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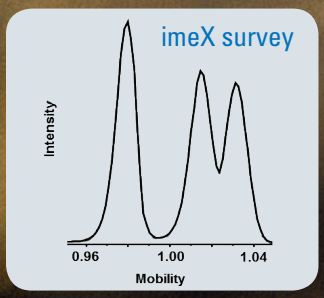

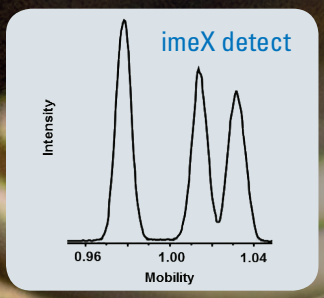

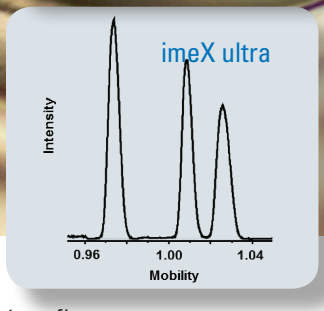

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EDITORIAL

Queridos socios de la SECyTA,

Termina un nuevo año y comenzamos 2024 cargados de nuevos retos e ilusiones. Este año hemos celebrado la XXII Reunión Científica de la Sociedad Española de Cromatografía y Técnicas Afines (SECyTA 2023), en S'Arenal, Palma de Mallorca, del 16 al 18 de octubre de 2023. Pudimos disfrutar de un entorno maravilloso con un tiempo magnífico donde combinamos la ciencia y la belleza del Mediterráneo en esta fantástica isla. Quiero dar las gracias a los Dres. Joan Grimalt (IDAEA CSIC, Barcelona) y Manuel Miró (Universidad de las Islas Baleares) y a todo el comité por su excelente organización.

En colaboración con la Sociedad Española de Espectrometría de Masas (SEEM) se impartió el día previo a la reunión el curso avanzado de espectrometría de masas: MOVILIDAD IÓNICA – ESPECTROMETRÍA DE MASAS PARA EL ANÁLISIS MEDIOAMBIENTAL Y ALIMENTARIO. La experiencia de los Profesores Encarna Moyano (Universidad de Barcelona) y Juan Vicente Sancho (Universidad Jaume I) en este ámbito junto con las aplicaciones prácticas que mostraron D. Pablo de la Iglesia (Waters) y D. Miguel Ángel Pérez (Bruker) permitieron a los participantes conocer los últimos avances en estas técnicas y profundizar en aspectos prácticos de gran interés.

En esta ocasión tuvimos la oportunidad de disfrutar las conferencias plenarias de destacados especialistas, como el Prof. Philippe Schmitt-Kopplin (Technical University of Munich), el Prof. Lars Wörmer (MARUM – Center of Marine Environmental Sciences, University of Bremen), el Prof. Pavel Kubáň(Czech Academy of Science), el Prof. Rafael Lucena (Universidad de Córdoba) y la Prof. Ana Agüera (Universidad de Almería), presentando investigaciones innovadoras en metabolómica aplicada a nutrición, espectrometría de masas de imagen aplicada a muestras fósiles, tratamientos de muestra automatizados en electroforesis capilar, dispositivos de microextracción en espectrometría de masas en condiciones ambientales, o perspectivas en el análisis de contaminantes emergentes en aguas. Una interesante novedad fue la realización de una mesa redonda sobre la problemática del helio debido a su escasez actual y a las limitaciones que esto plantea considerando su carácter esencial para el funcionamiento eficiente de columnas capilares en cromatografía de gases. Para ello, contamos con destacados especialistas de las casas comerciales Air Liquide, Carburos Metálicos, Agilent y LECO, a los que agradecemos sinceramente desde aquí sus valiosas aportaciones, incluyendo alternativas para su sustitución en los instrumentos.

Contamos además con 28 comunicaciones orales senior, 76 comunicaciones en formato póster y las contribuciones de los jóvenes socios, que presentaron 13 comunicaciones orales y algunos de los pósteres fueron seleccionados como presentaciones "flash". Como siempre, agradecemos la presencia de nuestras empresas patrocinadoras, que apoyaron activamente la celebración de SECyTA 2023, y que participaron en la exposición comercial y en las sesiones. De nuevo, un año más agradecemos a Bruker el patrocinio de los ***Premios José Antonio García-Domínguez, en su XVIII edición***. Este año se ha celebrado también la II edición del ***Premio SECyTA a la mejor Tesis Doctoral en Cromatografía y Técnicas Afines***, que se otorgó entre las tesis presentadas correspondientes al año 2022. Agradecemos sinceramente el trabajo del jurado que evaluó las Tesis presentadas por su esfuerzo y la difícil tarea de una selección compleja por la calidad de sus contribuciones. Enhorabuena a los premiados.

En 2024 celebraremos las reunión en Pamplona y esperamos que participéis activamente y fomentéis en vuestros jóvenes investigadores la asistencia a estas reuniones donde se hace ciencia en un ambiente distendido, fomentando la cooperación entre los grupos de investigación. En estas reuniones se pone de manifiesto que la mejora continua y la innovación en técnicas de separación y detección y en metodologías analíticas son la base del progreso, impulsándonos hacia soluciones para desafíos globales urgentes y abriendo nuevos horizontes en el descubrimiento científico.

Os deseamos todo lo mejor para 2024, mucho ánimo, buen trabajo y mucha suerte.

Un abrazo,

Ana M. García Campaña
Presidenta de SECyTA

ARTÍCULO

Application of chromatographic techniques in microplastics analysis and determination

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ABSTRACT

Microplastics (MPs) represent nowadays an increasingly pressing global environmental concern, owing to their capacity to adsorb chemicals from their surrounding environment as well as their intrinsic status as pollutants. In the first case, the sorption potential of MPs is affected by an interplay of factors, encompassing the polymeric composition of the MP, the characteristics of the surrounding matrix, and the specific contaminants involved. In recent years, the evaluation of pollutants sorption onto MPs through kinetic and isotherm studies has expanded the comprehension of the underlying mechanisms and the associated risks of these processes. Simultaneously, the importance of the determination of pollutants on environmental MPs has also gained significant prominence; however, publications have predominantly leaned on traditional extraction techniques, with a relatively limited number of instances in which alternative methods have been applied. With regards to the chromatographic techniques used for the determination of the different analytes, both liquid and gas chromatography techniques coupled to various detectors have been applied. Generally, gas chromatography has emerged as the dominant method for the analysis of environmental MPs, primarily due to the nature of the analytes involved (mostly, persistent organic pollutants). In contrast, liquid chromatography has found frequent applications in sorption studies. Moreover, recent advances in thermal analysis have yielded significant breakthroughs, as the combination of pyrolysis with gas chromatography coupled to mass spectrometry detectors has allowed the simultaneous determination and quantification of both the polymer and the pollutants/additives present in MPs. Furthermore, liquid chromatography with tandem mass spectrometry has also started to be used for the determination of MPs, although the number of publications is still limited. This article aims to provide an overview of the current status and recent advancements in the analysis and determination of MPs using chromatographic techniques.

Keywords: Microplastics, environment, pollutants, liquid chromatography, gas chromatography

1. INTRODUCTION

Plastics are one of the fundamental inventions of the last two centuries, due to their many advantageous properties that have made them nearly irreplaceable in many fields. From the last reported data on plastics global production, in the year 2022 an all-time high of 400.3 million tons was reached, with the main manufacturers being from Asia (China contributing to 32% of the global production, representing the biggest plastic producer in the world) [1]. Among the most common polymer types used, polypropylene (PP) (15.4%), linear- and low-density polyethylene (LL-, LDPE) (13.4%), polyvinylchloride (PVC) (9.1%) and medium density and high-density polyethylene (MD-, HDPE) (8.7%) plastics can be highlighted [1]. Nevertheless, out of all these materials, only 18.5% and 1.0% were recycled or bio-based, respectively, while 80.3% were directly obtained from fossil fuels [1]. As a result of the high production volume, and poor waste treatment strategies developed in most countries, it is not strange to observe the immense amounts of plastics that end up in landfills and that are eventually released into the environment [2]. Once there, plastics are affected by numerous phenomena that can alter their original properties, making them more susceptible to fragmentation (through physical, chemical, or biological degradation processes) [3]. The plastics resulting from these degradation processes can be classified into different categories based on the size of their largest dimension: macroplastics (> 25 mm), mesoplastics (25-5 mm), microplastics (MPs; 5 mm-0.1/1 μm), and even, if the fragmentation continues, nanoplastics (< 0.1/1 μm) [4]. Although all these categories would deserve an in-depth evaluation, in the following manuscript we will be focusing fundamentally on MPs, as they are very commonly found in the

environment and have shown several properties that have made them particularly damaging.

Over the years, many research articles have been published in the scientific literature, providing valuable information with regards to MPs and current problems surrounding them. From the snow in dormant volcanoes [5], to the highest altitudes on Earth [6], passing through some of the coldest [7, 8], and hottest regions [9] and even in the deepest parts of oceans and seas [10-12], MPs have been found in all environmental compartments [13]. Even the most inhospitable natural sceneries in which human presence is negligible, have succumbed to this generalized pollution, demonstrating the capability of MPs to travel very long distances through different pathways (rivers, great oceanic gyres, winds, etc.) [14, 15], making them a global scale issue. Additionally, MPs are not isolated, but instead they interact with their surroundings by binding with debris found in the environment and other natural elements (e.g., rocks, algae, etc.), forming new plastic complex formations such as plastiglomerates [16], pyroplastics [17], plasticrusts [18], anthropoquinas [19], or the recently called "plastitar" [20], a combination of MPs embedded in tar which can pose a new threat to the environment.

For a long time, the discussion surrounding MPs has been centered on their behavior as environmental pollutants and, therefore, on the need to develop suitable analytical methodologies for their determination in which MPs are considered as "analytes"; however, there is another layer of complexity that must be introduced, which is their ability to retain contaminants on their surface and, as a result, the need to analyze MPs (as "matrices") in order to determine such pollutants.

Through different mechanisms, chemical pollutants can be pre-concentrated in the polymer matrix and protected from degradation, prolonging their persistence in the environment [21]. Sorption in MPs occurs through two pathways: adsorption or absorption [22], although adsorption has been more frequently reported [23]. Figure 1 shows a simplified illustration of the different sorption mechanisms in MPs. To understand them, it must be acknowledged that these processes are influenced by multiple factors involving the nature of the MPs, their surrounding conditions, as well as the properties of the contaminant implicated. As an example, parameters such as the crystallinity of the MPs have shown to play an important role in sorption processes and must be

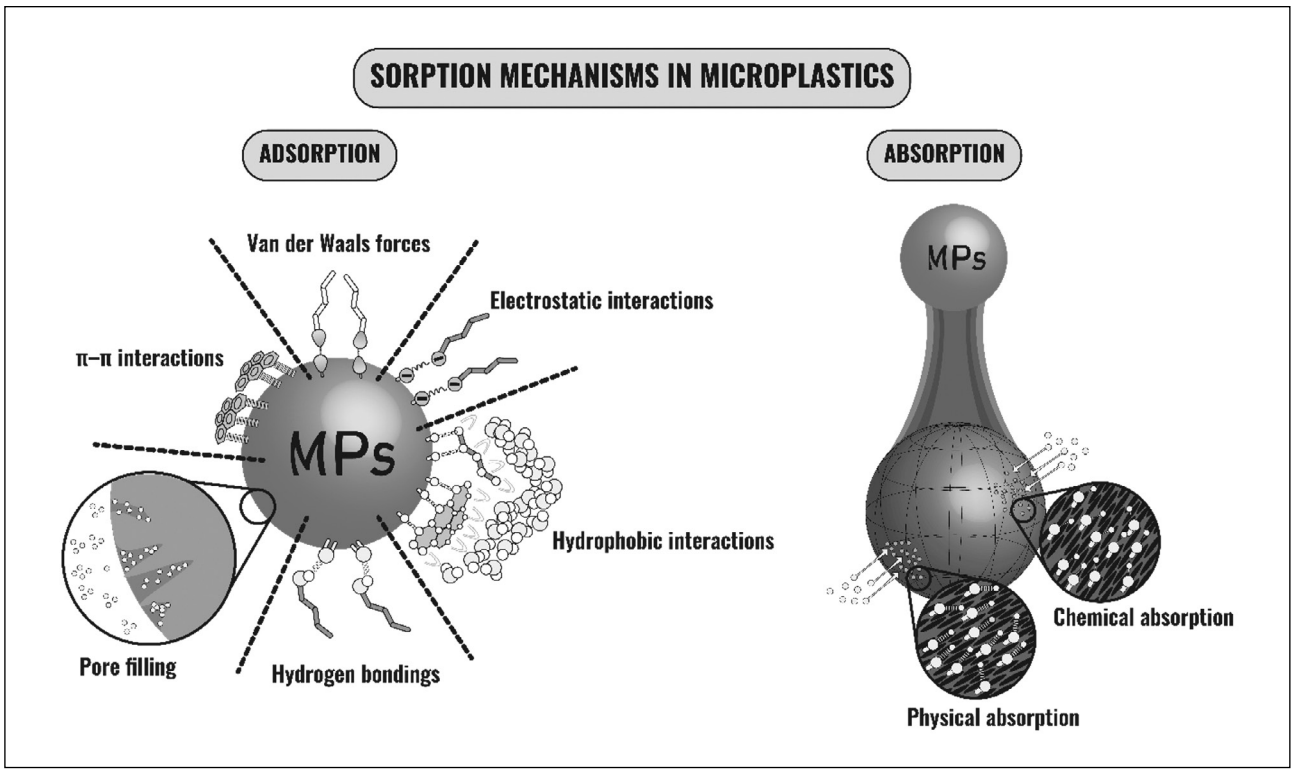

Figure 1. General types of interactions in adsorption and absorption mechanisms in MPs.

reckoned. Plastic polymers can be semi-crystalline (containing both crystalline and amorphous regions in their structure) or amorphous [24, 25]. When a polymer is semi-crystalline there is a reduction in the sorption rate and capacity, as a result of the higher energetic cost of the sorption process, especially if compared to amorphous polymers, where the larger free volume results in improved sorption of the contaminant [24]. Additionally, depending on the glass-transition temperature (T_g) of each polymer, amorphous regions will experience a transition from rubbery to glassy states, when temperatures are above or below T_g, respectively [24-26]. In the rubbery state, polymer chains will have increased flexibility, more mobility and larger free volume, which will favor absorption processes [27, 28]; on the other hand, in its glassy state, polymer chains will be more cohered, interactions between chains will be more frequent, and the resulting structure will be more condensed, which will ultimately lead to less chain mobility and smaller free volume, favoring adsorption interactions [22, 27].

