información relevante de la presencia de algunos polifenoles específicos o índices generales relacionados con familias de polifenoles. Esto es posible gracias a la estabilidad, reproducibilidad y sensibilidad de las señales obtenidas con los electrodos modificados que, además, permite procesar las muestras con un mínimo tratamiento de dilución y/o filtrado.

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**TAN-P48**

# DETERMINACIÓN DE HIDROCARBUROS POLICÍCLICOS AROMÁTICOS (PAH) EN PRODUCTOS ALIMENTICIOS APÍCOLAS Y CÁRNICOS POR CROMATOGRAFÍA LÍQUIDA A PARTIR DE PROTOCOLOS SENCILLOS DE EXTRACCIÓN EN FASE SÓLIDA (SPE) Y EXTRACCIÓN CON FLUÍDO SUPERCRÍTICO-$CO_2$ (SFE)

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Los hidrocarburos aromáticos policíclicos (PAHs) se caracterizan por ser una amplia familia de compuestos orgánicos que contienen dos o más anillos aromáticos en su estructura. Éstos representan una importante clase de compuestos muy tóxicos, cuya presencia ha sido ampliamente estudiada en diferentes matrices. Aunque el origen en el medio ambiente es muy diverso, éstos pueden formarse por pirólisis de materia orgánica a altas temperaturas, origen petrogénico, diagénesis de materia orgánica, biosíntesis de microorganismos y plantas, etc. 16 de ellos están incluidos en la lista de contaminantes prioritarios de la US EPA. Debido a estudios reportados en alimentos, en esta oportunidad hemos elegido benzo[a]pireno, benzo[a]fenantreno (criseno), benzo[a]antraceno y benzo[a]fluoranteno, considerados como los más peligrosos y recurrentes[1].
Normalmente las técnicas utilizadas para el análisis, son las cromatográficas con diferentes detectores. En este estudio se realizó extracciones en fase sólida (SPE) o con fluido supercrítico (SFE) y posteriormente se inyectó en un cromatógrafo con detector UV-VIS con arreglo de diodos y un detector de fluorescencia. Para el análisis de criseno (CHR) y benzo[a]pireno (BaP) en mieles comerciales y de origen artesanal como también en jamón comercial ahumado de diferentes procedencias se utilizó el detector UV-VIS.
Las mieles analizadas se almacenaron en oscuridad a 4°C. Fueron disueltas en agua a concentraciones de 150 g/L y disueltas con agitación a temperatura ambiente. Luego, 100,0 mL de disolución fue extraído en columnas C-18 de SPE por pasaje activo (forzado por émbolo) para luego recuperar en 1,0 mL de acetonitrilo (pasaje pasivo por goteo). Las muestras fueron analizadas mediante HPLC-UV-DAD para CHR (268 nm) y BaP (295 nm), previa obtención de los espectros de absorción UV. La fase móvil en gradiente estuvo compuesta por acetonitrilo-agua en diferentes proporciones. Se utilizó una columna RP-C18 para PAHs a 40°C. La cuantificación de las muestras se hizo mediante adición de estándar, obteniendo valores para CHR desde 0,90 hasta 14,92 µg/Kg, mientras que para BaP desde 1,19 hasta 18,89 µg/Kg. De la misma forma fueron analizadas las muestras de jamones, optimizando previamente la metodología de extracción con $CO_2$ supercrítico mediante programa multivariable, encontrando las siguientes condiciones óptimas: 60°C temperatura del horno, 1500 psi de presión, extracción estática de 30 min, extracción dinámica de 7 min y con una temperatura del restrictor de 40°C. A la celda se introdujeron 20 g de jamón seco y se extrajo según los parámetros descritos. El extracto se recibió en acetonitrilo a 4°C para luego mezclar vigorosamente, dejar reposar a temperatura ambiente y luego centrifugar. Se tomó la fase orgánica de la centrifugación (superior) y se aforó a 2,0 mL con acetonitrilo. Se obtuvieron valores entre 8,75 y 13,58 µg/Kg para CHR y de 0,25 y 12,57 µg/Kg para BaP en las muestras de jamón.
Según la regulación EC 208/2005 el límite permitido para carne ahumada (no especifica si incluye jamón) corresponde a 5,0 µg/Kg para BaP, mientras que, para miel, no se especifica ningún valor máximo permitido, existiendo valores para alimentos desde 1,0 a 10,0 µg BaP/Kg[2]. En este estudio se encontraron muestras que superan el máximo de BaP establecido para alimentos mediante la utilización de una técnica accesible (HPLC-UV-DAD) y sencillas preconcentraciones.

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**TAN-P49**

## SELECCIÓN Y SENSIBILIZACION DE LA REFLECTANCIA EN TECNICAS DE IMAGEN DIGITAL

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Las actuales tecnologías de imagen digital permiten simplificar los procesos de medida y control analítico hasta el punto de romper con mitos asociados a necesidades de grandes equipamientos para proporcionar altos niveles de prestaciones. Aplicaciones de equipos comerciales tales como cámaras digitales, teléfonos móviles, tabletas, etc, permiten conseguir respuestas de modo rápido y eficiente en un amplio rango de problemas. En particular la técnica Digital Image Colorimetry-DIC- [1] con captura de imágenes cromáticas mediante cámaras digitales comerciales ha ganado un gran interés en la comunidad analítica.

En el presente trabajo se ha estudiado la respuesta de una cámara digital que se aplica en la determinación de la amina biógena Putrescina (Put) mediante DIC. La medida de la cámara representa la captura de la reflectancia de un compuesto cromofórico, que es iluminado y registrado en detectores CCD. En tres canales seleccionados con filtros RGB. Por tanto, el resultado se ha justificado como el producto y convolución de tres procesos principales: interacción del iluminante sobre el compuesto cromofórico, reflexión de radiación dispersa y captura-lectura por el detector. La respuesta espectral de los tres procesos ha podido ser caracterizada e integrada para dar la respuesta final.

Como iluminante se han utilizado: lámparas pancromáticas blancas LED (de amplio espectro 400-700 nm), lámparas LEDs monocromáticas (de colores rojo, verde, azul [2]) y un laser azul de menor ancho de banda, cuyos espectros de emisión han sido previamente caracterizados. Así mismo la superposición de filtros de selección de radiación permite reducir el ancho de banda espectral efectiva.

Los productos cromofóricos medidos son el resultado de la oxidación de la Putrescina por la enzima DiaminoOxidasa, DAO que dan lugar a producción de ϒ-amino,butaraldehido. Su posterior reacción con el o-AminoBenzaldehido da un producto de adición de coloración amarilla. La respuesta espectral de la cámara digital, en sus tres canales ha sido también caracterizada experimentalmente. Su resultado final es expresado por el producto-convolución de los tres procesos mencionados.

De los mismos se evidencia que el empleo de fuentes de iluminación con bandas espectrales discretas permite ser mas selectivo tanto hacia la absorción como a la reflectancia del analito, y también posibilita una respuesta más sensible de canales específicos de las cámaras. Así, la determinación de Putrescina experimenta una ampliación en el rango de respuesta lineal hasta 50 mg/l. El procedimiento demuestra un aumento significativo de las prestaciones analíticas sin perder en eficiencia y rapidez de técnicas de las cámaras digitales.

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**TAN-P50**

## NOVEL USES OF MAGNETIC NANOPARTICLES IN BIOSENSING

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Magnetic particles are very frequently used in Analytical Chemistry as separation tool, in order to preconcentrate and isolate the analyte. Immunoseparation techniques allow to increase sensitivity and selectivity at the determination of biomarkers in complex biological fluids, toxins and allergenics in food matrix, or pathogens and pollutants in low concentrations in environmental samples. However, the properties of the magnetic nanoparticles could be tuned for novel sensing principles which will synergistically combine both the detection and separation, and this possibility has not been yet fully explored.

Our research group is developing lateral flow immunoassays by using superparamagnetic nanoparticles (SPNPs) as reporter label. These tests are based on the immobilization of capture antibodies or bio-reagents at the test line on a nitrocellulose membrane. The target analyte is immobilized at that zone, and the test is revealed through a labelled biomolecule (detection antibody). When SPNPs are used, and the strips are scanned on an AC eletromagnetic transducer, the increase of impedance (or frequency) can be used as analytical signal [1]. This effect seems to be related to the random oscillation of the magnetic moment of the nanoparticles at room temperature, which is unique to this type of magnetic nanoparticles. We have proved that it can be advantageously used for the development of a sandwich immunochromatographic assays for biomarkers[2].

On the other hand, it has been reported that magnetite nanoparticles could display peroxidase effect, which could be related to the balance between Fe (II) and Fe (III) at their surface. Peroxidase labels are widely used at immunoassays. They behave as catalysts of the oxidation of 3,3`,5,5´-tetramethylbencidine (TMB) in presence of $H_2O_2$. The blue color of the product of this reaction has allowed us to combine the optical signal (optical density, reflectance) with the electromagnetic information at the nitrocellulose strips.

In this work we have analyzed the properties of magnetic nanoparticles that have an influence of these sensing principles. Crystal structure, synthesis route, particle size, zeta potential and capping agent, have been studied in order to provide new insights on their analytical synergistic effects. We have developed a magneto immunosensor for the allergenic histamine in wine, which provides results in agreement with those of HPLC, but with no sample preparation (just filtering is required) and in 10 minutes.

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**TAN-P51**

# SELECTIVE LIGANDS FOR DEVELOPING ELECTROCHEMICAL SILVER NANOPARTICLES SENSORS

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The detection and quantification of silver nanoparticles (AgNPs) are usually carried out with very powerful analytical techniques such as electron microscopy, light scattering techniques, single particle inductively-coupled plasma mass spectrometry, or hydrodynamic chromatography techniques [1]. These techniques are usually coupled to separation procedures such as ultrafiltration, cloud point extraction, asymmetric field flow fractionation (AF4), capillary gel electrophoresis or density gradient centrifugation. This type of techniques allows the determination of nanoparticles up to 1 nm in size at levels of concentrations of ng $mL^{-1}$, although with very sophisticated instrumentation.

These analytical techniques mentioned above are often used as confirmation techniques. However, simpler screening analytical methods are increasingly required for allowing rapid detection, quantification, and even the characterization of AgNPs. Electrochemical (bio)sensors can contribute significantly in rapid, early detection, and accurate determination of the AgNPs, compared with more sophisticated instrumental techniques.

In this work, the design of new voltammetric sensors for the rapid detection and quantification of AgNPs has been studied. Electropolymerization procedures of monomers that gave rise to immobilized polymers on the working electrode, with functional groups capable of selectively retaining the AgNPs, have been addressed. For this purpose, we have tested the analytical possibilities of several monomers (thionine, o-phenylenediamine, L-lysine, and tiophene-3-carboxamide), which under certain electropolymerization conditions, form oligomers onto the working electrode with a large number of thiol and amino functional groups, capable of strongly retaining AgNPs.

The results of these sensors have been compared with the covalent modification of L-cysteine (*sticky electrodes*), and with bare (unmodified) voltammetric glassy-carbon electrodes, by using the technique of immobilized (nano)particles voltammetry.

These sensors have sensitivities in the order of sub mg $L^{-1}$, the size of the AgNPs (in the range 20-80 nm.) can be evaluated, and easily discriminate between ionic silver ions and the solid silver nanoparticles.

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**TAN-P52**

# 3D µPAD BASED ON CARBON DOTS-IODOACETYL CONJUGATES AS FLUORESCENCE PROBE FOR THE DETECTION OF GLUTATHIONE

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Glutathione (GSH) is present in nearly all cells and mediates numerous cellular processes and plays a crucial role in mediating a number of physiological and pathological processes. For these reason, it is significant of importance to develop a rapid, selective and sensitive method for the detection of glutathione. Recently, optical sensing approaches have aroused great interest owing to their intrinsic simplicity and high sensitivity among the various detection techniques [1]. Besides, the combination of nanomaterials with microfluidic paper-based analytical devices (µPADs) through the immobilization of them on the cellulose fibers let us the developing inexpensive portable sensing platforms for disease diagnostics and the point of care (POC) settings. Cellulose paper based analytical devices have demonstrated enormous potential for developing robust, inexpensive and portable devices for disease diagnostics. This work shows the developing of a functionalized 3D µPAD through the covalently immobilization of carbon quantum dots (CQDs) on cellulose fibers for visual determination of GSH.

The developed method of GSH determination involved the use of divinyl sulfone (DVS) to activate the hydroxyl groups of the cellulose paper [2], and subsequently to immobilize CQDs covalently through nucleophilic addition so, the paper analytical device presents high fluorescence intensity. Iodo acetic acid was using to functionalize the immobilized CQDs on the paper turn off the fluorescence to incorporate iodo atoms in the structure (CQDs-I) [3]. The presence of GSH can involve the substituted of -I by the –SH group of thiol meaning a significant fluorescence intensity increase.

The 3D µPAD consists of three separate layers of paper and one layer of absorbent pad. The different layers were laminated with a plastic film to protect the GSH to the atmospheric oxygen. The first layer consist in a sampling and transport area to move the GHS sample toward the functionalized paper, the second layer consist in the detection area where the CQDs are immobilized covalently on the paper disk, the third is a transport channel to sweep along the free iodide produced after reaction of GSH with the CQDs-I and the quarter area has an absorbent pad to collect the free iodide.

For assuring the perfect functioning of the device different parameters such as the concentration of CQDs immobilized, iodoacetic acid, pH value, the time to functionalize the CQDs with iodo acetic acid and reaction time to detect GSH. This sensing system has a good selectivity for GSH over other biological thiols, provides a wide linear range from 1 to 200 µM. To further evaluate the feasibility of the proposed method in the analysis of human urine. The accuracy of the urine samples analysis was tested by spiking a known amount of standard GSH with urine samples and calculating its recovery.

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**TAN-P53**

# DETECTION OF PESTICIDES BY ELECTROCHEMICAL SURFACE-ENHANCED RAMAN SPECTROSCOPY (EC-SERS).

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Over the last years, the development of new procedures has allowed the improvement of Raman spectroelectrochemical features. An interesting methodology consists of the combination of electrochemistry and Surface-Enhanced Raman Scattering (SERS) effect. Enhancement of the Raman signal displayed by Electrochemical Surface-Enhanced Raman Scattering (EC-SERS) is associated with two phenomena: (1) the interaction of the species with the electrode surface is potential-dependent and certain potentials increase the analyte adsorption on the electrode surface, and (2) the in-situ generation of fresh nanofeatures with SERS properties in presence of analyte.

Detection of pesticides in food is an essential safety issue for protecting human health. Currently, there is a clear demand in the development of new and easy methodologies for quick and sensitive detection of pesticides residues. The utility of EC-SERS in this field has been demonstrated by the detection of different pesticides as for example imidacloprid (Figure 1) by EC-SERS. In this case, the employed procedure consists of the in-situ generation of metal nanoparticles and pesticide detection in a unique experiment. Hence, EC-SERS allows the fast and simple detection of pesticides, opening new gates for future application in this field.

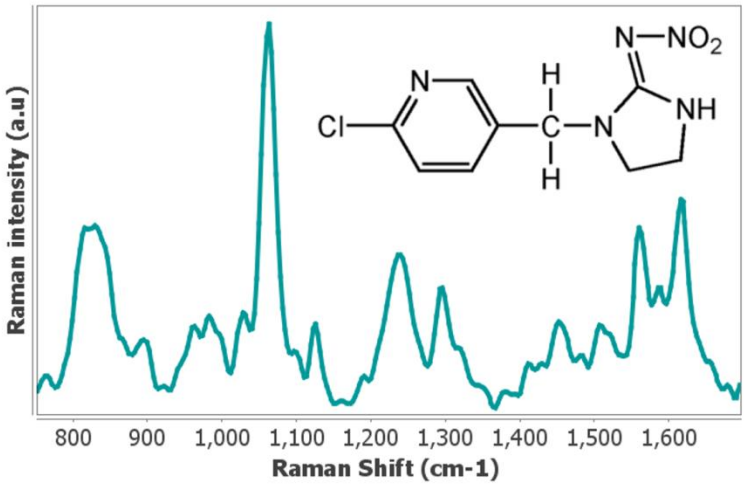


Figure 1. Raman spectrum of imidacloprid obtained by EC-SERS.


# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *TÉCNICAS DE SEPARACIÓN*

**TSE-P01**

## EVALUATION OF THE SELECTIVITY OF IONIC LIQUID-BASED GAS CHROMATOGRAPHIC COLUMNS

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Ionic liquids (ILs) have been used as stationary phases in gas chromatography (GC) due to their low vapor pressure, relative low bleeding and, in particular, because of their tailored selectivity. Up to now, a number of studies have reported on these positive features of IL-based GC phases for mixtures of compounds with different chemical structures. However, studies investigating on the selectivity of IL-based columns for complex mixtures of isomers, such as, for example, the polychlorinated biphenyls (PCBs), are still rare in the literature.

Topological or electrotopological descriptors have been frequently used in statistical studies trying to predict the GC retention of PCB congeners in specific stationary phases. However, the practical use of these approaches requires very accurate predictions due to the high number of congeners (209) and the frequent presence of co-elutions. On the contrary, the statistical estimation of the effects of simple structural PCB descriptors on the GC retention can be considered a more interesting approach, as it allows comparing the interactions of PCBs with different stationary phases and, consequently, describing the specific phase selectivity toward groups of compounds sharing a specific substructure.

In this study, the SLB-IL-60 (Sigma-Aldrich, Bellefonte, PA, EEUU) GC phase, was used as IL-based model phase. Using this column, programmed temperature retention times and retention indices of 59 PCBs representative of different groups of homologues and chemical substructures were used as dependent variables for the statistical valuation of the retention behavior of these analytes in this stationary phase. Meanwhile, the number and position of the chlorine substitutions in the ring (used as size and shape descriptors), and the number and position of the *orto*- substitutions describing interactions between the two rings, were used as independent variables. The software Statistica 7 (Statsoft, Tulsa, OK, EEUU) was used for the stepwise calculation of the regressions.