Apart from the previously commented issues, MPs in the environment, as already mentioned, suffer constant degradation through different mechanisms. This degradation overtime changes the surface of the MPs introducing modifications that can have important effects in sorption rate and capacity (i.e. oxygen atoms are introduced in their structure), as well as facilitating the retention of more hydrophilic compounds which would otherwise not be retained or at least hardly retained [3, 25, 28-30]. Additionally, higher specific surface area, which is affected by the shape and size as well as particle aggregation of the MPs, plays an important role in sorption, as it favors adsorption processes [22, 25]. With regards to the conditions of the surrounding medium in which MPs are present (i.e. sea water, soils, etc.), parameters such as pH, salinity, temperature, or dissolved organic matter (DOM), to name a few, as well as pollutant characteristics like hydrophobicity, pKa, molecular structure, etc., are extremely relevant and must be studied, since, for instance, pH, salinity and pKa can alter the electrostatic interactions between MPs and pollutants, generating new sorption dynamics [30]. Furthermore, salinity, biofouling, and DOM can also alter the available surface area of the plastic, and foster aggregation and agglomeration [30].

Even though the first publications highlighting the presence of plastics in the environment date back to the 1970s [31, 32], it has only been in recent years that MP-retained pollutants have gained more attention, with the number of publications in this field increasing. In this sense, further research is needed for the development of more efficient extraction methods, since traditional techniques are still present on a big portion of the total publications in this field [21] and the families of compounds that have been studied —especially in environmental samples— are constrained [23].

Focusing on the number of review articles published until this date, there is a wide range of topics that have been thoroughly assessed, pointing out many of the problematics surrounding MPs. In this sense, reviews have discussed the bioaccumulation, ecotoxicological effects, sorption and migration mechanisms, and transport of pollutants in MPs, as well as all the different methodologies developed for the determination of the pollutants retained in these plastic particles as well as the MPs themselves [21, 24, 25, 28, 30, 33-38]. In this sense, Atugoda [30] explored the factors influencing the MP-assisted vector transportation of pharmaceuticals and personal care products, leading to their widespread presence in water. As highlighted in the review, hydrophilic compounds show greater affinity for polar and amorphous polymers, leading to increased adsorption on weathered MPs, with both hydrophobic and electrostatic interactions governing these sorption mechanisms. Santana-Viera et al. [35] also developed a thorough investigation on the different methodologies developed for the analysis of pollutants retained in MPs as well as the occurrence in oceans. In this regard, researchers highlighted the common use of traditional techniques (e.g., Soxhlet), the overall fixation on persistent organic pollutants (POPs) and the widespread use of gas chromatography (GC) for environmental sample analysis, something also mentioned in other review articles [21]. Menéndez-Pereira et al. [28] also assessed many of the factors influencing pollutants sorption on MPs, as well as the interaction of the pollutants retained in the MPs with the biota and the respective ecotoxicological effects. In this sense, as indicated in the review, there are evidence that these effects cannot be overlooked, but further research is still required to fully understand the severity of the problem at hand. With this objective in mind, the use of chromatographic techniques is of great importance. Since the nature of pollutants that can be evaluated expands through many different families with different physicochemical properties, it is not strange that both liquid chromatography (LC) and GC techniques (coupled to the wide range of commercially available detectors)

have been used for sorption studies, as well as for pollutants determination in real samples [23].

Additionally, the advancements in thermal techniques have led to the development of hyphenated techniques, in which pyrolizers are combined with GC-mass spectrometry (MS) systems, allowing the simultaneous determination of the additives/pollutants present in the MPs, as well as determining the composition of the polymer [39] which can also be achieved by thermogravimetric analysis (TGA) coupled with MS [40]. Furthermore, new strategies employing different approaches for the depolymerization of MPs and the use of LC combined with tandem mass spectrometry (MS/MS) detectors for quantification/identification have also gained importance, although the number of publications in this realm is limited [41, 42].

This article pretends to provide an overview of the current status and recent advancements in the analysis and determination of MPs. A detailed discussion is conducted on the application of chromatographic techniques in sorption studies and the analysis of environmental samples. Additionally, the article also highlights the latest developments in chromatographic methods for the determination of MPs.

2. SORPTION STUDIES IN MICROPLASTICS

Understanding the mechanisms involved in the retention of pollutants in different polymers and matrices is an important knowledge that on many occasions is not properly addressed or completely ignored. In the case of MPs, understanding sorption processes provides valuable insights that can be used to determine the real risks that can entail certain contaminants when they are retained on the MP and exposed to different environmental conditions, as well as when they are ingested by an organism. As it has already been discussed, such processes are influenced by a wide number of factors that involve the MPs, the surrounding medium and the pollutants, such as crystallinity, T_g, pH, salinity, pKa, etc. The combination of all of these factors results in different sorption dynamics that can be studied using chromatographic techniques.

After a close inspection of the scientific literature, it is clear that a wide variety of compounds have been studied [23, 43], being pharmaceuticals and antibiotics the most extensively researched. Nonetheless, other compounds including polycyclic aromatic hydrocarbons (PAHs) and their derivatives, polychlorinated biphenyls (PCBs), pesticides, antibacterial agents, perfluoroalkyl substances (PFASs), among others, have also been evaluated [23, 43]. To quantify these compounds, the technique of choice has been LC paired with a variety of detectors (including, diode array —DAD—, fluorescence —FD—, ultraviolet —UV—, MS, or MS/MS) and using in most cases C_{18} columns [23]. In fewer instances, GC has been employed, often coupled with either an electron capture detector (ECD), a flame ionization detector (FID), or MS detectors and employing (5%-phenyl)-methylpolysiloxane (in most cases), phenyl arylene or 6% cyanopropylphenyl polydimethylsiloxane columns [23].

Overall, many of these studies have indicated that adsorption is the most common sorption mechanism, which highlights the surface nature influence on the sorption phenomena in MPs [23, 43]. Table 1 compiles some publications regarding sorption studies in MPs. For instance, in a publication by Wang et al. [44] the sorption behavior of five pesticides was studied in PE films (< 5 mm) using high-performance LC (HPLC) coupled to a MS/MS detector. In this study both kinetic and isotherm studies were performed, revealing that the pollutants adjusted better to pseudo-second order and Freundlich models, leading to the conclusion that the main force driving sorption were hydrophobic interactions. Another publication by Liu et al. [45] evaluated the sorption behavior of 17β-estradiol (an estrogen) on environmental MPs (polyamide —PA—, polycarbonate —PC—, polymethylmetacrilate —PMMA—, PVC HDPE, LDPE, and LLDPE) in three different matrices (Milli-Q water, seawater, and artificial seawater) using also ultra-high-performance LC (UHPLC) with a MS/MS detector. Researchers concluded that hydrophobic partition was the main mechanism dominating sorption. Other examples are the works of Puckowski et al. [46], or Wu et al. [47] which evaluated the sorption behavior of 9 pharmaceuticals (i.e., 5-fluorouracil, ciprofloxacin, enrofloxacin, fenbendazole, flubendazole, methotrexate, nadolol, norfloxacin, and propranolol) in PP, LDPE, HDPE, and PVC (seawater and distilled water as matrices) and 5 bisphenols (i.e., bisphenol A, bisphenol S, bisphenol F, bisphenol B, and bisphenol AF) analogues in PVC (deionized water), respectively. Both studies evaluated sorption kinetics and isotherms using HPLC-DAD and HPLC-MS systems, respectively, for the determination of the analytes, and highlighted that the

Table 1. Examples of sorption studies on microplastics in different types of matrices.

Microplastic polymer, shape, and size	Analytes	Matrix	Determination technique and column	Kinetic/isotherm models studied	Comments	Reference
PP, PS, LDPE, and HDPE Pellets 2-5 mm	Levonorgestrel	Milli-Q and seawater	HPLC-UV C_{18} (100 mm × 4.6 mm, 3.5 µm)	Kinetics: PFO, PSO, InPD Isotherms: Langmuir, Freundlich, Temkin, Linear, Dubinin-Radushkevich, Redlich-Peterson	Sorption was driven by electrostatic and hydrophobic interactions.	[48]
PE and PS - 0.250-0.260 mm	2 PCPs (N,N-diethyl-meta-toluamide, and triclosan), 5 pharmaceuticals (carbamazepine, caffeine, diclofenac, ibuprofen, torasemide), and 8 pesticides (atrazine, diazinon, carbendazim, 2-methyl-4-chlorophenoxyacetic acid, mecoprop, tebuconazole, terbutryn, propiconazole), 1 flame retardant (tris(2-chloroisopropyl)-phosphate), 1 PAH (phenanthrene), 1 bisphenol (4-nonylphenol), and 1 corrosion inhibitor (benzotriazole)	Ultrapure water	GC-MS Phenyl arylene (30 m × 0.025 mm × 0.25 µm)	Kinetics: - Isotherms: Linear*	*Other models were studied but not specified	[49]
PE - 0.250-0.280 mm	2 PCPs (4-methylbenzylidene camphor, and triclosan), 1 pharmaceutical (carbamazepine) and 1 hormone (17α-ethinylestradiol)	Aqueous solution*	HPLC-DAD C_{18} (75 mm × 4.6 mm, 2.6 µm)	Kinetics: Studied but not specified Isotherms: Linear	*Containing $CaCl_2$ (0.01 mol/L)	[50]
PE Pellets -	3 pharmaceuticals (propranolol, sertraline, and sulfamethoxazole)	Ultrapure water	UHPLC-MS/MS C_{18} (100 mm × 2.1 mm, 1.7 µm)	Kinetics: PFO, and PSO Isotherms: Linear and Freundlich	Desorption experiments were carried out.	[51]
PE, PS, and PVC - 0.015-0.250 mm	2 PFASs (perfluorooctanesulfonate, and perfluorooctanesulfonamide)	Aqueous solution	UHPLC-MS/MS C_{18} (50 mm × 2.1 mm, 1.7 µm)	Kinetics: Studied but not specified Isotherms: Linear	Hydrophobic interaction was the main sorption mechanism.	[52]

DAD: diode array detector; GC: gas chromatography; HDPE: high-density polyethylene; HPLC: high-performance liquid chromatography; InPD: intra-particle diffusion; LDPE: low-density polyethylene; MS/MS: tandem mass spectrometry; PAH: polycyclic aromatic hydrocarbon; PCP: personal care product; PE: polyethylene; PFAS: perfluoroalkyl substance; PFO: pseudo-first- order; PP: polypropylene; PS: polystyrene; PSO: pseudo-second-order; PVC: polyvinyl chloride; UHPLC: ultra-high-performance liquid chromatography; UV: ultraviolet.

sorption mechanisms were influenced by electrostatic forces and hydrophobic interactions in both cases and, distinctly, ionic strength and noncovalent bonds, respectively. In another publication by Jiménez-Skrzypek et al. [48] the sorption behavior of levonorgestrel was evaluated in four types of MPs (PP, polystyrene —PS—, LDPE, and HDPE) in two different matrices (Milli-Q water and seawater) using a HPLC-UV system for the determination of the analyte. Overall, researchers carried out both kinetic and isotherm studies observing clear changes in the sorption mechanisms between the two matrices and the different types of polymers. Figure 2 shows the kinetic models plot obtained for levonorgestrel in the different MPs in seawater. As indicated by the researchers, three different kinetic models were evaluated, observing that each type of polymer showed different sorption behavior, with PP showing a best fit to intra-particle diffusion model (determination coefficient –R^2– of 0.928), LDPE to pseudo-second order model (R^2 of 0.988), and PS and HDPE to the pseudo-first order model (R^2 of 0.960 and 0.963, respectively).