Interestingly, the proposed model provided equally valid regression values ($R>0.996$) using retention index and retention time values. It was also demonstrated to provide valid values (i.e., $R>0.99$) in both pseudo-isotherm and programmed conditions. Application of the optimized model to PCB retention data obtained using polar (SLB-IL-76 and SuplecoWax-10), semi-polar (DB-17) and non-polar (DB-5 and HT-8) stationary GC phases resulted also on satisfactory-to-acceptable regression fittings. More importantly, the statistical comparison of the PCB retention behavior in the five evaluated phases demonstrated that those based on ILs showed a completely differentiated selectivity through PCBs compared to the other more conventional stationary phases evaluated.

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**TSE-P02**

# DETERMINATION OF GLUCOSINOLATES IN BEE POLLEN USING SOLID PHASE EXTRACTION AND ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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The increasing number of publications concerning bee pollen analysis in the last decade demonstrates the rediscovery of this product due mainly to its associated biological activities, for instance, those relating to its antimicrobial, anti-mutagenic, antioxidant, or anti-inflammatory properties. According to the number of publications, phenolic compounds, proteins, vitamins, and carbohydrates could be considered the most representative bioactive compounds of bee pollen. However, in the last few years, attention has also been drawn to a family of compounds, namely, glucosinolates, which are plant secondary metabolites in the order Brassicales; these have toxic or repellent effects on a variety of plant pests, but they are also becoming significant for human nutrition due to their preventive role in health, chiefly in terms of their breakdown products. Both the quality and the number of glucosinolates differ among plant species and subsequently in their pollen. As a result, monitoring glucosinolate content in bee pollen could be of great interest not only from a nutritional but also from a botanical point of view, as such compounds could be used as biomarkers to identify the origin of the samples.

The main goal of this work has consisted in developing a new method by using ultra high performance liquid chromatography coupled to a mass spectrometer with a quadrupole-time of flight analyzer for the determination of fifteen glucosinolates in bee pollen. The proposed sample treatment involved a solid-liquid extraction with hot water, followed by a solid phase extraction (with a weak anion exchange sorbent ($NH_2$) for separation and concentration of the analytes from bee pollen. The chromatographic separation was carried out in gradient elution mode with a Luna Omega $C_{18}$ column and a mobile phase composed of a mixture of acetonitrile (0.1% formic acid) and water (0.1% formic acid) at a flow-rate of 0.2 mL/min. Finally, the analytical performance of
the proposed method was evaluated and then applied to assay intact GLSs in commercial bee pollen samples from different Spanish regions.

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**TSE-P03**

# MEJORA DE LA EFICIENCIA DE SEPARACIÓN EN AF4 MEDIANTE EL USO DE UN CANAL DE DIMENSIONES REDUCIDAS: APLICACIÓN A LA CARACTERIZACIÓN DE NANOPARTÍCULAS DE DIÓXIDO DE TITANIO

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La caracterización de nanomateriales basada en técnicas de separación, como el Fraccionamiento en Flujo mediante Campo de Flujo Asimétrico (AF4), suele suponer una serie de problemas recurrentes en términos de resolución y recuperaciones, lo que se traduce en una falta de metodología aplicable robusta [1].

En AF4, optimizar una separación significa un incremento de la resolución en un tiempo de análisis razonable, sin comprometer por ello sus valores de recuperación (fracción eluida respecto a la inyectada). Sin embargo, factores como la resolución o el tiempo de análisis no suelen ser complementarios, y la mejora de uno de ellos no es sinónimo de unas condiciones adecuadas para otros elementos que determinan la separación del sistema [2]. El incremento de la fuerza de campo aplicado, por ejemplo, supone una mejora en la resolución, pero es una limitación en términos de recuperación, pues conlleva una mayor interacción entre las nanopartículas (NPs) y la membrana de permeación del canal [3].

Con el objetivo de optimizar la separación para la caracterización de dióxido de titanio, uno de los nanomateriales más empleados en la actualidad, se propone el uso de un canal de dimensiones reducidas. En este trabajo se estudiaron las implicaciones que supone el empleo de un canal de menor longitud respecto a uno convencional, tanto a nivel teórico como experimental.

En primer lugar, se analizaron las variables que determinan la separación en AF4 para ambos canales, y se modelizó su comportamiento con estándares de tamaño de poliestireno (PS NPs), en términos de tiempos de retención, eficiencia, resolución y recuperación. A partir de estos resultados, se comprobó que el uso de un canal de menor longitud permite mejorar la eficiencia de la separación, manteniendo los valores de recuperación y tiempos de retención. Esta metodología se aplicó posteriormente a la caracterización de $TiO_2$ NPs, procedentes de diferentes productos comerciales, poniéndose de manifiesto la capacidad del canal de menor longitud para la separación e identificación de dos poblaciones de NPs de dióxido de titanio de diferente tamaño: sin agregar (en torno a 8 nm) y agregadas (>30 nm), presentes en la misma muestra.

Este trabajo ha sido cofinanciado al 65% por el Fondo Europeo de Desarrollo Regional (FEDER) a través del programa Interreg V-A España-Francia-Andorra (POCTEFA 2014-2020). Proyecto OUTBIOTICS EFA 183/16.

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**TSE-P04**

# ESTUDIO DE PICOS CROMATOGRÁFICOS DE COMPUESTOS QUIRALES UTILIZANDO FUNCIONES GAUSSIANAS MODIFICADAS

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La descripción de los perfiles de los picos cromatográficos se ha estudiado ampliamente, habiéndose propuesto un gran número de funciones matemáticas [1]. Entre ellas, se ha destacado la exactitud alcanzada con los modelos gaussianos modificados [2,3], que describen la desviación de un pico gaussiano ideal como un cambio en la varianza o desviación estándar del pico con el tiempo:

$$h = H_0 e^{-\frac{1}{2}\frac{(t-t_R)^2}{f(t-t_R)}}$$

Los modelos gaussianos modificados son, en realidad, una familia de funciones de diferente complejidad con una gran flexibilidad para ajustar picos cromatográficos en un amplio intervalo de asimetrías y formas. Sin embargo, en ocasiones, puede producirse un crecimiento no controlado de la señal fuera de la región de elución, lo que obliga a utilizar distintas estrategias que evitan este inconveniente. Una posibilidad es combinar el modelo gaussiano modificado con una ecuación que añada una cola exponencial.

Gran parte de los fármacos más utilizados en la actualidad son compuestos quirales, cuyos enantiómeros pueden tener diferentes propiedades farmacológicas y toxicológicas. Por ello, la cuantificación de la fracción enantiomérica, tanto en muestras comerciales como ambientales, es de gran importancia [4]. Este trabajo estudia las propiedades de los picos obtenidos en la separación de fármacos con actividad quiral, mediante cromatografía líquida utilizando columnas enantioselectivas. El objetivo es conocer las características diferenciadoras de los picos de los compuestos quirales para su reconocimiento. El estudio se realiza también con propósitos de deconvolución de los picos de los dos enantiómeros, cuando éstos no se han resuelto completamente. El procedimiento desarrollado permite la cuantificación de la fracción enantiomérica de las muestras analizadas.

A lo largo del estudio, se comparan las prestaciones de los modelos LMG (Linear Modified Gaussian), PVMG (Parabolic Variance Modified Gaussian) y PLMG (Parabolic-Lorentzian Modified Gaussian), con las proporcionadas por sus variantes que añaden colas exponenciales, para realizar el ajuste conjunto de los picos.

**Agradecimientos:** Este trabajo ha sido financiado por los Proyectos CTQ2015-70904-R y CTQ2016–75644-P del Ministerio de Economía, Industria y Competitividad.

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**TSE-P05**

# ESTUDIO DE LA DISPERSIÓN DE PICO EN ELUCIÓN EN GRADIENTE: UN ENFOQUE A PARTIR DE LA TEORÍA DE PLATOS

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En cromatografía líquida, la elución en gradiente se aplica a muestras que contienen solutos con una amplia gama de polaridades, para reducir el tiempo de análisis, mejorar la eficacia y aumentar la capacidad de pico [1]. La elución en gradiente ha sido ampliamente estudiada teóricamente tanto desde el punto de vista de la retención como de la dispersión de los picos [2]. La mayoría de los estudios se han basado en modelos cinéticos de dispersión como el modelo de transporte [3]. El modelo de platos se ha aplicado en menor medida, a pesar de ser la base para introducir los conceptos de altura de plato y eficacia cromatográfica. Según el modelo de platos, la columna se divide en infinidad de secciones iguales llamadas platos y a lo largo de la elución, la fase móvil se transfiere de un plato al siguiente en volúmenes infinitesimales, produciendo una mezcla entre la fase entrante y la fase del plato receptor. El modelo de platos implica un ensanchamiento adicional del pico debido a este efecto convectivo.