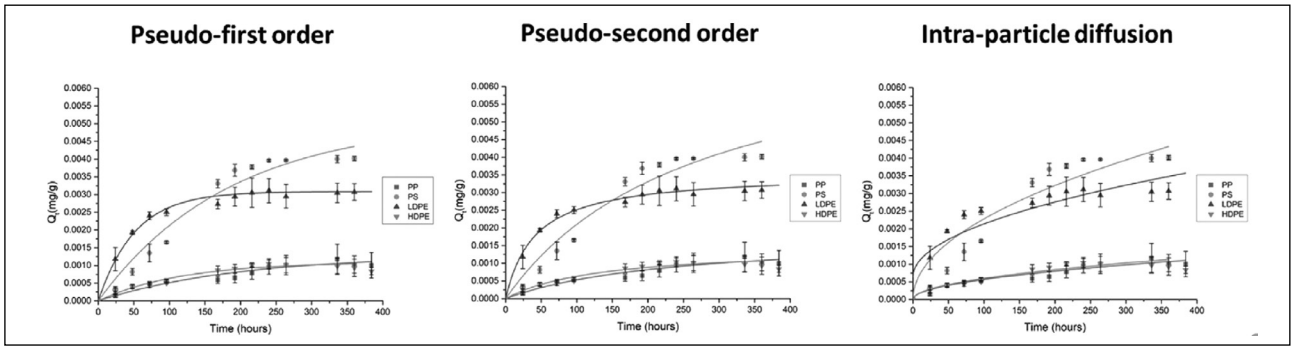

Figure 2. Kinetic models plot for levonorgestrel sorption in MPs in a seawater matrix. Reprinted from Jiménez-Skrzypek et al. [48] with the permission of Elsevier.

3. DETERMINATION OF POLLUTANTS IN ENVIRONMENTAL MICROPLASTICS

As a result of the sorption behavior previously commented, from an analytical point of view, MPs can be considered as analytical matrices, and as such, they can be analyzed to determine the contaminants retained from their environment. In this sense, the treatment that MPs receive as samples does not differ too much from those used for other matrices. Nevertheless, due to the novelty of the field, more advances still need to be made. On the one hand, more efficient extraction techniques need to be developed and applied, as there are many publications that use traditional techniques (e.g., Soxhlet extraction, etc.) in which Green Analytical Chemistry Principles are widely disregarded [23, 35]. On the other hand, from the various articles found in the literature, higher diversity in the extraction techniques needs to be implemented, since most of these publications rely on techniques like ultrasound-assisted extraction (UAE) or accelerated solvent extraction (ASE) with solvents that can potentially dissolve a good amount of the plastics [21, 53]. For this reason, the use of Hildebrand and Hansen solubility parameters [53] becomes expedient to assess the compatibility of the polymers with the solvents used. Moreover, a distinct prevalence of POPs, such as PAHs (mainly high-priority PAHs designated by the United States Environmental Protection Agency), PCBs and organochlorinated pesticides, is evident in most publications. In contrast, other categories of pollutants, like emerging organic contaminants, have received limited attention up to this date [21]. Table 2 compiles different publications on the realm of environmental MPs analysis.

Due to the widespread analysis of POPs, it is not strange that GC has been the most common technique used in this type of studies, although LC has also been employed [23, 35]. In terms of detection systems, MS has been widely utilized, followed by ECD, or UV, DAD, FD and MS/MS in LC analysis [21, 35]. Concerning the composition of MPs, the most commonly studied polymer types include PE, PS, and PP (the ones mostly produced nowadays and, therefore, the ones more present in the environment), with additional polymers like PA, PVC, polyurethane (PU), polyethylene terephthalate (PET), among others, having also been analyzed in different publications [21, 35]. Regarding the shape of MPs, pellets have been a focal point in numerous publications, although others like fragments, fibers, or foams have also been assessed. It is important to note that determining which MP shapes are best suited for contaminant analysis remains unclear, given the vast variability in the shapes, sizes, and porosity of MPs found in the environment, as well as the altering composition and sorption behavior caused by weathering, which highlights the importance of evaluating all these parameters during the optimization of the extraction methods [21]. Finally, focusing on the environmental compartment in which these MPs were collected from, the majority of the analyzed samples have consisted of marine MPs, particularly those collected from beach sand and seawater, nonetheless, those from river water, coastal sediments, among others have also been examined [21]. As an example, Pannetier et al. [61] determined the presence of various pollutants including 26 pesticides, 19 PAHs and 13 PCBs in PP, PS, PE, and ethylene vinyl acetate (EVA) MPs (collected from beach samples). The extraction method employed an initial grinding step (600 µm) and was followed by an initial extraction of the pollutants from the MPs (100 mg) using dimethyl sulfoxide (1 mL) and shaking (16 hours). After that, the analytes were ex-

Table 2. Examples of some works dealing with the determination of contaminants sorbed onto environmental microplastics.

Microplastic polymer, size, and amount	Collection site	Analytes	Extraction technique	Determination technique	LODs and LOQs	Comments	Reference
PE, PP, PS and PVC 1-5 mm 4 g	Beach sand	PAHs, OCPs, and PCBs	UAE (15 mL of DCM:hexane, 1:9 v/v, 15 min)	GC-MS	LODs: - LOQs: 0.3-0.5 ng/g	Fractionation with alumina column was required. Pollutants were found at concentrations between 1.9-3814 µg/kg	[54]
- - 0.3 g	Beach sand	Pharmaceuticals	UAE (7.5 mL of MeOH, 10 min)	UHPLC-MS/MS	LODs: 0.25-15.8 µg/kg LOQs: 1.20-49.5 µg/kg	Pollutants were found at concentrations between 34.0-111 µg/kg	[55]
- 0.25-5 mm -	Beach sand	PAHs, OCPs, and PCBs	Soxhlet (acetone:DCM 1:1 v/v, 24 h)	PAHs and PCBs: GC-MS OCPs: GC-ECD	LODs: 0.05-1.14 µg/kg LOQs: -	Fractionation with silica gel column was required. Pollutants were found at concentrations between 1.96-1509 µg/kg	[56]
- 0.25-1 mm 1.0 g	Beach sand	PAHs and OCPs	UAE (2x10 mL of hexane, 30 min)	OCPs: GC-ECD PAHs: GC-MS	LODs: - LOQs: -	Fractionation with alumina and silica gel column was required. Pollutants were found at concentrations between 1.6-2051.2 µg/kg	[57]
PE, PP, PS and PET - 0.1 g	Seawater	PAHs and PCBs	Stirring (2x10 mL of DCM:heptane 1:1 v/v, 24 hours)	PCBs: GC-MS PAHs: UHPLC-FD	LODs: - LOQs: 0.08-6.5 µg/kg	Pollutants were found at concentrations between 0.13-$1.56 \cdot 10^4$ µg/kg	[58]
PE and PP 2-6 mm 1.0 g	Beach sand	PFASs	UAE (10 mL of MeOH, 1 hour)	HPLC-MS/MS	LODs: 0.3-150 ng/kg LOQs: 1.1-500 ng/kg	Pollutants were found at concentrations between 0.01-0.18 µg/kg	[59]
- - 0.3 g	Beach sand	UV-stabilizers and UV-filters	UAE (3 mL of MeOH, 30 min)	UHPLC-MS/MS	LODs: 0.01-0.69 µg/kg LOQs: 0.02-2.29 µg/kg	Pollutants were found at concentrations between 1-4031 µg/kg	[60]

DCM: dichloromethane; ECD: electron capture detector; FD: fluorescence detector; GC: gas chromatography; HPLC: high performance liquid chromatography; LOD: limit of detection; LOQ: limit of quantification; MeOH: methanol; MS: mass spectrometry ; MS/MS: tandem mass spectrometry; OCP: organochlorine pesticide; PAH: polycyclic aromatic hydrocarbon; PCB: polychlorinated biphenyl; PE: polyethylene; PET: polyethylene terephthalate; PFAS: perfluoroalkyl substance; PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; UHPLC: ultrahigh performance liquid chromatography; UAE: ultrasound assisted extraction; UV: ultraviolet

tracted and preconcentrated using a stir-bar sorptive extraction (SBSE) approach, the analytes were determined through thermal desorption combined with a GC-MS/MS system. Other examples are the works of Llorca et al. [59] in which PFASs were determined in MPs pellets (PP, and PP; collected from beach samples)

through UAE with MeOH (10 mL; 1 hour) combined with a UHPLC-MS/MS system for the determination of the analytes, or Chen et al. [57] that determined the presence of PAHs in MPs (PE, PP, PS, PA and PVC) using a combination of vortex (60 seconds) and UAE (15 min) using hexane (2 × 1 mL).

4. THE USE OF CHROMATOGRAPHY IN MICROPLASTICS IDENTIFICATION AND QUANTIFICATION

Even though conventional spectroscopic techniques, like Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy (including their microscopy versions), are still very popular for the identification of MPs, the introduction of thermal analysis techniques like pyrolysis-GC-MS (Pyr-GC-MS) are gaining attraction, mainly as a result of the fact that mass quantification can be developed. This approach thermally decomposes the macromolecules that constitute the MPs (generally under low pressure under an inert atmosphere, using a quartz tube to hold the samples and heating them at temperatures between 500-1400 °C) into molecules with lower molecular weight that can be chromatographically separated in a GC column and properly determined through MS [13, 62]. Furthermore, the use of Pyr-GC-MS allows the simultaneous determination of the contaminants present in a MP, also the additives of the plastic particles, as well as providing the composition of the MP polymer, allowing its identification [63].

Nevertheless, it is important to take into consideration that thermal decomposition techniques have some inherent limitations. On the one hand, these techniques are destructive [62], and they do not offer insights into particle attributes (shape, size, color, etc.). Furthermore, Pyr-GC-MS is also constrained by the diameter of thermal desorption tubes (generally, 1.5 mm), and the size of the MPs themselves, which must be manually handled and placed in the pyrolysis tube (typically, the weight of the MPs must be higher than 100 mg or their size larger than 100 µm) [64, 65]. Additionally, the simultaneous determination of various MPs is a challenge, making it necessary to carry out a sequential analysis of individual MPs, increasing the time needed to complete the analysis [66]. Also, Pyr-GC-MS may not perfectly distinguish between specific plastic subtypes, such as PS, cross-linked PS, and expanded PS or HDPE and LDPE [65]. In addition to these challenges, it is essential to consider factors that can result in poor reproducibility, including sample inhomogeneity, matrix effect, slow transfer of the pyrolyzate to the chromatographic column, and the complexity of interpreting pyrograms [62]. On the other hand, from a technical point of view, various complications may arise when dealing with compounds of high molecular weights [67]. For this reason, regular maintenance becomes of capital importance, as there is the risk of condensation of fractions from the degradation of heavier polymers with high boiling points or high molecular weight (greater than 300 °C or 400 g/mol, respectively) in the capillary between the pyrolysis chamber and the GC, which can lead to several issues (cross-contamination, blockages, etc.) [68]. As an example of the application of this technique, Fries et al. [64] used Pyr-GC-MS for the simultaneous determination of MPs composition and organic plastic additives (MPs were collected from marine sediments). Overall, additives were desorbed at 350 °C in a thermal desorption tube, that had been previously conditioned at 40 °C for 60 min and after obtaining this first chromatogram, pyrolysis was carried out at 700 °C for 60 seconds to identify the MP polymer. Another work by Krauskopf et al. [69] developed an off-line Pyr-GC-MS method for the determination of MPs (PP, PVC, PE, PS, and PET) collected from river sediments. The proposed method proved to be highly effective for quantifying PP and PS, while PE showed slightly lower specificity and reproducibility. However, PVC and PET analyses presented inconsistencies, requiring additional analyses to ensure result reliability. Other example is the work of Käppler et al. [66] in which the composition of MPs was successfully determined. In the first case, particles and fibers compositions (PS, PVC, PET, PE, PP, polyvinyl acetate —PVAc—, EVA, polytetrafluoroethylene —PTFE— and alkyd resin; collected from river sediments) were determined using a microfurnace-Pyr-GC-MS technique, heating at 590 °C for 60 seconds. Additionally, researchers were also able to determine 8 additives present in the samples. Fischer et al. [70] also applied the same technique (microfurnace-Pyr-GC-MS; 590 °C) for the determination of the composition of the MPs (PE, PP, PS, PET, PVC, PMMA, PC, PA-6 and methyl-dimethyl-diisocyanate-PU; collected from seawater, sea salt, and muddy sediment). Finally, in the work of Gregoris et al. [71] an early exploration into the direct use of Pyr-GC-MS for identifying polymers present in atmospheric aerosols is developed. As indicated by the researchers, the results obtained show the effectiveness and viability of the method, but a more in-depth investigation is essential to comprehensively assess polymer content in atmospheric aerosols. Figure 3