En trabajos anteriores [4,5], aplicamos el modelo de platos para describir el ensanchamiento del picos en elución isocrática teniendo en cuenta la transferencia de masa lenta, la difusión longitudinal tanto en la fase móvil como en la fase estacionaria, y la dispersión extra-columna. En esta comunicación presentamos un enfoque general para describir la dispersión en elución en gradiente aplicando el modelo de platos. La ecuación general obtenida ha sido:

$$\frac{d\sigma_l^2}{dl} - \frac{2}{u}\left(\frac{\partial k_l}{\partial t}\right)_l \sigma_l^2 = \frac{H}{u^2}(1+k_l)^2$$

Esta ecuación se ha aplicado a tres tipos de gradiente, en función de la existencia de una rampa de factor de retención a lo largo de la columna, ya que éste es el origen del factor de compresión. Se consideraron los siguientes gradientes: (i) gradiente de referencia sin rampa en el cual el factor de retención varía con el tiempo de manera idéntica en toda la columna, (ii) gradiente de fase estacionaria en el que la naturaleza de la fase estacionaria varía continuamente en el interior de la columna, dando lugar a una rampa de factor de retención constante con el tiempo, y finalmente, (iii) gradiente de fase móvil, que produce una rampa de factor de retención que varía con el tiempo. Las ecuaciones obtenidas se validaron comparando los resultados con los obtenidos mediante la simulación del perfil de pico, utilizando el cálculo numérico para resolver el sistema de ecuaciones diferenciales que describe la elución del gradiente según el modelo de platos.

**Agradecimientos:** Este trabajo ha sido financiado por el Proyecto CTQ2016–75644-P del Ministerio de Economía, Industria y Competitividad.

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**TSE-P06**

# MEASURING THE VOLUME PHASE RATIO IN CHROMATOGRAPHIC COLUMNS: RELEVANCE FOR HILIC

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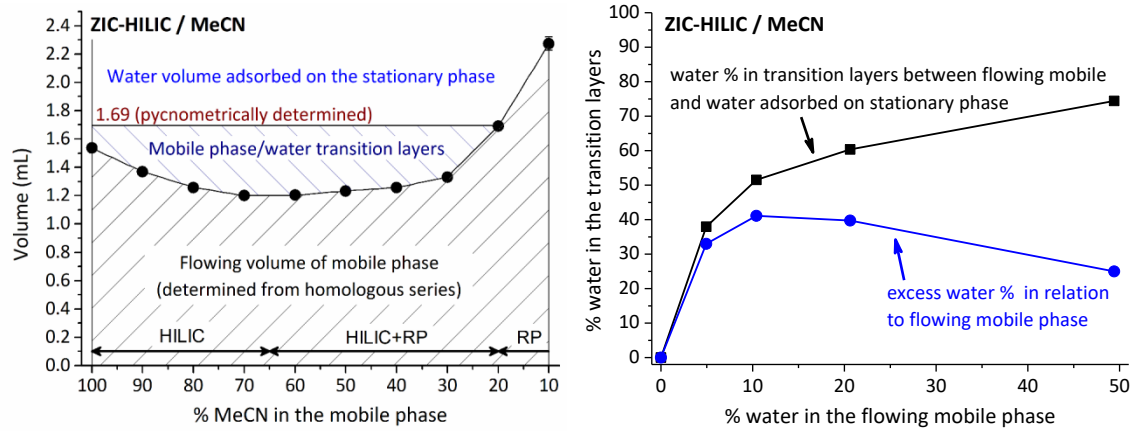
The main retention mechanism in hydrophilic interaction liquid chromatography (HILIC) is based on the partition of solutes between the bulk mobile phase (MP) and a water enriched layer that is semi-immobilized on the stationary phase (SP). The thickness of the water layer depends on the support and the functionalization of the SP, but especially on the water content and composition of the MP [1-3]. In the present work four columns with SPs made of silica support but different functionalization have been studied (zwitterionic sulfobetaine (ZIC-HILIC) and phosphorylcholine (ZIC-cHILIC), aminopropyl (Luna NH2) and pentafluorophenyl (Kinetex F5)) using MP containing acetonitrile (MeCN) or methanol (MeOH) as organic modifiers.

Hold-up volumes determined from chromatographic measurements account for the volume of the flowing mobile phase, as long as part of the solvent is partially immobilized as transition layers between SP and bulk MP. These hold-up volumes can be accurately determined at any MP composition by means of a homologous series approach derived from the Abraham's solvation model, providing additionally information about the predominant mode of retention (reversed-phase or HILIC) [4]. The total content of MP inside the column (flowing and immobilized) can be pycnometrically determined, and solvent weight can be related to volume and composition due to sufficiently different densities of water and organic solvents (MeCN and MeOH).

For the zwitterionic and the aminopropyl columns and when MeCN is used as organic solvent, the transition layers between the bulk MP and the water adsorbed on the SP are enriched in water in relation to the flowing mobile phase. On the contrary, the pentafluorophenyl column shows a slight enrichment in MeCN. However, these differences in composition are negligible in MP containing MeOH instead of MeCN.

Concerning the observed retention mode, reversed-phase or HILIC, both mechanisms can take place depending on the MP composition, with the only exception of the pentafluorophenyl column. Hydrophilic mode predominates in organic solvent-rich MPs and reversed-phase in water-rich MPs, and in between these two extreme compositions a mixed retention mode takes place. However, boundary MP compositions between retention modes depend on the organic solvent nature and column employed.

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**TSE-P07**

# STUDY OF DIFFERENT CHIRAL STATIONARY PHASES FOR THE ENANTIOMERIC SEPARATION OF SEVERAL FLUOROQUINOLONES USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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In the last decade, supercritical fluid chromatography (SFC) has demonstrated to be a powerful technique in the enantioseparations arena. Due to the unique properties of supercritical fluids, it offers several advantages such as short equilibration times, high efficiencies, high resolutions in short analysis times and the upscaling feasibility. Among the different chiral stationary phases employed, those based on polysaccharide derivatives and macrocyclic antibiotics, have shown a wide applicability and a high degree of success [1, 2].

In this work, six different chiral stationary phases, five derived from natural polysaccharides and one derived from the macrocyclic antibiotic teicoplanin, were checked for the enantiomeric separation of three fluoroquinolones: ofloxacin, flumequine and lomefloxacin, using supercritical fluid chromatography (SFC). The effect of different organic modifiers, as well as pressure and temperature, was also studied. The best results were obtained using the cellulose based columns, especially the Chiralcel OD-H and methanol or ethanol as organic modifiers. Good baseline enantioresolutions were achieved for ofloxacin and flumequine, but only a partial resolution for lomefloxacin. These compounds showed a high retention on the Lux Cellulose 2 column, which made necessary the use of a mixture methanol/water (90/10) as modifier, obtaining a clear improvement in the enantioresolution of ofloxacin and flumequine. The amylose based chiral columns provided resolution just for flumequine, obtaining the best results with the Chiralpak AD and Lux Amylose 2 columns. Finally, the teicoplanine derived column did not provided any kind of enantioresolution for these compounds.

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**TSE-P08**

# EVALUATION OF ASYMMETRICAL FLOW FIELD FLOW FRACTIONATION FOR STUDY THE STABILITITY OF SILVER NANOPARTICLES: EFFECT OF DILUTION

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Nowadays, the development of methods to monitor nanomaterials is one of the crucial research area. More specifically, particle size and stability analysis are key elements since the NPs properties depends on these parameters. Among different nanomaterials, silver nanoparticles (AgNPs) have become useful tools for a wide variety of applications taking into account their properties and versatility [1]. For this reason, asymmetrical flow filed flow fractionation (AF4) has been proposed as a powerful technique to characterize and to study the stability of AgNPs aqueous dispersions [2].

In the present work, AF4 coupled to UV-Vis-DLS detectors in series has been evaluated for stability studies of citrate- capped AgNPs dispersions. First, experimental parameters such as mobile phase or cross flow rate were optimized. The results have been compared with the results obtained for AuNPs. Sodium azide to pH 9.2 was selected as best mobile phase. The stability of bulk dispersions of AgNPs (20, 40 and 60 nm) and also, of their dilutions with water have been tested. Fractograms showed a profile variation as a function of time for the diluted dispersions, which is related with a loss of AgNPs concentration in the several size dispersions. The results indicated that the dependence of the signal with time was more intense for AgNPs than for AuNPs, which can be correlated with their stability. In addition, the stability decreased with particle size. Finally, under the optimal conditions, an AgNPs mixture with different particle sizes was analysed with successful results.

Therefore, AF4 coupled to UV-Vis-DLS showed the lower stability of aqueous AgNPs dispersions as a function of time compared with AuNPs, which can be relevant for their applications. The proposed methodology is an alternative to extent the practical application of AgNPs.