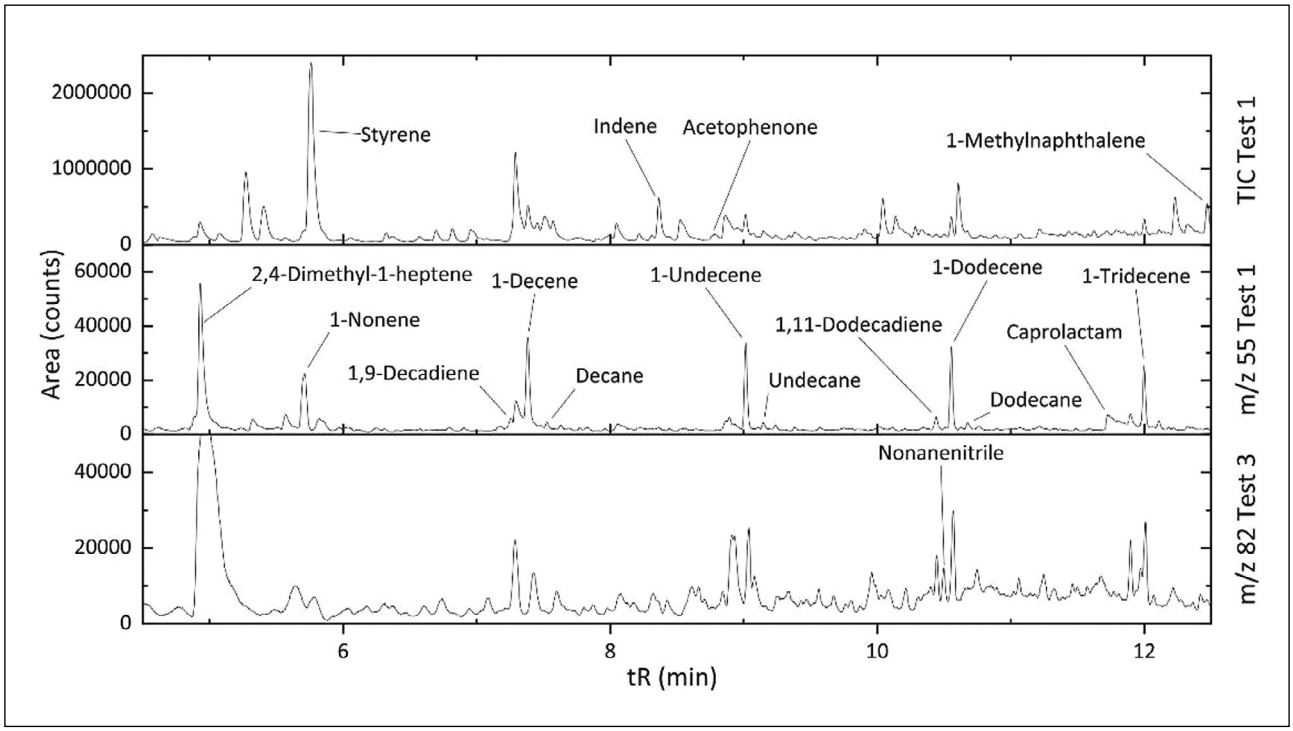

Figure 3. Pyrogram obtained for a house dust standard reference material (SRM2585; National Institute of Standards and Technology). Reprinted from Gregoris et al. [71] with the permission of Elsevier.

shows a pyrogram obtained for a house dust standard reference material studied in this publication.

More recently, some publications have applied novel approaches for the determination of the composition of MPs through LC coupled to MS/MS detectors [42]. The process involves sample extraction, purification, and depolymerization before injection into the LC system. Initial steps include extracting and purifying MPs with solvents, followed by depolymerization to break down the MPs into monomers, dimers, and oligomers, and finally, identification and quantification of these smaller molecules through MS/MS [41, 42]. Despite the novelty, the characteristics of this approach still require further improvements, as there are several issues regarding negative effects on the MS source, as incomplete degradation of substances generated during depolymerization have the potential contaminating the ionization source, leading to potential damage to the device [42]. Moreover, akin to Py-GC-MS, this technique is intrinsically destructive as it mandates the depolymerization of macromolecules prior to analysis [41]. Consequently, it does not provide information regarding the morphotype of the MPs (size, color, and shape). As an example, in the work of Wang et al. [72] LC-MS/MS was successfully applied for the determination of PC and PET MPs. Overall, researchers employed an alkali-assisted thermal hydrolysis to depolymerize the MPs (pentanol or butanol system), and after injection, the MPs were quantified using the concentrations of the depolymerized building block of the polymer. The proposed method was applied to samples from different complex matrices (marine sediments, sea salt and rock salt, indoor dust, digestive residues in mussel and clam, and sludge), and observed that in the digestive residues of a clam, concentrations of 63.7 mg/kg for PC and 127 mg/kg for PET were present. Another publication by Peng et al. [73] developed a method for the determination of PA-6 and PA-66 in a wide range of samples (marine sediment, freshwater sediment, fishery sediment, fish guts and gills, sludge, and indoor dust) using an acidic-thermal depolymerization approach with sulfuric acid. MPs were extensively identified in various matrices (indoor dust, sludge, marine sediment, freshwater sediment, fishery sediment, and fish guts and gills), exhibiting concentrations ranging from 0.725 to 321 mg/kg. Furthermore, elevated levels of PA-66 were observed in indoor dust and fish guts and gills, highlighting the clear potential for human exposure through both dust ingestion and dietary pathways.

It is also worth mentioning that other studies have also implemented the use TGA coupled to MS detectors (TGA-MS). In this process, gaseous degradation products are directly introduced into the MS detector without undergoing chromatographic separation through GC [40]. As an example, David et al. [74] developed a method for the analysis of PET MPs in soils via TGA-MS. As highlighted by the authors, TGA-MS provides a cost-effective approach with reduced sample preparation requirements. Additionally, the technique flexibility allows various heating rates and sample sizes (up to 1000 mg), addressing sample heterogeneity commonly found in soils. This cost-effectiveness and adaptability make TGA-MS a valuable supplementary tool, complementing established analytical methods [74]. In other works, the capability of this technique to differentiate different types of polymers (PP, PE, PS and PVC) in suspended matter was also proven [75, 76]; although the differentiation of PP and PE required model calculations [40]. Overall, the successful application of these technique opens new important approaches for the determination of MPs in environmental samples, in combination with the already well-established methods.

Aside from the previously mentioned techniques, there are additional MS-based methods, such as single particle-inductively coupled plasma MS, atmospheric solids analysis probe MS, or isotope ratio MS, which show potential in this field, although they are currently in a developmental stage [42].

5. CONCLUSIONS

The degradation of plastics in the environment results in the formation of small particles known as MPs. For a long time, the environmental problems surrounding MPs have been neglected, but in recent years, there has been an increase in the interest of the general public and the scientific community in this topic. These particles are not only a pollutant by themselves; due to their nature, these polymeric matrices possess properties that favor the sorption of a wide array of organic pollutants. Chromatographic techniques are powerful tools that are used for the determination of a wide range of pollutants sorbed onto MPs and are extremely useful in studies involving MPs. Since contaminants with different physicochemical properties have been studied in MPs, it is not strange to observe that both GC and LC techniques are commonly used. Nevertheless, when it comes to sorption studies, LC approaches have been more common in the scientific literature, while in environmental MPs analysis GC has been the preferred option. The advancements in thermal analysis techniques like Pyr-GC-MS systems are extremely valuable, as they allow for the determination of the additives present in the MPs, as well as elucidating the type of polymer. Although up to this date the number of research articles with respect to MP-retained pollutants is limited, it is to be expected that more publications will be presented in recent years, providing new valuable information. Furthermore, new approaches employing LC-MS/MS systems for the determination of MPs after a complete depolymerization, have started to be successfully applied, nevertheless, the number of publications in this line of research is limited, with few available examples in the scientific literature.

Conflict of interest

Authors declare no conflict of interest.

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XXII REUNIÓN CIENTÍFICA DE LA SECyTA (51.ª REUNIÓN CIENTÍFICA DEL GCTA)

Entre los días 16 y 18 del pasado mes de octubre se celebró la XXII Reunión Científica de la Sociedad Española de Cromatografía y Técnicas Afines (SECyTA) en S'Arenal de Palma (Mallorca). La reunión fue organizada y presidida por Joan Grimalt (IDAEA.CSIC) y Manuel Miró (Universidad de las Islas Baleares) y contó con el apoyo de otros miembros de la propia Universidad, del IDAEA-CSIC y de la Junta de SECyTA, así como de la SEEM (Sociedad Española de Espectrometría de Masas).

La reunión tuvo como preámbulo el *Curso avanzado de espectrometría de masas: movilidad iónica – espectrometría de masas para el análisis medioambiental y alimentario* que se desarrolló el domingo 15 de octubre, a cargo de los profesores Encarnación Moyano, de la Universidad de Barcelona, y Juan Vicente Sancho, de la Universidad Jaume I de Castellón.

Los temas cubiertos por esta reunión científica abarcaron: 1) el desarrollo de los fundamentos teóricos en la separación en columna; 2) las técnicas acopladas y ómicas; 3) la miniaturización y automatización; 4) imaging; 5) los métodos verdes de separación; 6) la quimiometría, y 7) análisis aplicado a medio ambiente, toxicología, ciencia forense, alimentos y nutrición.

El amplio y denso programa científico contó con cinco atractivas conferencias plenarias a cargo de científicos de prestigio internacional. El primer día comenzó con la presentada por el **Prof. Lars Wörmer** (MARUM – Center of Marine Environmental Sciences, University of Bremen, Alemania) con el título *Reading the fine print: mass spectrometry imaging of molecular fossils in geological simples*. Por la tarde, el **Prof. Philippe Schmitt-Kopplin** (Technical University of Munich, Alemania) presentó la conferencia titulada *High resolution tailored metabolomics in the food-nutrition-health continuum.* El segundo día, pudimos escuchar la exposición del **Prof. Rafael Lucena** (Universidad de Córdoba, España) que versó sobre *Biopolymer-based sorptive phases into stainless steel needles: microextraction and ambient mass spectrometry analysis in a single device*. El último día, contamos con dos conferencias plenarias. La primera corrió a cargo del Prof. **Prof. Pavel Kubáň** (Czech Academy of Science, Chequia) con el título *Capillary electrophoresis as an all-in-one tool for the fully autonomous pretreatment and analysis of dried blood spots*; la segunda se tituló *Target and suspect analysis of contaminants of emerging concern in water reuse practices: challenges and future perspectives*, impartida por la **Prof. Ana Agüera** (Universidad de Almería, España), con la que concluyó el tercer y último día de la reunión.

Aparte de las 5 excelentes conferencias plenarias, en esta Reunión se presentaron 28 comunicaciones orales, 76 comunicaciones en formato de póster, así como 13 comunicaciones orales presentadas por jóvenes investigadores.

Igualmente, hay que destacar la exposición comercial en la que las empresas más destacadas en instrumentación analítica pudieron atender e interactuar con los asistentes e igualmente participaron con presentaciones orales sobre instrumentación.

Como viene siendo habitual, en la tarde del segundo día del congreso, se celebró la Asamblea General de la SECyTA, que se desarrolló con la exposición de los diferentes informes de la presidenta, el secretario y el tesorero. También se recordó a los socios que pueden enviar a los editores del boletín artículos científicos, así como información sobre congresos a los que han asistido, resúmenes de tesis presentadas, homenajes, premios, etc. Además, se hizo pública la sede de la próxima reunión de SECyTA 2024, que se celebrará en Pamplona y se dio paso a Elena González Peñas, miembro de la Junta de la Junta de la Sociedad y profesora de la Universidad de Navarra, quien encabezará su organización.

La Cena de Gala tuvo lugar tras la Asamblea General en el Hotel Playa de Palma Palace, justo al lado del hotel sede de la Reunión, con muy buen ambiente y una cena copiosa que animó a los asistentes.

Durante la ceremonia de Clausura y como es habitual, se llevó a cabo la entrega de la edición XVII de los premios "José Antonio García Domínguez" a las dos mejores comunicaciones orales y en formato de póster de jóvenes investigadores, premios patrocinados por la empresa Bruker. De igual forma, también se celebró la entrega de la 2.ª edición del Premio a la Mejor Tesis Doctoral SECyTA para aquellas tesis presentadas a lo largo del año 2022 y cuyos ganadores se conocieron

el 6 de octubre a través de correo desde la secretaría, así como de la web y las redes sociales. Solo resta agradecer a los organizadores su gran trabajo y dedicación, así como reseñar el interés despertado y la gran calidad de las presentaciones que conformaron el programa científico de esta reunión.

El Comité Editorial de *CTA*

XVIII EDICIÓN DE LOS PREMIOS "JOSÉ ANTONIO GARCÍA DOMÍNGUEZ"

Como en ocasiones anteriores, dentro de la XXII Reunión Científica de la SECyTA (51.ª Reunión Científica del GCTA) celebrada en Mallorca del 16 al 18 de octubre de 2023, se concedieron los premios *José Antonio García Domínguez* en su XVIII edición. Estos premios están patrocinados por la empresa Bruker y se otorgan a las dos mejores comunicaciones orales y las dos mejores en formato de póster de las presentadas por jóvenes investigadores. Una vez se reunieron los jurados encargados de fallar los premios en sus respectivas modalidades, unánimemente se tomaron los acuerdos con las siguientes concesiones:

1.er Premio a la mejor comunicación oral (800 €)

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

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

Acknowledgements

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2.º Premio a la mejor comunicación oral (600 €)

APPLICATION OF HOMEMADE SILICA-BASED ZWITTERIONIC ION-EXCHANGE MATERIALS FOR THE EXTRACTION OF PHARMACEUTICALS FROM ENVIRONMENTAL WATER SIMPLES

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The use of sorptive techniques is the preferred way to extract analytes from liquid samples, being the solid-phase extraction the common technique. The development of new materials that can be applied to solid-phase extraction is one important research area [1]. One problem is the extraction of ionic and ionizable compounds, there are commercial solutions that allow the extraction of cationic or anionic analytes (Oasis MCX, Strata X-AW…), however, the simultaneous extraction of both type of analytes is not possible with commercial sorbents. In this sense, in the present study, three homemade silica-based zwitterionic ion-exchange materials were synthesized through sol-gel reactions. After the functionalization, the sorbents had quaternary amines and sulfonic groups, allowing them to perform strong anion and cation-exchange interactions.