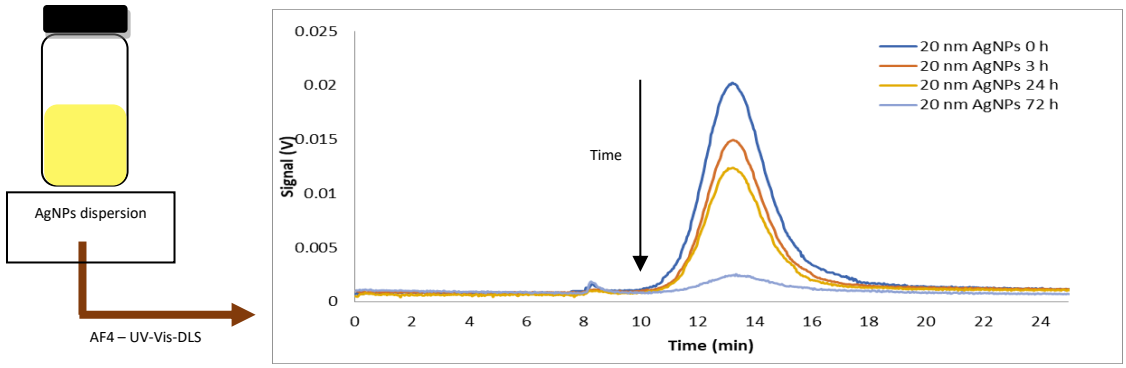


Figure 1: Fractograms of AgNPs dispersion (dilution 1:8, size 20 nm) in function of time.

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**TSE-P09**

## IMPRINTED CORE-SHELL BEADS DEVELOPED UNDER RAFT TECHNOLOGY AS CHIRAL STATIONARY PHASES FOR THE RESOLUTION OF DRUG ENANTIOMERS

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In recent years, interest for chiral separation has attracted considerable attention. It is widely known that biochemical processes happening in living organisms is based on specific stereoselective interactions between reacting molecules and catalysts [1]. In this light, drug enantiomers can behave differently showing opposite pharmacological and toxicological properties. Accordingly, chiral resolution of drug racemates is crucial, particularly in the pharmaceutical field, and that is why research on this topic is gaining special interest over the last years. In the pharmaceutical industry, strict regulations of the authorities exist concerning chiral compounds and the determination of individual enantiomers is definitely determinant for quality control and stability studies [2, 3].

Molecular imprinting technology has proved effective to prepare artificial receptors that present selective recognition capability comparable to that presented by natural ones such as enzymes or antibodies. This benefit has been exploited in developing materials capable of binding a target compound with higher affinity in contrast to structural analogues. This may found particular interest in chiral separation, with the aim of developing new chiral stationary phases (CSP) for enantiomer resolution. MIP based CSP present several advantages, such as mechanical and chemical stability, low material costs, ease of preparation and predetermined selectivity for the target enantiomer. However, in many cases baseline separation of enantiomers is not achieved due to peak broadening and tailing effects typically associated with imprinted polymers developed under traditional bulk synthesis [3]. To minimise these drawbacks, use of surface imprinting technology has received considerable attention. Particles prepared under this method show spherical morphology, higher binding capacity and faster mass transfer, which is translated into narrower chromatographic peaks [4].

In this work, core-shell particles developed by RAFT technology (reversible addition-fragmentation chain transfer) are presented as stationary phase for the chromatographic resolution of chiral drugs. In a first stage, the cores were synthesised through thermally initiated RAFT polymerisation using divinylbenzene as monomer. This allowed for obtaining spherical micro-sized particles with pendant functional groups capable of reinitiating controlled radical polymerisation through UV radiation. Secondly, prepared cores were coated with a molecularly imprinted layer (known as shell) using itaconic acid as functional monomer and ethylene dimethacrylate as cross linker without the need of any other initiator. The obtained sorbent was used as CSP for the chiral resolution of drugs, exhibiting high selectivity for the target enantiomer (separation factor >1.5-2.0, resolution >1). The methodology presented here could be exploited for the development of CSP for other racemic drugs to easily obtain new columns to resolve drug racemates by liquid chromatography.

### Acknowledgements
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**TSE-P10**

# CROMATOGRAFÍA "MIXED-MODE" EN LA SEPARACIÓN DE ANTIBIÓTICOS GLUCOPÉPTIDOS: DESVELANDO LA COMPOSICIÓN DE LA TEICOPLANINA

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La cromatografía de modo mixto o "*mixed-mode*" es aquella que utiliza fases estacionarias capaces de retener los analitos a través de más de un tipo de interacción, de forma que la separación cromatográfica no puede atribuirse a un único mecanismo de retención[1]. Tradicionalmente, la presencia de interacciones secundarias era considerada perjudicial para la separación cromatográfica, por lo que habitualmente se buscaba su eliminación. Sin embargo, en los últimos años, se han desarrollado fases estacionarias con capacidad para retener los analitos por interacciones secundarias, e incluso terciarias, reproducibles gracias a un diseño cuidadoso en la introducción de diferentes grupos funcionales en la fase estacionaria, así como a la mejora de los procesos de síntesis de dichas fases estacionarias.

Los antibióticos glucopéptidos son una familia de fármacos, de origen microbiano, cuya estructura química está basada en cadenas peptídicas no ribosomales policíclicas y glucosiladas.[2] Consideradas por la OMS como medicinas esenciales, estos antibióticos suponen una alternativa para el tratamiento de infecciones por bacterias gram-positivas resistentes a otros antibióticos. La elevada polaridad y la presencia de múltiples cargas en los antibióticos glucopéptidos dificultan, si no imposibilitan, su determinación mediante la cromatografía clásica RPLC-MS, especialmente en muestras complejas donde una separación eficiente es de vital importancia. Es por ello por lo que estos compuestos suponen un candidato ideal para el estudio y aplicación de nuevas tendencias cromatográficas como "*mixed-mode*", técnica que, en nuestro conocimiento, no han sido aplicada a esta clase de compuestos.

En este trabajo, se ha abordado la separación mediante cromatografía "*mixed-mode*" de teicoplanina (Fig. 1), antibiótico glicopéptido semisintético formado por 5 compuestos mayoritarios (teicoplanina $A_2$-1 a $A_2$-5) y 4 minoritarios (teicoplanina $R_S$-1 a $R_S$-4). De las diferentes fases estacionarias de tipo *"mixed-mode"* estudiadas, la que mostró los mejores resultados es la basada en una combinación de residuos $C_{18}$ y pentafluorofenilo (PFP). Esta fase estacionaria mixta, $C_{18}$-PFP, no solo permitió una mejor retención y separación de los diferentes compuestos que forman la teicoplanina, sino que incluso reveló la existencia de otros compuestos, nunca antes descritos, y que permanecían ocultos debido principalmente a las pobres separaciones cromatográficas mediante las que se había estudiado este antibiótico anteriormente.

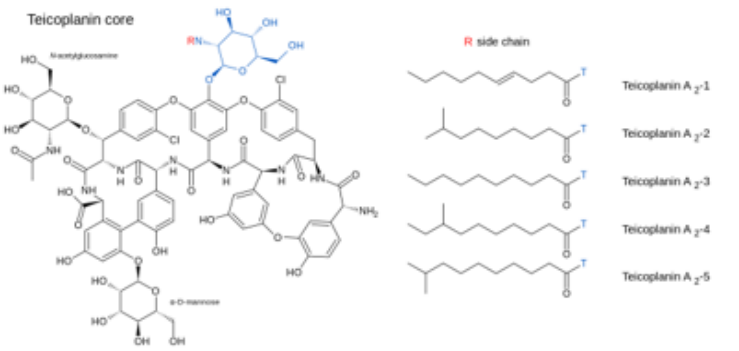

Fig. 1. Estructura y compuestos mayoritarios del antibiótico glucopéptido teicoplanina

Estos resultados ponen de manifiesto la relevancia de las nuevas tendencias cromatográficas, como las fases estacionarias "*mixed-mode*", que permiten abordar problemas hasta ahora no resueltos vinculados a analitos polares y cargados en los campos clínico, ambiental y alimentario.

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**TSE-P11**

## DEVELOPMENT AND VALIDATION OF NEW METHODS FOR DETERMINING SPINETORAM RESIDUES IN HONEY

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Beekeeping is an activity that plays a very important role for both the environment, as it ensures the biodiversity of a wide range of crops and wild plants, and for humans, due to the production of honey, bee pollen, beeswax, royal jelly and propolis, as well as additives for the pharmaceutical industry. Honey in particular is a natural product of great value on account of its nutritional properties and therapeutic applications, and the increase in consumption in the last few years have made it one of the most widely consumed bee products However, during this period residues of contaminants, such as antibiotics and insecticides, have been detected in honey samples from different countries, resulting perhaps from agricultural treatments applied on nearby crops or from beekeeping practices. Spinetoram, which is a reduced risk bio-insecticide of the spinosyn family, is a mixture of two components, 3'-O-ethyl-5, 6-dihydro-spinosyn J (major component) and 3'-O-ethyl spinosyn L (minor component), whose common names are spinetoram J and L, respectively. As a derivative of a compound of natural origin, spinetoram implies less environmental risk than many systemic and/or synthetic insecticides, such as organophosphate and pyrethroids, potentially making this compound a real alternative to the latter. Nevertheless, the use of this insecticide can pose a risk to beneficial insects like bees, mammals or fresh water invertebrates, and the consumption of foods containing spinetoram can have an adverse effect on the consumer´s health. Therefore, it is clear that a monitoring of spinetoram residues in foods, and honey in particular, is required.