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

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

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

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1.[er] Premio al mejor póster (400 €)

DEVELOPMENT OF A HPLC-DAD-TOF MS METHODOLOGY FOR THE AUTHENTICATION OF DAMIANA (*TURNERA DIFFUSSA*) EXTRACTS

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Damiana (*Turnera diffussa*) is an endemic shrub from Mexico, that has been historically used in traditional herbal medicine throughout the world, mainly as a sexual stimulant or aphrodisiac, being one of the most used herbs in this type of formulations [1]. Moreover, it has been also described as a traditional remedy against stomachache, lung diseases related to tobacco abuse, bladder and kidney infections, etc. [2] and it is

now widely consumed as food supplement (FS). FS for sexual enhancement are some of the most adulterated at present using drugs intended for the treatment of erectile dysfunction [such as phosphodiesterase-5 (PDE-5) inhibitors] [3]. However, the development of analytical methods to detect such adulterations in damiana formulations has hardly been addressed. Therefore, in this work, a new analytical method to detect these potential frauds has been proposed.

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

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

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2.º Premio al mejor póster (300 €)

OCCURRENCE AND SPATIAL DISTRIBUTION OF PHARMACEUTICALS IN MEDITERRANEAN INTERMITTENT RIVER BASINS

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

five countries was investigated. The studied river basins from France and Algeria reported lowest concentrations and statistical analysis were performed to study the potential differences per CECs class between countries, and the most remarkable class were the industrial compounds where significant differences between Tunisia and the other four countries were observed. Regarding specific contaminants, caffeine concentrations were outstanding in Tunisia compared to the rest of countries, and Italy presented remarkable concentrations of antihypertensives, with ibersartan and valsartan acid concentrations being statistically different from the rest of the studied countries.

Acknowledgements

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ACTA DE LA 23.ª ASAMBLEA GENERAL DE LA SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES (SECyTA)

La 23.ª Asamblea General de la SECyTA, que contó con la asistencia de 72 socios, se celebró el día 17 de octubre de 2023, a las 18:30 h, en el Hipotels Convention Center (Avinguda de Fra Joan Llabrés, 20, S'Arenal, Mallorca) con el siguiente orden del día:

1. Lectura y aprobación, si procede, del acta de la reunión anterior.
2. Informe de la Presidenta.
3. Informe del Secretario.
4. Informe del Tesorero.
5. Ruegos y preguntas.

Desarrollo de la sesión y acuerdos adoptados

En primer lugar, la Presidenta da la bienvenida a todos los asistentes a la 23.ª Asamblea General de la SECyTA y expresa su más sincero agradecimiento a los miembros de los Comités Científico y sobre todo Organizador de la XXII Reunión Científica de la SECyTA por el excelente trabajo realizado. Además, la Presidenta también comenta a los socios la satisfacción de poder celebrar en esta edición en Mallorca los 51 años de la constitución del Grupo de Cromatografía y Técnicas Afines, precursor de la SECyTA. Asimismo, tendremos el honor y el privilegio de tener entre nosotros a miembros destacados que formaron parte de esta Sociedad, como el Dr. Xavier Guardino y la Dra. María Teresa Galcerán, que nos acompañaran posteriormente en la cena de Gala del congreso.

1. Lectura y aprobación del acta de la Reunión anterior

La presidenta indica a los asistentes que el borrador del Acta de la 22.ª Asamblea General de la SECyTA celebrada el año pasado en Almería se puso a disposición de todos los socios para que pudiera ser consultada en la web de la SECyTA. En este momento la Presidenta pregunta si alguno de los asistentes quiere hacer alguna modificación al Acta o si algún socio quiere que se lea el Acta en su totalidad. Al no haber ninguna intervención por parte de los socios presentes, se procede a aprobar el Acta de la 22.ª Asamblea General de la SECyTA.

2. Informe de la Presidenta

En su informe, la Presidenta trató los siguientes temas.

2.1. *Celebración de la 22nd Scientific Meeting of Spanish Society of Chromatography and Related Techniques – S'Arenal 2023*

La Presidenta da las gracias a los Dres. Joan Grimalt y Manuel Miró, como Chair y Co-Chair de la *XXII Scientific Meeting of Spanish Society of Chromatography and Related Techniques*, a los miembros de su grupo de investigación y a todos los integrantes del Comité Organizador y Científico de esta reunión científica por el excelente trabajo realizado para poder ofrecer un programa científico de calidad a la altura de las expectativas de los

socios, todo ello complementado con un programa social con el que ya estamos disfrutando de la localidad de S'Arenal de la ciudad de Palma de Mallorca.

En esta edición, se han recibido un total de 122 comunicaciones, distribuidas en 28 orales ordinarias, 13 orales de jóvenes investigadores, que optan al premio José Antonio García Domínguez, y 76 pósteres, de los cuales 17 han sido seleccionados para su presentación en formato flash, además de 5 conferencias plenarias. El número total de inscritos en esta edición (alrededor de 134 congresistas) es ligeramente inferior al alcanzado en reuniones anteriores (Almería, 180 inscritos; Granada 2018, 190 inscritos; Sevilla 2016, 180 inscritos; reunión conjunta SECyTA-SEEM, celebrada en Castellón en 2015, 185 inscritos). En este sentido, la Presidenta indica que seguimos manteniendo un nivel de participación similar al conseguido en ediciones anteriores.

Respecto a las becas de inscripción y ayuda de viaje concedidas para facilitar la asistencia de los jóvenes investigadores a esta edición de la SECyTA-2023 se han concedido 27 becas de inscripción (250 €) y 27 ayudas de viaje (175 €), por un importe total de 11.475 €.

La Presidenta también agradece el importante apoyo de las casas comerciales para poder realizar esta Reunión Científica. Agradece a Leco, Agilent y Bruker su participación como sponsor gold, Jasco y Thermo como silver, y Air Liquide, Carburos Metálicos y Waters como bronze. No obstante, el número de casas comerciales ha disminuido sensiblemente respecto a la reunión de Almería debido a que algunas casas comerciales importantes no han participado en la Reunión.

Como en años anteriores, se ha gestionado con la revista *Journal of Chromatography A* la creación de un volumen especial virtual dedicado a recoger los trabajos presentados en este congreso denominado como "SECyTA2023.Mallorca", para dar la posibilidad a los socios la presentación de sus comunicaciones en formato de artículo. Los trabajos presentados deberán seguir el proceso habitual de revisión por pares para su aceptación. La Presidenta recuerda que la fecha límite para el envío de trabajos es el 31 de marzo de 2024. Todos los asistentes a este congreso han recibido por email las instrucciones para el envío de los trabajos y estas instrucciones están también disponibles en la web de la reunión. Si hubiese alguna extensión de la fecha límite para el envío de los artículos se comunicaría a los socios y se actualizaría también en la web de la reunión.

2.2. *Cierre económico de la XXI Reunión SECyTA2022*

La Presidenta informa que una vez finalizada la XXI Reunión Científica de la SECyTA, celebrada en Almería del 25 al 27 de octubre de 2022, se ha recibido por parte de los organizadores de la Reunión, el balance económico final.

Respecto al balance económico, cabe señalar que ha sido positivo con un valor neto de 13.713,81 €. En todo caso el tesorero nos podrá dar información más detallada del cierre económico del congreso. La Presidenta agradece a la Dra. Ana Agüera y a la Dra. Patricia Bolaños y a todo el Comité Organizador de SECyTA2022 el trabajo que hicieron y el éxito de la reunión, tanto desde el punto de visto social, científico y ahora también económico.

La Presidenta aprovecha para informar que el volumen especial virtual de la reunión SECyTA 2022, ya se puede consultar en la web de la revista *Journal of Chromatography A*, en el apartado de "Article collections". El número total de artículos aceptados hasta el momento es de 3, un número que sigue siendo bajo. Desde la Junta queremos seguir impulsando la publicación en esta revista científica de los trabajos presentados en nuestras reuniones. Creemos que mantener la relación con la revista *Journal of Chromatography A* supone un prestigio para nuestra sociedad y sirve para dar a conocer la investigación que se realiza en nuestro país.

2.3. *Segunda edición Premios SECyTA a Tesis Doctorales*

La Presidenta informa que en la Reunión del año pasado inauguramos los premios a las mejores Tesis Doctorales leídas por nuestros socios jóvenes en el año anterior. La Presidenta comenta que propone eliminar lo de "mejor" Tesis Doctoral, ya que le consta que según el Jurado han sido todas muy buenas. El Jurado ha estado constituido por el Dr. Javier Santos que ha actuado como presidente, la Dra. Begoña Jiménez Luque que ha sido la secretaria, la Dra. Ana Agüera y el Dr. Rafael Lucena como vocales. El Jurado no tenía trabajos ni publicaciones en colaboración con los socios que presentaban sus Tesis Doctorales. En esta segunda edición, finalmente, se han presentados siete Tesis leídas en 2022. El fallo de los Premios ya se anunció a los socios y están las actas del Jurado en la página web y se entregarán los Premios durante la ceremonia de clausura de SECyTA2023. La Presidenta lamenta el retraso en comunicar el fallo, por lo que los

premiados no podrán asistir a la misma, pero recogerán el premio sus directores de Tesis.

La Presidenta anima a los socios jóvenes a que participen en la próxima edición que, con las sugerencias del jurado y el bagaje adquirido en esta segunda edición, permitirán que los premiados puedan asistir, sobre todo si se encuentran de estancia post-doctoral en el extranjero.

La Presidenta felicita a los premiados de las tesis presentadas en 2022: la Dra. Leticia Lacalle Bergeron y el accésit es para el Dr. Adrián Gutiérrez Serpa.

La Presidenta también informa que se ha actualizado la web de la SECyTA para recoger estos nuevos premios a Tesis Doctorales, además de los Premios José Antonio García Domínguez. Aprovecha para dar las gracias al Dr. Mario Fernández por su colaboración en la actualización de la web, así como de las redes sociales, Facebook, Twitter y recientemente Instagram.

2.4. *Boletín especial 50 aniversario*

La Presidenta comenta que en el volumen 43, número 2 del Boletín especial del 50 aniversario, la Dra. María Teresa Galcerán presenta un artículo muy interesante de la creación de la SECyTA desde el GCTA (Del GCTA a la SECyTA: una visión personal). Asimismo, el Dr. Emilio Gelpí presenta un artículo sobre los métodos pioneros de cromatografía de gases en columnas capilares (Algunas reminiscencias de tiempos pasados), y anima a los socios a que lean estos artículos que son historia viva de la Cromatografía en España.

La Presidenta también fel icita a los editores del Boletín, M.ª Luz Sanz, Ana Cristina Soria, Ana Isabel Ruiz y Mario Fernández, por el trabajo realizado para la edición de los Boletines del 50 aniversario.

2.5. *Participación de SECyTA en Farmaforum junto otras sociedades*

La Presidenta indica que, a propuesta de D. Bernabé Bodas, de Sciex, se había ofrecido a SECyTA la participación junto con otras sociedades en un evento conjunto, en principio con el espíritu de las antiguas JAI, dentro de Farmaforum, una reunión que se ha llevado a cabo en IFEMA Madrid del 20 al 21 de septiembre de 2023, dentro del sector farmacéutico, cosmético y nutracéutico…

Han participado distintas sociedades (SECyTA, SEQA, SEEM, SESMet, SEProt), además de D. Bernabé Bodas, de Sciex. Estuvieron presidentes, vicepresidentes, secretarios de distintas sociedades. Se realizó una jornada de los diferentes grupos de las distintas sociedades que presentaron sus líneas de investigación de interés para el mundo farmacéutico (10 minutos).

La Presidenta presenta a modo de resumen, algunos aspectos positivos, como favorecer *networking*, dar a conocer el trabajo de los miembros de la Sociedad para establecer colaboraciones, etc. Al final, se convirtió en pasar una jornada con colegas de distintas sociedades. Quizás haber realizado una reunión compartida, pero en un entorno distinto, hubiera sido más provechosa.

Respecto a aspectos negativos, las salas eran abiertas, sin techo y con paneles, por lo tanto, había mucho ruido del exterior y los asistentes necesitaron el uso de auriculares para escuchar a los conferenciantes. Con este formato no se produjo la interacción esperada con la empresa y quizás se tendría que reformular el formato. Los presidentes de las distintas sociedades han decidido valorar a nivel de Juntas de Gobierno la experiencia de Innovaforum, recogiendo también la opinión de los socios, para evaluar si esta experiencia se repite el próximo año en el mismo foro o por el contrario se intenta llevar a cabo algún tipo de reunión cada tres años de las Sociedades, pero en otro entorno, donde haya ese contacto entre las Sociedades, que era positivo pero por diferentes motivos se fue disolviendo.

2.6. *Organización de Reunión Científica Internacional por parte de SECyTA*

La Presidenta comenta que el año pasado se planteó a nivel de Asamblea la iniciativa de que la SECyTA volviera a organizar un Congreso Internacional, como ya se hizo en 2010 con el ISC en Valencia y en 2019 con el Iberian Meeting en Santiago de Compostela.

La Presidenta comenta que la SECyTA es una entidad con capacidad para organizar un Congreso Internacional y entre los congresos que nos podrían interesar como sociedad de cromatografía serían el ISC y el HPLC. Por lo que respecta al ISC la primera fecha libre sería el 2028, mientras que para el HPLC sería en 2029, que coincidiría con el 30 aniversario del HPLC99 que también se organizó en Granada por el Dr. Gelpí.

Respecto al ISC, la Presidenta comenta que ya contactó con el Dr. Michael Lammerhöffer, miembro del comité permanente de ISC y que le pareció una

buena idea. El Dr. Lammerhöffer también está en el comité organizador de HPLC2023, y nos indicó que quizás mejor empezar por ISC, que tiene un tamaño medio, en lugar del HPLC, que es un congreso de más de 1.000 asistentes. Por lo tanto, en la Asamblea de la SECyTA celebrada en Almería en 2022, se decidió optar a la candidatura de ISC2028.

La Presidenta asistió a HPLC2023 en Düsseldorf (Alemania) y presentó la candidatura a ISC2028 por parte de SECyTA para realizarlo en Granada. Se presentó la Sociedad, los eventos organizados, los contactos internacionales de sus miembros, etc., así como también la sede del Congreso, la ciudad de Granada. Se presentó a los miembros del Comité permanente de ISC en junio durante HPLC2023 y hasta septiembre no se notificó que se había ganado la candidatura, aunque tampoco conocemos si había otros candidatos.

Las próximas ediciones de ISC serán en 2024 Liverpool y en 2026 en Praga, mientras que para 2028 tendremos ISC en Granada, con SECyTA2028 dentro de ISC, como ya se hizo en 2010 en Valencia. Todavía no hay fechas decididas, se discutirá en Junta de Gobierno, pero septiembre-octubre sería adecuado para ISC. El congreso se celebraría en el Palacio de Congresos de Granada, como en SECyTA2018. Es una sede relativamente económica, cerca del centro, con hoteles próximos.

2.7. *Premios y reconocimientos a socios*

La Presidenta comenta que distintos socios de la SECyTA han recibido distintos premios:

El Dr. José Carlos Díez-Masa recibió la Medalla de Honor de la SECyTA durante la Cena de Gala de la XXI Reunión Científica de la SECyTA en reconocimiento a su brillante trayectoria profesional, a su contribución al desarrollo de las técnicas de separación en nuestro país y a su implicación en nuestra Sociedad.

La Dra. Rosa Ventura Alemany ha sido recientemente distinguida como mejor dirigente por la Asociación Catalana de Dirigentes del Deporte (ACDE). La distinción reconoce su labor al frente del Laboratorio Antidopaje de Catalunya. Desde SECyTA queremos felicitarla muy sinceramente por su éxito y su consolidada trayectoria científica.

Finalmente, la Presidenta lamenta comunicar el fallecimiento de nuestro socio José Luis Bernal Yagüe, Profesor Emérito de la Universidad de Valladolid.

2.8. *Organización de la próxima Reunión Científica SECyTA 2024*

La Presidenta comenta que aunque se presentará con más detalle durante la ceremonia de Clausura, ya tenemos sede para la Reunión de 2024. Las organizadoras serán las Dras. Elena González-Peñas y Elena Lizárraga y se celebrará probablemente en Pamplona en septiembre-octubre de 2024.

2.9. *Propuesta organización de la próxima Reunión Científica SECyTA 2025*

La Presidenta comenta que ha recibido una propuesta por parte de la Dra. Rosa Maria Marcé de realizar la reunión Científica de 2025 dentro de Euroanalysis, que se celebrará en Barcelona del 31 de agosto al 4 de septiembre de 2025. La Dra. Marcé forma parte del comité organizador de esta reunión y plantea esta posibilidad a la Junta, y la traslado a la Asamblea.

La Presidenta, antes de abrir el debate a los socios, presenta diferentes puntos, tanto positivos como negativos. Entre los positivos, tendríamos una sede para el 2025, tendríamos la reunión en un foro internacional. Entre los negativos, el punto más difícil, cómo articular nuestra reunión dentro de Euroanalysis, un congreso más genérico de Química Analítica, y no solo de cromatografía, como será en el caso de ISC2028. Otro punto a discutir, es cómo se realizaría la gestión económica entre las Sociedades participantes.

La Presidenta cede la palabra a la Dra. Núria Fontanals, compañera de la Dra. Marcé, por si quiere añadir algo más sobre este tema. La Dra. Fontanals comenta que no se pide un compromiso ni obligación a la SECyTA, sólo que valore si podría articular la reunión de la SECyTA dentro de este foro de Euroanalysis.

El Dr. Jordi Díaz toma la palabra y comenta que quizás se podría articular de un modo parecido a como se realizó la reunión de 2013 en Tenerife, que se realizó a continuación del ITP, con un pequeño solapamiento de parte social y científica. Aunque en este caso, ITP también es un congreso dedicado a técnicas separativas (electroforesis).

La Presidenta recuerda que en Granada en 2018 también se solapó una sesión con la Reunión de la GRASEQA, aunque esto implica realizar inscripciones separadas.

La Dra. Marta Lores toma la palabra y pregunta si la duración de Euroanalysis son cinco días. Porque en

ese caso será difícil que se solapen los congresos ya que no iríamos a un formato de 5 + 2. La Reunión debería ser dentro de los cinco días de Euroanalysis, con los problemas que ello conlleva. Una posible solución sería tener sesiones comunes y sesiones en paralelo.

La Presidenta comenta que la Junta evaluará las distintas opciones e informará a los socios, pertinentemente.

Finalmente, la Presidenta informa que el punto de Ruegos y Preguntas al final de la asamblea, se realizarán unas presentaciones de SECyTA 2024, SEEM2025 y la Escuela Europea de Metabolómica, por sus respectivos organizadores.

3. Informe de la Secretaría

En su informe, la Secretaría trató los siguientes temas.

3.1. *Socios de la SECyTA*

El Secretario de la SECyTA, Dr. Juan V Sancho, informa que, desde la última Asamblea General celebrada el 26 de octubre de 2022 en Almería, hasta hoy, 17 de octubre de 2023, se han recibido un total de 32 altas y 28 bajas. Tras cuatro ediciones (2012-2015) con balances negativos en número de socios, y dos ediciones (2016-2017) con balances positivos, se revierte el cambio de tendencia observado de ligeras bajadas en las ediciones anteriores (2018-2021), y en esta edición (2023) se consolidan los balances netos positivos, aunque de solo +4 socios. En el listado actual de Secretaría el número de socios a día de la celebración de esta Asamblea es de 519 socios. El Secretario exhorta a los socios a que se animen a promocionar la Sociedad entre sus colegas, amigos, compañeros de departamento, estudiantes, etc., para hacer más grande, aun si cabe, la Sociedad Española de Cromatografía y Técnicas Afines.

De las 28 bajas, 11 corresponden a bajas estatutarias (debido a que el socio en cuestión lleva tres impagos consecutivos) y 5 corresponden a jubilaciones de socios. El resto de bajas (12) engloban las bajas anuales habituales, típicamente por cambios profesionales donde las técnicas cromatográficas ya no son tan importantes.

Al respecto de las bajas, el Secretario recuerda, aunque supone que también lo hará el Tesorero, que el socio que desea darse de baja, lo comunique a Secretaría o Tesorería a principio de año (enero o febrero) para que ya no se le pasen las cuotas y no genere gastos innecesarios por devolución de recibos. Además, como se puede observar prácticamente el 50% de las bajas son por tres impagos, con los consiguientes gastos innecesarios de gestión. El Secretario comenta que quizás sería hora de modificar esta baja estatutaria, pero el Tesorero replica que no es fácil modificar los estatutos.

3.2. *Ayudas concedidas por la SECyTA*

Se han concedido un total de 13 ayudas (de 500 € cada una) para la asistencia a congresos internacionales, se nota la disminución del impacto de la pandemia, distribuidos de la siguiente forma:

- 33rd SETAC Europe Annual Meeting, April 30th – May 4th, 2023. Dublin, Irlanda (5 ayudas).
- 25th International Symposium on Advances in Extraction Technologies (ExTech2023), July 18th-21st Tenerife, España (2 ayudas).
- 29th International Symposium on Electro and Liquid Phase Separation Techniques (ITP2023), September 13rd-17th 2023. Rome-San Felice Circeo, Italia (2 ayudas).
- 41st International Conference on Environmental and Food Monitoring (ISEAC41), November 20th-24th 2023. Amsterdam, Países Bajos (2 ayudas).
- 9th International Conference on Food Chemistry and Technology (FCT2023), November 27th – 29th 2023. Paris, Francia (2 ayudas).

En este año la inversión en ayudas para que nuestros socios jóvenes puedan asistir a eventos internacionales se ha incrementado notablemente (de 5 ayudas en 2022 a 13 ayudas en 2023) al ir desapareciendo paulatinamente las restricciones por la pandemia.

Se han concedido un total de 6 ayudas (de 250 € cada una) para la asistencia a congresos patrocinados por nuestra Sociedad, distribuidos de la siguiente forma:

- 51st International Symposium on High Performance Liquid Phase Separations and related Techniques (HPLC2023), June 18th-22nd 2023. Düsseldorf, Alemania (2 ayudas).
- 25th International Symposium on Advances in Extraction Technologies (ExTech2023), July 18th-21st Tenerife, España (4 ayudas).

Por lo tanto, en este año la inversión en ayudas para que nuestros socios jóvenes puedan asistir a eventos patrocinados también se ha incrementado

notablemente, aunque no se ha vuelto a niveles de 2017. En cualquier caso, la política de becas debería revisarse en función de la disponibilidad presupuestaria de la Sociedad.

En el caso de la presente Reunión, la Sociedad ha concedido un total de 27 becas de inscripción a la XXII Reunión Científica de la SECyTA (que han supuesto un total de 6.750 €) y 27 ayudas de viaje (4.725 €) a jóvenes investigadores socios de la SECyTA que se desplazan desde fuera de Mallorca. De nuevo, se pone de manifiesto el esfuerzo de la Sociedad para que sus socios estudiantes puedan asistir y difundir los resultados de sus investigaciones, ya que a nivel global teniendo en cuentas todas las ayudas concedidas, ha supuesto una inversión en 2023 de 19.475 €, ligeramente inferior a la del año pasado (23.220 €), quizás por efecto del 50 aniversario del GCTA.

3.3. *Comunicación electrónica con los socios*

El Secretario informa que desde la dirección de correo secretaria@secyta.es los socios han recibido a lo largo de este año 2022 hasta 16 diferentes envíos de comunicación por parte de Secretaria con novedades, cursos, congresos, ofertas de trabajos, etc. Se recuerda a los socios que sigan utilizando la dirección secretaria.secyta@gmail.com para altas, preguntas, cuestiones, etc.

3.4. *Número especial del Journal of Chromatography A*

Como ya ha mencionado la Presidenta, desde Secretaría también se recuerda a los asistentes que se pueden enviar los trabajos presentados a la Reunión actual como artículos a publicar en un Volumen Virtual Especial de la revista *Journal of Chromatography A*, como se ha venido haciendo en los años anteriores. En esta ocasión los editores serán Joan Grimalt y Manuel Miró. Las instrucciones para el envío de los artículos, así como la fecha límite (31 de marzo de 2023) están indicados en la página web del congreso. Se anima a los socios a que envíen los trabajos a publicar ya que los números de los últimos dos años ha sido relativamente bajo (3 publicaciones) para el número de comunicaciones presentadas a la Reunión, aunque la edición 2019 (prepandemia) se había producido un repunte (11 publicaciones) que debemos intentar consolidar.

4. Informe del Tesorero

En el informe del Tesorero se trataron los siguientes asuntos.

El Tesorero de la SECyTA, Dr. Jordi Díaz Ferrero, presenta el estado de cuentas y el balance de ingresos y gastos desde la pasada Asamblea General celebrada en Almería el 26 de octubre de 2022, pero del período del 01-07-2022 al 30-06-2023, ya que como se comentó en la pasada Asamblea, las cuentas se han de aprobar por la misma según los Estatutos hasta el 30 de junio, fecha anterior a la Asamblea, ya que el ejercicio económico se cierra en esa fecha. Así mismo, se debe presentar un balance de pérdidas y ganancias. Sin embargo, el Tesorero también presenta un balance de entradas y salidas de caja como el que ha venido presentando en las últimas asambleas. Se presenta también el balance económico de situación del ejercicio 2022/2023 que ha preparado el asesor fiscal, siendo éste positivo.

Al final de su intervención, el Tesorero aborda algunos temas generales, y en primer lugar comenta que a fecha actual todo el dinero de la Sociedad está en cuenta corriente y que como sociedad científica no deberíamos contratar fondos de inversión y correr riesgos con el dinero de nuestros socios. Por otra parte, el Tesorero comenta los esfuerzos por minimizar las comisiones bancarias, y en estos momentos estamos bien ya que hay una persona física con la cual negociar y relacionarse.

Respecto al tema de los impagados, este año ha habido unos 9, lo que nos genera gastos bancarios. Por lo tanto, el Tesorero recuerda a los socios que si en lugar de no pagar la cuota correspondiente al año en curso se avisase a la Sociedad de la baja correspondiente antes de pasar al cobro de las cuotas (habitualmente a final del primer trimestre), se haría un favor a la Sociedad.

La Presidenta indica a los socios que han solicitado las ayudas que, para no alargar la Asamblea, se acerquen al lugar de la sala donde se encuentran el Secretario y el Tesorero para recibir el cheque bancario correspondiente, toda vez que ya han finalizado sus respectivos informes.

5. Ruegos y preguntas

En este punto, como ya indicó anteriormente la Presidenta, se realizarán unas breves presentaciones de los próximos eventos de interés para los socios.

En primer lugar, las Dras. Elena González-Peñas y Elena Lizárraga presentan SECyTA2024 en Pamplona, que tendrá lugar en la Universidad. Se visionan dos

vídeos sobre la ciudad de Pamplona, y sobre la Universidad, sede de la Reunión. Las organizadoras invitan a todos los socios a asistir a SECyTA2024 y comunicarán las fechas cuando estén disponibles, pero seguro que no serán del 6 al 14 de julio.