In this study, the feasibility of two different methods has been evaluated for the determination of spinetoram J and L residues in honey from different botanical origins using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (quadrupole time-of-flight) and liquid chromatography coupled to mass spectrometry (single quadrupole). Chromatographic analysis was performed in all cases on with the same $C_{18}$ based column (Kinetex® EVO) and mobile phase components (acetonitrile and ammonium formate), which were employed in different percentages accordingly to the methodology employed. Meanwhile, a sample treatment based on solid phase extraction with polymeric cartridges has been proposed, as this has proven efficient in terms of recovery (82% and 94%) and absence of a matrix effect. Validation of the method showed that all the parameters studied complied with existing European legislation, with quantification limits well below the MRL established for this insecticide in bee products. Moreover, results also showed that a similar analytical performance was achieved using both methodologies. Finally, several honey samples were analyzed, and spinetoram residues were not found in any of the cases.

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**TSE-P12**

# ANALYSIS OF TERPENIC COMPOUNDS IN MICROSAMPLES OF NATURAL RESINS BY CAPILLARY LIQUID CHROMATOGRAPHY

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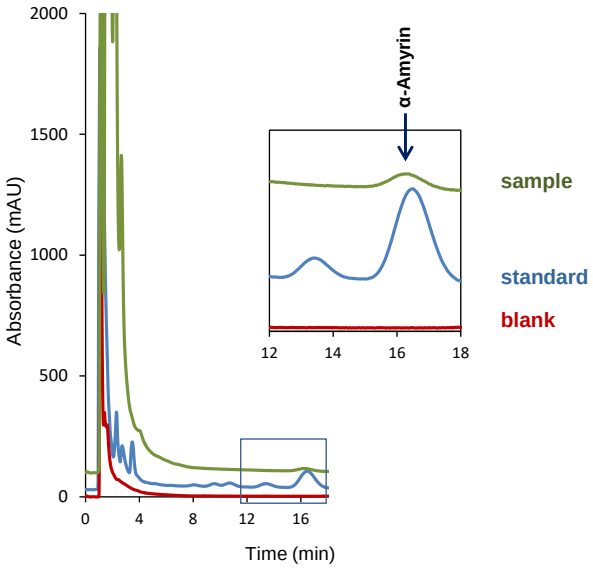
The analysis of natural resin samples is a very complex task due to the high number of compounds that maybe present, and the wide variety of their chemical properties (volatilities, polarities). The lack of chromophores of the predominant compounds, triterpenes, is also a limitation, especially if microsamples are going to be analysed; this may be the case of the characterization of archaeological samples treated with resins. For these reasons, most chromatographic studies are limited to establish the chemical fingerprinting of the resin [1].

A method is described here for the separation and quantification of terpenic compounds typically used as markers in the chemical characterization of resins, which uses capillary liquid chromatography coupled to UV detection. The monoterpene limonene and the triterpenes lupeol, lupenone, β-amyrin and α-amyrin have been selected as model compounds. Sample treatment, separation and chromatographic conditions have been optimized in order to make possible the analysis of volatile and non-volatile analytes in a single chromatographic run, with the adequate sensitivity to be applied to the analysis of microsamples. The proposed method shows suitable linearity, accuracy and precision within the 0.5-10.0 μg $mL^{-1}$ concentration interval, and limits of detection in the 0.1-0.25 μg $mL^{-1}$ range. The reliability of the proposed method has been tested by analyzing three natural resins, white copal, copal in tears and ocote tree resin. Percentages of the triterpenes in the range 0.010-0.16 % were measured using 10-15 mg of the samples, whereas the most abundant compound limonene (≥ 0.93 %) could be determined using 1 mg of the resins. As an example, in the figure is shown the chromatogram obtained for white copal resin with percentage of (0.020 ± 0.002) % of α-amyrin.

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**TSE-P13**

## ANALYSIS OF TRANSTHYRETIN IN AMYLOID DISEASES BY ON-LINE IMMUNOAFFINITY SOLID-PHASE EXTRACTION CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY

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Transthyretin (TTR) is a homotetrameric protein known to misfold and aggregate as stable insoluble fibrils causing different types of familial amyloidotic polyneuropathies (FAP). Among them, FAP-I, caused by a single amino acid substitution of valine for methionine at position 30 of the TTR sequence (Met 30), is the most common [1,2]. Methods used to analyze TTR proteoforms in serum samples typically require an off-line immunoextraction before separation and characterization by liquid chromatography-mass spectrometry (LC-MS) [3].

In this study, as an alternative to these traditional methods, we present immunoextraction, preconcentration, detection and characterization of TTR from human serum samples by on-line immunoaffinity solid-phase extraction capillary electrophoresis mass spectrometry (IA-SPE-CE-MS) using immunoaffinity sorbents with polyclonal antibodies [4] and Fab' antibody fragments [5]. The optimization and validation of both methods are described, and advantages and disadvantages compared. Furthermore, the applicability to screen for FAP-I characteristic TTR proteoforms is demonstrated analyzing serum samples from healthy controls and FAP-I patients.

Overall, these results show the great potential of IA-SPE-CE-MS for the analysis of biomarkers in biological fluids, minimizing sample handling and increasing the analysis throughput, while overcoming the complexity of the sample matrix and the typically poor concentration limits of detection of microscale separation techniques.

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Acknowledgments:
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**TSE-P14**

## MICROEMULSION LIQUID CHROMATOGRAPHY VERSUS HIGH SUBMICELLAR LIQUID CHROMATOGRAPHY FOR THE ANALYSIS OF β-BLOCKERS

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β-Blockers are basic compounds that in the usual pH range in conventional HPLC form cationic species, which yield broad and asymmetrical peaks, due to the interaction with the free silanols in the stationary phase. In the literature, the chromatographic performance of these separations has been enhanced with the assistance of secondary equilibria, involving mobile phases containing ionic liquids [1], and particularly, surfactants forming micelles [2].

There is an extensive study on the use of solutions of surfactants above the critical micellar concentration to analyse basic compounds. The best surfactant for these separations is sodium dodecyl sulphate (SDS). The peak shape is improved yielding symmetrical peaks. However, the cationic basic compounds are strongly attracted to the stationary phase, modified by the anionic SDS, which increases the retention excessively. This forces the addition of a relatively high amount of organic solvent to get practical times. In these conditions, micelles are not formed, and in fact, a new chromatographic mode with particular characteristics is achieved, which has been called “High Submicellar Liquid Chromatography (HSLC)” [3].

We have recently studied the possibility of analyzing β-blockers using microemulsions as mobile phases, in the so-called Microemulsion liquid chromatography (MELC) [4]. Microemulsions are transparent coloidal solutions, thermodynamically stable, where equivalent amounts of immiscible liquids, such as water and an apolar solvent, can coexist thanks to the presence of a surfactant, usually SDS. A solvent (such as butanol or pentanol) that acts as co-surfactant, is often needed to stabilise the microscopic oil droplets. The surfactant plays a major role in the stability of the microemulsions and in the separation performance, but the oil choice is also important, having a great effect in the distribution of the solutes between the mobile and stationary phases, and in the chromatographic selectivity. The choice of co-surfactant and mobile phase temperature also determine the separation performance.

In this work, we compare the performance of both chromatographic modes (HSLC and MELC) for the analysis of mixtures of 7 β-blockers (propanolol, oxprenolol, atenolol, acebutolol, metoprolol, carteolol, and timolol), using a mobile phase of SDS and acetonitrile (HSLC), and a microemulsion of SDS, octane and n-butanol (MELC). Both modes yield good peak shape and appropriate retention times for basic compounds, giving rise to competitive procedures. However, MELC has the advantage of using a very small amount of organic solvent.

**Acknowledgement:** This work has been founded by CTQ2016–75644-P project of the Ministry of Economy, Industry and Competitiveness.

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**TSE-P15**

# SEPARACIÓN DEL BITUMEN Y CRUDO DE PETRÓLEO EN FRACCIONES POLARES ENRIQUECIDAS EN DISTITOS TIPOS DE METALOPORFIRINAS

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La extracción de metaloporfirinas a partir de bitúmenes y crudos permite tener una mejor comprensión acerca del origen de los sedimentos y la evolución de la materia orgánica. Los crudos pesados de Venezuela presentan altas concentraciones de vanadio y níquel, principalmente en la forma de porfirinas. Las petroporfirinas más abundantes que se han aislado son los complejos de vanadio tipo DPEP, aunque también se han encontrado otros tipos minoritarios: ETIO y Di-DPEP, entre otros. Desde un punto de vista analítico, las metaloporfirinas poseen un espectro UV-Vis muy característico. Estos espectros exhiben la denominada banda de ¨Soret¨ en la región del ultravioleta cercano entre 380 y 420 nm, así como dos bandas adicionales de menor intensidad ubicadas en la región del visible a 510 y 600 nm, denominadas "$\alpha$" y "$\beta$" respectivamente.

En el estudio de las metaloporfirinas del petróleo es de primordial importancia el modo de extraer los concentrados adecuados para su caracterización. Así, en 1997, Granadillo [1] optimizó el método SARA para separar a partir de un crudo pesado los maltenos (saturados y aromáticos) de los componentes polares (resinas y asfaltenos) usando unas mezcla de tolueno/*n*-hexano (1:1 v/v) y dimetilformamida/etanonitrilo (1:2 v/v) como eluyentes, además de desarrollar un registro de espectros UV-Vis para la identificación cualitativa de metaloporfirinas. En la presente investigación se tratará de separar distintos tipos de porfirinas de vanadio y níquel presentes en el crudo pesado Boscán (Venezuela), mediante una modificación del método SARA de Granadillo, usando *n*-pentano, una mezcla de diclorometano y *n*-pentano (1:1 v/v) y acetona como eluyentes, y posterior sublimación al vacío de los componentes polares variando las temperaturas entre 100 y 240 °C, para luego caracterizar las diferentes fracciones obtenidas con las técnicas UV-Vis y ICP-AES de acuerdo con Sugihara y Bean [2].

Los espectros UV-Vis del crudo total y de los componentes polares presentaron la bandas Soret, alfa y beta a 411, 532 y 574 nm, respectivamente. A su vez, en la fracción sublimada entre 100 y 120ºC se observa un ligero "hombro" en una longitud de onda de 406 nm (Soret) y dos ligeras bandas en 534 y 572 nm (alfa y beta), lo cual indica que la cantidad de porfirinas que se logró aislar en este rango de temperatura fue pequeño. En la fracción sublimada entre 120 y 210ºC, se observó las mismas bandas, sin apenas desplazamiento batocrómico, pero con una intensidad mayor, esto indica que en este rango de temperaturas la cantidad de petroporfirinas que se logró aislar fue más elevada. Por el contrario, En la última fracción sublimada (210-240ºC), se observó que la banda Soret tuvo un desplazamiento batocrómico de 406 a 409 nm, que puede atribuirse a un tipo diferente de metaloporfirinas.

Comparando estos resultados con los que obtuvo Granadillo, podemos decir que son similares, aunque los datos obtenidos en el presente trabajo son más precisos, indicando que la metodología empleada en este caso fue más efectiva. En cuanto al tipo de metaloporfirinas aisladas, en base la posición de la banda Soret en las fracciones sublimadas (406-409 nm) y a los análisis de V y Ni, cabe decir que mayoritariamente se separaron petroporfirinas con Ni (100-120ºC) y vanadil-porfitinas (120-240ºC).

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**TSE-P16**

# DESARROLLO DE UN POLÍMERO DE IMPRONTA MOLECULAR SELECTIVO A ÁCIDO FÓLICO PARA SU DETERMINACIÓN EN EXTRACTOS DE ALIMENTOS UTILIZANDO EXTRACCIÓN EN FASE SÓLIDA DISPERSIVA Y LC-MS

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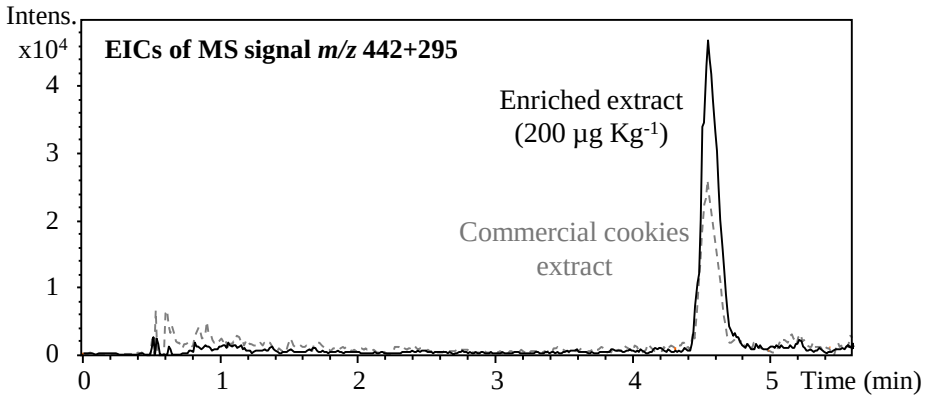
El ácido fólico (FA) es un compuesto que pertenece al grupo de las vitaminas B (vitamina $B_9$) de gran importancia nutricional. Existen diferentes metodologías para llevar a cabo su determinación de manera precisa en diferentes matrices y, en todas ellas, se hace necesario incluir una etapa de extracción previa a su determinación, lo que mejora la sensibilidad de las mediciones y reduce los posibles efectos matriz. Tanto la extracción en fase sólida (SPE) como técnicas relacionadas que utilizan polímeros de impronta molecular (MIP) permiten una purificación simple y efectiva, así como una preconcentración del FA presente en alimentos y muestras biológicas. En este trabajo se ha desarrollado un nuevo MIP para la extracción selectiva de FA en matrices alimentarias. Para ello, se han estudiado diversas combinaciones de monómeros (ácido metacrílico (MAA), 4-vinilpiridina (4VPy) y cloruro de vinilbencil trimetilamonio (VBTMAC)) y entrecruzadores (etilenglicoldimetacrilato (EGDMA) y divinilbenceno (DVB)) en diversos porógenos. Las isotermas de absorción muestran que la mayor afinidad se consigue usando VBTMAC como monómero funcional y EGDMA como entrecruzador. Posteriormente, se optimizó la relación FA:VBTMAC:EGDMA (1:25:250) y se determinaron sus propiedades de unión, tanto cinéticas como de equilibrio, determinando una alta afinidad (2.5 mmol $g^{-1}$) y una elevada relación MIP/NIP (superior a 37).




Posteriormente, se usó este MIP para la extracción de FA en lechuga y galletas enriquecidas mediante extracción en fase sólida dispersiva (DSPE) y su ulterior análisis por LC-MS, obteniendo una recuperación y repetibilidad apropiadas (≥ 79,50% y ≤ 13,15 (% RDS), respectivamente).

Agradecimientos. Este trabajo ha sido financiado por el programa People Programme (Marie Curie Actions, Multi-ITN) del 7 programa Marco de la Unión Europea (grant agreement n° 608104 (EUROMBR)) y el MINECO (proyecto CTQ2014-53442-P).

**TSE-P17**

# RAPID MAGNETIC DISPERSIVE SOLID PHASE EXTRACTION TO PRECONCENTRATION/DETERMINATION OF Cd AND Pb IN AQUEOUS SAMPLES

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A new magnetic dispersive solid phase extraction (MDSPE) method and graphite furnace atomic absorption spectrometry (GFAAS) have been combined for the analysis of Cd and Pb in environmental samples. For the preconcentration, a shell structured $Fe_3O_4$@graphene oxide nanospheres was synthetized and characterized. The material was suspended in the ionic liquid 1-n-butyl-3-metilimidazolium tetrafluoroborate [BMIM][$BF_4$], the obtained stable colloidal suspension is named ferrofluid. GO presents excellent adsorbent properties for organic species due to the presence of the electronic π system. For this reason, the organic ligand [1,5-bis-(2-dipyridyl) methylene] thiocarbonohydrazide (DPTH) was used in order to form organic complexes of Cd and Pb. Once the DPTH ligand has been added to sample, the ferrofluid was injected and finely dispersed in the sample solution in order to extract the formed chelates (Fig. 1). The complete adsorption of the chelates took place within few seconds then, the solid was separated from the solution with the aid of a strong magnet. Cd and Pb ions were desorbed from the material with 1 mL of acid nitric 5% solution and quantified by GFAAS. All experimental and instrumental variables were optimized.

The analytical performances of the optimized method were: EF (Enrichment factor): 200 with LODs (detection limit): 0.005 and 0.004 µg $L^{-1}$ and LOQs (determination limit): 0.017 and 0.013 µg $L^{-1}$, for Cd and Pb, respectively. The reliability of the developed procedure was tested by relative standard deviation (% RSD), which was found to be < 5%. The accuracy of the proposed method was verified using certified reference materials (SLRS-5, SPS-SW2, and BCR-723) and by determining the analyte content in spiked aqueous samples. Sea waters and tap water samples collected from Málaga (Spain) were also analysed. The determined values were in good agreement with the certified values and the recoveries for the spiked samples were around 100% in all cases.

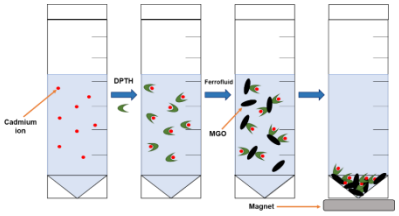


Fig. 1 Magnetic solid phase extraction process.