En segundo lugar, el Dr. Esteban Abad y la Dra. Encarna Moyano presentan SEEM2024 donde se celebrará el 25.º aniversario de la Sociedad Española de Espectrometría de Masas (SEEM), que se quiere compartir con todas las Sociedades hermanas, entre ellas la SECyTA. El evento será un encuentro de ciencia, de celebración y de compartir las experiencias de los últimos años. La Dra. Moyano respecto a la fecha de creación de la SEEM, recuerda que en 1998 en la red temática de Masas de la Generalitat de Catalunya se propuso la creación de una Junta gestora de una Sociedad de Masas que pudiera organizar el congreso internacional IMSC en el año 2000 en Barcelona. Por lo tanto, la Junta de la SEEM optó por el 1999 como punto medio para definir la creación de la misma y celebrar el 25 aniversario en 2024, también en Barcelona, siendo la sede el Paraninfo de la Universidad de Barcelona del 26 al 28 de junio de 2024, ubicado en la Plaza Universitat, en pleno centro de Barcelona. También se celebrará el típico curso pre-congreso en la Facultat de Química. Se está preparando el programa, pero se promete una reunión memorable, y se invita a los socios de SECyTA a que participen de la misma.

En tercer lugar, Luca Narduzzi de la Universidad de Granada y miembro del comité organizador de la European School of Metabolomics 2024 nos presenta esta escuela interesante para los socios jóvenes trabajando en el campo de la metabolómica. La escuela tendrá lugar en Granada del 22 al 26 de abril de 2024 y constará de formación, *workshops* así como actividades culturales y de *team-building*.

A continuación, se abre el turno de ruegos y preguntas aunque en primer lugar la Presidenta cede la palabra al Dr. Mario Fernández, uno de los editores del Boletín de la SECyTA para que informe a los socios sobre el mismo.

En nombre de los editores del Boletín, el Dr. Mario Fernández recuerda a los socios que el Boletín es la revista de la Sociedad y que tenemos que sentirla como propia y estar orgullosos de publicar en ella y de poder contribuir con nuestros trabajos. El Dr. Fernández recuerda que hay secciones que no se incluyen en algunos números porque no hay aportaciones de los socios, como la sección de reseñas bibliográficas. Además, también recuerda a los socios jóvenes que redactar un artículo para el Boletín también es un trabajo formativo, y que no hay ningún problema en publicarlo en inglés, si les resulta más sencillo. También recuerda a los socios jóvenes la sección de resúmenes de Tesis Doctorales defendidas por los socios, que empezó muy fuerte cuando se lanzó pero que en los últimos números ha descendido, y seguro que no es porque los socios no presenten Tesis Doctorales, como ya hemos visto en la participación del Premio SECyTA a Tesis Doctorales. Así que anima a los socios presentes que envíen sus resúmenes al Boletín cuando defiendan sus Tesis, un o dos meses antes de junio o diciembre para que aparezcan en el Boletín correspondiente.

La Presidenta da las gracias a todos los editores del Boletín, Mario Fernández, Mari Luz Sanz, Ana Cristina Soria y Ana Isabel Ruiz por todo el trabajo excelente que hacen para producir unos Boletines tan interesantes para los socios.

La Dra. Mercedes de Frutos pide la palabra para dar las gracias a Manuel Miró, pero también se queja por el poco tiempo que han estado los pósteres expuestos. Hay que pensar en formatos alternativos para hacer más atractivas las sesiones de pósteres.

El Dr. Joan Grimalt recuerda que hay que traer el *xiulell* a la Cena del Congreso.

En este punto del orden del día y a la vista de que no hay más ruegos ni preguntas ni más asuntos que tratar, la Presidenta da por finalizada la 23.ª Asamblea General de la SECyTA a las 20:00 horas del citado día, de todo lo cual doy fe como Secretario y firmo la presente con el VºBº de la Presidenta.

S'Arenal, 17 de octubre de 2023

Fdo.:
Juan Vicente Sancho Llopis
Secretario de la SECyTA

VºBº:
Ana María García Campaña
Presidenta de la SECyTA

PREMIO SECyTA A LA MEJOR TESIS DOCTORAL EN CROMATOGRAFÍA Y TÉCNICAS AFINES

2.ª edición del Premio SECyTA a la mejor Tesis Doctoral en Cromatografía y Técnicas Afines

El pasado 21 de marzo de 2023, la Junta de la SECyTA aprobó la convocatoria de la 2.ª edición de los Premios SECyTA a la mejor Tesis Doctoral en Cromatografía y Técnicas Afines, información que fue puesta en conocimiento de todos los socios mediante correo electrónico, web y redes sociales el día 1 de mayo, así como en el boletín de la Sociedad (*CTA* **44**_1, 21).

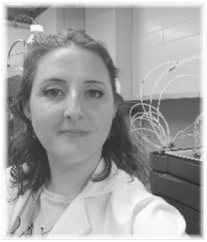
Una vez llevado a cabo todo el proceso descrito en las bases, el jurado creado para tal efecto emitió su veredicto el día 6 de octubre de este año, en el que se "el elevado carácter innovador y calidad científica de todas las tesis doctorales presentadas a esta edición de los Premios". El veredicto final fue el siguiente:

Convocatoria año 2022

Premio SECyTA a Tesis doctorales leídas en 2022 concedida a la Tesis titulada *Mass Spectrometry based Untargeted Metabolomics in Food and Health Area*, defendida por la **Dra. María Leticia Lacalle Bergeron**, dirigida por los Dres. Juan V. Sancho Llopis y Tania Portolés Nicolau, y presentada en la Universidad Jaume I, en 2022.

Asimismo, teniendo en cuenta la elevada calidad científica y la aportación innovadora en Cromatografía y las técnicas afines del resto de Tesis Doctorales presentadas, se acuerda conceder el *accésit* que prevé la convocatoria de esta 2.ª edición en su punto 1 a la Tesis doctoral titulada *Advances in Microextraction Techniques using Novel Materials*, defendida por el **Dr. Adrián Gutiérrez Serpa**, dirigida por las Dras. Ana I. Jiménez Abizanda y Verónica Pino Estévez, y presentada en la Universidad de La Laguna, en 2022.

Resumen tesis Premio Mejor Tesis SECyTA 2.ª edición del Premio, convocatoria de 2022



Mass spectrometry based untargeted metabolomics in food and health area

Autora: **Leticia Lacalle Bergeron**

Directores: Dr. Juan Vicente Sancho Llopis y Dra. Tania Portolés Nicolau

Grupo de investigación: Química Analítica en Salud Pública y Medioambiente (Q-AMS), Instituto Universitario de Plaguicidas y Aguas (IUPA), Departamento de Química Física y Analítica, Universitat Jaume I de Castellón. Fecha de la defensa: 12 de mayo de 2022.

En esta Tesis Doctoral se ha evaluado la aportación de la metabolómica no dirigida en combinación con técnicas analíticas avanzadas basadas en el acoplamiento de separaciones cromatográficas con espectrometría de masas tanto de baja (MS) como de alta resolución (HRMS) en distintos estudios relacionados con el campo de la alimentación, la salud y la comunicación química intra-especie. Además, se evaluaron las ventajas que aportan los instrumentos de última generación basados en separación por movilidad iónica en combinación con espectrometría de masas de alta resolución (IMS-HRMS) en la elucidación de compuestos desconocidos.

Por un lado, se hace una revisión bibliográfica extensa del estado del arte de las técnicas estrategias más empleadas en cada etapa del flujo de trabajo de la metabolómica no dirigida. Por otro lado, se aplicó y evaluó la aproximación de la metabolómica no dirigida con distintas técnicas analíticas avanzadas en diferentes campos del conocimiento.

En el ámbito de la alimentación, se realizó un estudio cuyo objetivo era encontrar marcadores volátiles que confirmaran la diferencia entre productos pesqueros ahumados con diversas técnicas de procesado y que culminó satisfactoriamente en un modelo de clasificación. Por otra parte, se estudiaron las ventajas que la nueva combinación IMS-HRMS podía aportar para el descubrimiento de nuevos biomarcadores dietético, concretamente para la identificación de marcadores de ingesta de naranja a corto y medio plazo. Por último, se estudió el efecto de compuestos bioactivos como el resveratrol y el pterostilbeno en la esteatosis hepática. Además, se evaluaron las distintas herramientas para facilitar la identificación de biomarcadores gracias a la implementación de instrumentos IMS-HRMS.

Por otra parte, se aplicó la metabolómica no dirigida para el descubrimiento de compuestos relacionados con la comunicación intra-especie, un campo de estudio hasta ahora poco abordado con esta metodología hasta el momento. Concretamente para la identificación de compuestos emitidos por crías de ratón que indujeran a una activación del instinto maternal. Se estudió por separado los compuestos de naturaleza más volátil y aquellos de carácter menos volátil, adaptando las técnicas de análisis a cada una de ellas.

Resumen tesis Accésit Mejor Tesis SECyTA 2.ª Edición del Premio, convocatoria de 2022

Advances in Microextraction Techniques using Novel Materials

Autor: **Adrián Gutiérrez Serpa**

Directores: Dras. Ana I. Jiménez Abizanda y Verónica Pino Estévez

Grupo de investigación: Materials for Chemical Analysis ULL (MAT4LL), Departamento de Química Analítica, Universidad de La Laguna.

Fecha de la defensa: 31 de marzo de 2022.

Las tendencias actuales en la preparación de muestras analíticas se centran en el desarrollo de nuevas técnicas de extracción miniaturizadas para realizar análisis más rápidos, sencillos y sostenibles. A pesar de todos los avances logrados durante las últimas décadas en cuanto a instrumentación analítica cada vez más sensible, los esfuerzos en la etapa de preparación de muestra son todavía necesarios, ya que es imprescindible desarrollar procedimientos medioambientalmente limpios que permitan determinar analitos traza en muestras complejas minimizando el tiempo de análisis y los procesos tediosos.

El desarrollo de estas técnicas de microextracción ha ido de la mano de la incorporación de nuevos materiales como fases extractantes en sustitución de los convencionales. Entre los diferentes materiales novedosos, cabe destacar la utilización de nanopartículas metálicas (NPs) y las redes metal-orgánicas (MOFs), como materiales adsorbentes de gran interés en la preparación de muestras analíticas dada su alta relación superficie/volumen (para NPs metálicas) o área superficial (para MOFs), estando estas propiedades directamente relacionadas con su impresionante capacidad de adsorción.

En esta Tesis Doctoral se incorporaron sorbentes metálicos basados en NPs y MOFs como materiales extractantes unidos a una variedad de soportes/dispositivos en diferentes técnicas de microextracción basadas en sorbentes: microextracción en fase sólida dispersiva (μ-dSPE), microextracción en fase sólida en fibra (SPME), SPME in-tube y microextracción de película delgada (TFME).

Con respecto a las NP metálicas, se han preparado NPs de oro y plata AgNP siguiendo un procedimiento bottom-up utilizando agentes de reducción alternativos en condiciones suaves. Estos adsorbentes se sintetizaron, caracterizaron e incorporaron como nuevos revestimientos de SPME en fibra. Además, se desarrolló un novedoso soporte trenzado-SPME como alternativa a los soportes convencionales de SPME. El desempeño de estas fibras se comparó con las fibras comerciales de SPME disponibles, observándose una mayor afinidad de los analitos hacia los recubrimientos basados en NPs.

Con respecto a los MOF, se (i) usaron como revestimiento sobre la superficie de las microesferas de sílice formando microesferas de tipo core-shell para usarse en μ-dSPE. Además, los MOFs se han usado adheridos a las paredes internas de capilares de sílice fundida para usarse como dispositivos SPME *in-tube*. Finalmente, los MOFs han sido incorporados en un polímero para la preparación de membranas de matriz mixta (MMM) basadas en MOF para su uso en TFME. La incorporación de MOF en estos diferentes formatos y técnicas de microextracción permitió lograr una alta capacidad de adsorción y extracción para la determinación de analitos a niveles de trazas en muestras complejas.

Los sorbentes, dispositivos y métodos de microextracción propuestos, se combinaron con técnicas cromatográficas, siendo los métodos resultantes optimizados, validados y aplicados al análisis de muestras reales, demostrando su adecuado desempeño analítico. Además, se realizó una evaluación verde de los métodos propuestos para discutir las ventajas y debilidades de los métodos de microextracción basados en los sorbentes desarrollados en esta Tesis Doctoral.

PREMIOS A SOCIOS

Premio MOTIVEM en la categoría de Ciencias

El grupo NUTRIMAT, integrado por los estudiantes Manel Feltrer, Lara Lis, Roser Payà, Pau Peiró y Luis Alberto Toca, bajo la coordinación del profesor Héctor Martínez Pérez-Cejuela, miembro de la SECyTA y reciente doctor en el grupo de investigación CLECEM adscrito al Departamento de Química Analítica de la Universitat de València (UV), recibió el primer premio en la décima edición del concurso MOTIVEM en la rama de ciencias. Esta competición es promovida por la Universitat de València en colaboración con ADEIT y dirigida por el Vicerrectorado de Innovación y Transferencia. Los premios cuentan con el respaldo de la Generalitat Valenciana y reciben el apoyo de la Fundación La Caixa a través de CaixaBank.