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# *XXII REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA*

***Valladolid 18-19 julio 2019***

## *COMUNICACIONES PÓSTER*

## *"LAST MINUTE"*

**LAST-P01**

## DETECCIÓN DE COMPUESTOS ORGÁNICOS MEDIANTE LIBS EN ROCAS DE INTERÉS EN EXPLORACIÓN PLANETARIA. APLICACIONES EN ASTROBIOLOGÍA

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La integración de instrumentos LIBS en misiones de exploración planetaria es una realidad desde hace ya unos años, siendo una tecnología conducente a la obtención de información multielemental en las distintas rocas y minerales existentes en la superficie de Marte. Su gran eficacia ha sido demostrada en muchos de los trabajos publicados hasta la fecha por los equipos de investigación participantes en la misión Mars Science Laboratory (MSL). Uno de los objetivos primordiales de dichas investigaciones radica en la detección de posibles bioindicadores [1], así como en la identificación y discriminación mediante LIBS de compuestos orgánicos, tarea que puede llegar a ser compleja ya que, entre otros aspectos, se trata de una técnica muy sensible a las condiciones ambientales (composición de la atmósfera y presión existente) [2] [3].

El presente trabajo ha buscado por un lado, evaluar el efecto de la atmósfera existente en el planeta rojo (rica en $CO_2$ con 7mb de presión media) en la formación de plasmas inducidos por láser a partir de la ablación de muestras formadas por matrices inorgánicas dopadas con compuestos orgánicos seleccionados. Por otro lado, a partir de las diferentes huellas espectrales obtenidas en dichas condiciones y mediante la aplicación de técnicas quimiométricas adaptadas, se estudian las posibilidades de identificación de dichos referentes orgánicos.
Las cuatro sustancias orgánicas seleccionadas por su interés como posibles indicadores o precursores de vida fueron adenina, glicina, pireno y urea. Para llevar a cabo el experimento se hizo uso de un sistema LIBS de laboratorio acoplado a una cámara de presión. Las muestras se prepararon en forma de pellets, utilizando tanto $CaCO_3$ como $CaSO_4$ como matrices inorgánicas. Los parámetros estudiados como variables fueron las intensidades de emisión de las principales señales recogidas en los espectros LIBS, tanto procedentes de especies atómicas (C, H, N y O) como de especies moleculares (CN y $C_2$, principalmente).
Además, se establecieron tanto los umbrales de ablación como los límites de detección de las diferentes especies de emisión para cada dopante orgánico. Estos últimos fueron calculados tanto para atmósfera de $CO_2$ como de aire, y para dos regímenes de fluencia diferente.
Es de destacar que las especies de emisión de carbono (C, CN, $C_2$) presentaron valores de fluencia umbral menores en ambiente de $CO_2$ que en atmósfera de aire, evidenciando la contribución del gas en la respuesta espectral de emisión. Además, el límite de detección de especies emisoras calculado para la matriz de $CaCO_3$ con un contenido variable de material orgánico fue inferior al 14% (wt/wt) en las condiciones ambientales de atmósfera de $CO_2$.

Los resultados obtenidos pusieron de manifiesto que es posible discriminar los dopantes orgánicos estudiados con respecto a la matriz utilizada mediante el uso de Análisis de Funciones Discriminantes, teniendo en cuenta la influencia de la naturaleza de la atmósfera presente.

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**LAST-P02**

## ATMOSPHERIC PHOTOIONIZATION SOURCE COUPLED TO A DMA-MS FOR THE ANLYSIS OF ATMOSPHERIC NON-POLAR CONTAMINANTS

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A field-free Atmospheric Pressure Photoinization (APPI) source has been designed specifically for the analysis of gas-phase analytes and airborne particles. Analyte residues are collected upon re-useable Tenax-GR coated fiberglass/stainless steel filters using a high volume sampling (HVS) apparatus. After the sampling, the filters are thermally desorbed (TD) into a carrier gas stream directed to the ionization source. Unique ion source architecture provides fully enclosed photoionization and extended field-free reaction regions with a small internal volume, allowing for a total source gas flow rate below 1 litre per minute. The generated ions are introduced into a narrow-band mobility filter (SEADM - P5 DMA) coupled to the atmospheric interface of a Sciex 3200 Qtrap mass spectrometer. The instrument gain has been calculated using standards of Dioxins, Furans and Polycyclic Aromatic Hydrocarbons (PAH´s). Finally, the analyzer was used to monitor the change in airborne PAH isobar concentrations within an underground parking facility throughout three hour period. 1.9 $m^3$ of air were sampled using the HVS at consecutive 10 min intervals. Traffic and PAH levels spike at around 3 pm as employees depart.

**LAST-P03**

## DETERMINATION OF BETAINES IN BEE POLLEN BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION-MASS SPECTROMETRY

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Betaines are quaternary ammonium compounds, which have a widespread distribution in the plant and animal kingdoms, produced by specific biosynthetic pathways involving the exhaustive nitrogen methylation of amino and imino acids. These substances tend to accumulate in the cytoplasm and intercellular fluids where they exert protective functions on the structures of proteins, nucleic acids, and cell membranes in response to plant abiotic stresses, such as reduced availability of water, high soil salinity, hypoxia, cold, and freezing. Plants express characteristic patterns and levels of betaines according to their species. In addition, there is a growing interest in betaines as natural compounds potentially beneficial to human health, as these compounds may also aid with digestive function, heart health, liver function and detoxification, fat loss, and muscle mass improvement. Thus, the determination of betaines in bee pollen could be an appropriate measure not only to ascertain its presence, which has a significant botanical interest, but also from a nutritional point of view. It must be remarked that to the best of our knowledge no study has been published determining their presence in bee pollen.

In this study, a new method has been proposed to determine betaines in bee pollen by means of liquid chromatography coupled to a single quadrupole mass detector equipped with an electrospray ionization source, which was operated in positive mode. For this purpose, an efficient and selective sample treatment has been proposed, with average analyte recoveries higher than 80% and absence of the matrix effect. This involved a solvent extraction and a further dilution of the extracts. Chromatographic analysis was performed on a HILIC based column. The mobile phase consisted of acetic acid (0.1%) in water and acetonitrile, with a flow-rate of 0.5 mL/min in gradient elution mode. The method was fully validated and the data demonstrated that it is consistent, reliable and has a wide linear range of applicability. Finally, the proposed method was applied to betaine analysis of commercial bee pollen samples from different Spanish regions.

**Acknowledgements**
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**LAST-P04**

# WASTEWATER ANALYSIS TO ESTIMATE HUMAN EXPOSURE TO PLASTICIZERS: DEVELOPMENT OF AN ANALYTICAL METHOD FOR PHTHALATE AND NON-PHTHALATE PLASTICIZER METABOLITES

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Plasticizers are high-production volume chemicals added to plastic materials to improve their flexibility and softness. Their use as additives, i.e. not chemically bonded to the organic polymer, facilitates their release into the surrounding environment, turning them into ubiquitous contaminants and representing a potential hazard for human beings. Among the different classes of plasticizers, phthalates (diesters of the phthalic acid) cover the great majority of the global market. However, the increased concern on their negative effects has led to the appearance of new alternatives: terephthalates, benzoates, adipates, or diisononyl cyclohexane-1,2-dicarboxylate (DINCH), among others [1]. Traditionally, human exposure to contaminants has been assessed through the measurement of the parent compounds and their metabolites in urine. A relatively new approach known as wastewater-based epidemiology (WBE) reveals the analysis of wastewater as a complementary tool to human biomonitoring. WBE is based on the concept that wastewater is a largely diluted and integrated sample of urine of a whole community and, thus, it can be used to correlate the levels of the biomarkers found in it with the exposure of a given population to a certain contaminant [2,3].

In this line, this study was aimed at optimizing and validating a new method to determine the main urinary metabolites of 21 plasticizers (including phthalates, terephthalates and DINCH) in wastewater. Analytes were solid-phase extracted on mixed-mode reversed phase-anion exchange sorbents Oasis MAX and extracts analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry using a 1.8 µm-particle size column Restek Biphenyl. Calibration was performed by the internal standard method. Parameters providing the best method performance were carefully optimized and the final method was validated in terms of trueness, precision and limits of detection and quantification. Percentages of recovery varied between 81% and 136% in ultrapure water spiked with all the analytes at 50 ng/L, and between 74% and 130% in wastewater spiked at 500 ng/L. Relative standard deviations were below 21% in all cases. Limits of quantification ranged from 0.4 ng/L to 4.4 ng/L. Stability tests conducted in wastewater demonstrated that all metabolites were stable at room temperature for at least 48 h at 4 ℃. Similarly, studies performed with the precursor plasticizers showed that only dimethyl terephthalate degraded naturally to methyl terephthalate, avoiding the use of the latter as biomarker of exposure in WBE.

The application of the method to analyse 24 h composite raw wastewater samples collected during one week in Santiago de Compostela (Spain) allowed to detect a higher presence of short-chain phthalate metabolites (<6 C atoms in the ester alkyl chain) followed by long-chain phthalate and terephthalate metabolites.

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