El programa tiene como objetivo fomentar la colaboración entre docentes y estudiantes para desarrollar proyectos innovadores relacionados con los Objetivos de Desarrollo Sostenible (ODS). El proyecto presentado por el grupo NUTRIMAT se enfoca en crear filtros biodegradables a partir de residuos de paja de arroz, provenientes de los campos de arroz cercanos al parque Natural de la Albufera. La celulosa, modificada por hidrólisis básica, se modificaba con aminos cuaternarios con capacidad de retener el exceso de fertilizantes presentes en las aguas (fosfatos y nitratos), con el propósito de abordar la eutrofización observada en la laguna del Parque Natural. La propuesta impulsa un modelo de economía circular, ya que los filtros saturados pueden ser desechados en los campos de cultivo para reutilizar el exceso de nutrientes capturados por ellos.

La entrega de premios tuvo lugar el 6 de julio de 2023 en la sede de la fundación ADEIT de la Universitat de València.

https://www.adeituv.es/noticias-entrega-premios-motivem-2023/

NUEVOS SOCIOS

2065
Muñoz Bustamante, Carolina
Instituto de Diagnóstico Ambiental y Estudios del Agua (IDAEA)-CSIC
Jordi Girona, 18-26. 08034 Barcelona

2068
Galindo Luján, Rocío del Pilar
Antoni Botey, 11. 08917 Badalona (Barcelona)

2069
Lucena Rodríguez, Rafael
Cantueso, 38. 14012 Córdoba

2070
Miró Lladó, Manuel
"Departamento de Química Universidad de las Illes Balears"
Carretera de Valldemossa, Km 7.5
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2071
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CONGRESOS CELEBRADOS

41st International Conference on Environmental & Food Monitoring

The "International Association of Environmental Analytical Chemistry" (IAEAC) aims to promote and maintain scientific excellence in the field of environmental analytical chemistry, focusing on the proper application of methodologies to assess substances significant for both humanity and the environment. IAEAC initiatives are designed to facilitate the exchange of the latest research findings in environmental matters and provide training in advanced analytical technologies.

After a period of absence related to the pandemic, the International Conference on Environmental & Food Monitoring was held at Novotel Amsterdam City in Amsterdam, Netherlands, for 5 days, from November 20 to 24. The first day involved conference registration with a warm welcome to all participants, providing an opportunity for networking and preparing materials for their contributions, such as posters, displayed in the lounge area.

On Tuesday, November 21, the meeting began with an opening ceremony by Marja Lamoree from Vrije Universiteit and Hans Mol from Wageningen University, both partner universities of the conference. There were seven morning plenary sessions on suspect and non-target screening, with Merixell Gross from the Catalan Institute for Water Research delivering a notable lecture on the identification of emerging organic contaminants in greywater emitted from ships using a comprehensive LC-HRMS target and suspect screening approach. After lunch, there were four more plenary sessions on the same theme, featuring Manuel Garcia Jaramillo from Oregon State University discussing the use of LCMS analysis and suspect and non-target screening to assess the efficiency of Hybrid Electrodialysis-Forward Osmosis (ED-FO) in water reuse. Shorter parallel sessions of 15 minutes were organized in the afternoon, including four sessions on food fraud and authenticity and five sessions on chemicals, metabolites, and transformation products. The day concluded with another plenary talk by Jean-Philippe Antignac from the French National Institute of Agronomic Research for a Coherent and sustainable development of agriculture, food, and the environment, on the partnership for the assessment of risks from chemicals. Commercial booths from companies like Restek, Bruker, Shimadzu, and Waters, among others, were available during breaks, along with posters from many attendees categorized by topics. It is worth noting significant presence of posters focused on per-and polyfluoroalkyl substances and on the analysis of non-specific environmental pollutants, such as wastewater and urines.

On Wednesday, November 22, the day started with plenary sessions by Beate Escher from the Helmholtz-Centre for Environmental Research on Chemical cocktails threaten the environment and human health and Bart Koelmans from Wageningen University on Advances in microplastic exposure, fate, effects and risks. Subsequently, there were six plenary sessions on risk assessment and modeling, with a noteworthy presentation by Kishore Kumar Jagadeesan from the University of Bath on a cutting-edge R-based tool for enhanced environmental risk assessment of active pharmaceutical ingredients in wastewater under the PERK initiative. After lunch, Sciex and CTC Analytics hosted two sales sessions on advances in MS technology for food analysis. This was followed by two parallel sessions, one on environmental contaminants and residues in food and the other on the analysis of micro/nanoplastics. In the former, Silvia Borrull from Rovira i Virgili University and SECyTA member presented her work on the occurrence of high-volume production chemicals in highly consumed seafood species and the evaluation of dietary intake and risk characterization.

On Thursday, November 23, the conference began with plenary sessions by Charlotta Turner from Lund University on Advancing green and sustainable analytical chemistry for Environmental and Food Analysis and Gaud Dervilly from Oniris, INRAE, Laberca, on strategies for characterizing the consumer's chemical exposome. After breakfast, nine plenary sessions were held, six on effect-directed analysis and in vitro assays for mixture evaluation, and three on analysis and sampling. Notable was the presentation by Maria Margalef Jornet from Vrije Universiteit Amsterdam on effect-directed analysis in the environment-food-human continuum to identify chemicals with Transthyretin binding properties. Later, two parallel sessions were organized, one on materials in contact with food and the other on toxic chemicals, metabolites, and trans-

formation products. In the latter theme, Reyes García from Universitat Rovira i Virgili and SECyTA member delivered a presentation on passive sampling of high production volume chemicals and polycyclic aromatic hydrocarbons in outdoor air samples. Application and risk assessment evaluation. At the end of the day, a dinner was held on an exclusive boat from Rederij 't Smidtje Canal Cruises, providing a unique experience of dining and cruising through the canals of Amsterdam for 3 hours, despite the cold weather.

On the final day of the congress (Friday, November 24), seven plenary sessions were held on sampling strategies and *in situ* detection. Marleen Voorhuijzen from Wageningen University and Research led one of these sessions, generating much anticipation due to her controversial title, "combatting cannibalism", which discussed the early detection of processed animal proteins in poultry feed. After the presentations, a small awards ceremony was held to recognize the best oral presentations and posters by selected novice researchers, sponsored by Analytical and Bioanalytical Chemistry and IAEAC.

The winners of the Roland W. Frei Award, presented by ISEAC for the best oral presentations, were Ids Benjamin Lemmink from Wageningen University and Research for "Towards rapid *in situ* detection of atropine in cereals" in the field of food, and Jaimy de Schepper from the Free University of Amsterdam for "The contribution of PFAS to the disruption of thyroid hormone activity in Dutch waters: A comparison between two in vitro bioassays with chemical analysis" in the field of the environment.

The winners of the Best Poster Award, presented by Analytical and Bioanalytical Chemistry, were Sven-Oliver Herter from the Bundesanstalt für Materialforschung und -prüfung for "Synthesis and application of isotopically labelled reference standards for the mass spectrometric quantification of ergot alkaloids in foodstuff" in the food category, and Denice Van Herweden from the University of Amsterdam for "Modular open-access and open-source Julia HRMS toolbox" in the environmental category.

In addition, participants in ISEAC-41 will have the opportunity to submit their work for publication in a special issue of *The International Journal of Environmental Analytical Chemistry* (IJEAC). Finally, the location of the next congress in Aveiro, Portugal, from June 9 to 13, 2025, was announced. Participation in this congress was a pleasant experience where a high representation of Spanish universities was personally observed. Despite the weather, enjoyable days were spent in the capital of the Netherlands.

Silvia Borrull Riera
Reyes García Garcinuño
Química Analítica I Química Orgánica,
Facultad de Química
Universitat Rovira I Virgili

25th International Symposium on Advances in Extraction Technologies (ExTech). (ExTech – Tenerife 2023)

The Pre-symposium Course "Solid-phase microextraction. Insights, present and future" with a total of 8.5 hours, held on July 18th, 2023, was given at Arona, Tenerife, Canary Islands, Spain. It consisted of 4 different sessions: The 1st session "SPME fundamentals and applications" was given by Prof. Janusz Pawliszyn (University of Waterloo, Canada) in which different theoretical approaches of SPME were introduced, followed by applications of SPME. The 2nd session, "Direct SPME-MS", was given by Wei Zhou, PhD (University of Waterloo, Canada) regarding the coupling of SPME with direct MS. The 3rd session, "Strategies of using microextraction in clinical and biomedical research", was given by Prof. Barbara Bojko (Nicolaus Copernicus University, Poland). Finally, the 4th session, "On-site extraction", was given by Prof. Rafael Lucena (University of Córdoba, Spain).

After the pre-symposium course, the opening ceremony took place followed by the opening plenary lecture entitled "Think big but design small and efficient, a green path of microextractions" was given by Prof. Stig Pedersen-Bjergaard (University of Oslo, Norway) and Prof. Janusz Pawliszyn (University of Waterloo, Canada).

The second day of the symposium started at 9:00 a.m. with two parallel sessions consisting of four keynote lectures and nine oral communications. The session dedicated to "New extraction methods" was

opened by Prof. José Manuel Herrero-Martínez (University of Valencia) with a keynote lecture on the advantages of smart materials for low-cost supports in the field of sample preparation while the closing keynote lecture covered new miniaturized approaches for the analysis of low-availability samples and was delivered by Prof. Alberto Chisvert (University of Valencia). The parallel morning session was devoted to "Novel sorbents and Solid-Phase Extraction configurations" and started with a lecture given by Prof. Milton Rosero-Montero (University of Caldas, Colombia) on the synthesis of clay-based nanocomposites and their application in solid-phase extraction. This session was closed by Prof. María Llompart (University of Santiago de Compostela) with a lecture on sustainable miniaturized sample preparation strategies to assess the impact of microplastics from recycled tire crumb rubber. Poster session and the sponsors exhibition could be enjoyed during the coffee break and the talks were resumed with two parallel sessions where six young oral communications and two keynote lectures. In these sessions, on-site extraction devices and microextraction methodologies for characterization of complex samples were covered in the keynote lectures delivered by Prof. Rafael Lucena (University of Córdoba) and Prof. Emmanuela Gionfriddo (University of Toledo, USA), respectively.

After lunch, the Poster Session 1 continued parallel to a Sponsors exhibition in the Espejos room. During this short period of time, we had the possibility to solve any doubts that had remained during the morning session. This was followed by a Discussion Table on Green Metrics in the Auditorium which was moderated by Renata Wietecha-Posłuszny from Jagiellonian University (Poland) and the panellists were: Marek Tobiszewski, Yolanda Moliner-Martínez, Torsten C. Schmidt, and Frank Michel. After the end of the round table discussion, the Young Oral Session 3 and 4 began in parallel at Auditorium and Atenas room respectively, where two keynote lectures and nine young oral communications were carried out. Both sessions started with two interesting keynote lectures; on the one hand, "Omics analysis of natural products is going greener: Opportunities from new materials combined with microextraction techniques" delivered by Cecilia Cagliero in the Auditorium and, on the other hand, "Aptamer-based sorbents for the selective extraction of molecules and ions at trace levels in complex samples" given by Valérie Pichon. The young oral session 3 began with "Miniaturized QuEChERS combined with HPLC-MS/MS for determination of atropine and scopolamine in leafy vegetables" and finished with "Towards high throughput analysis using 96-well plate SPE for residue monitoring in food control" that were conducted by Lorena González-Gómez and Ane Arrizabalaga-Larrañaga. Young oral session 4 started with "A green ionic liquid-based three-phase partitioning system as a simple miniaturized platform for the analysis of human saliva" by Raúl González-Martín and finished with the lecture entitled "Evaluating new generations of magnetic ionic liquids as extraction platforms for organic compounds present in biological fluids" of María J. Trujillo-Rodríguez. From 06:15 p.m. onwards, the day's talks were over and we were able to enjoy the wonderful beaches of Tenerife.

On Thursday, the session began at 9:00 a.m. The morning of Thursday, July 20th, included 6 keynotes, 8 oral communications, one plenary communication and 6 young oral communications, which were distributed in two rooms. The plenary lecture was given by Professor Jared L. Anderson from Iowa State University, who explained how to design and synthesise ionic liquids, magnetic ionic liquids and polymeric ionic liquids. Prof. Anderson also demonstrated applications of these materials within the field of sample preparation. The keynotes discussed solid-phase microextraction with headspace, automated flow-based methodologies for sample treatment in the pharmaceutical field, hybrid organic and inorganic extraction media based on niobium, tantalum and other transition metal oxides for capillary microextraction, new 3D-printed bioselective sorbents, micro-extraction techniques for the extraction of volatile organic compounds from faecal samples for the diagnosis and prevention of colorectal cancer or different applications of the matrix solid-phase dispersion technique. In the oral communications, there were several presentations from different trading house such as Bruker or Milestone. The rest of the oral communications included very diverse presentations on different novel sample preparation strategies. In addition, during the coffee break sessions of the day, different posters were presented. There were a total of 4 topics: bioanalysis, *in-vivo* analysis, metabolomics and natural products (Topic 1), food analysis (Topic 2), green analytical chemistry (Topic 3) and new extraction phases (Topic 4). A total of 89 posters were presented in the topics described on Thursday, 20 July.

After a delicious lunch, there was an hour for the poster exhibition, and the oral sessions continued at 03:45 p.m. The main topics of the afternoon sessions were advanced microextraction strategies and green analytical chemistry. There were two keynote lectures. Myriam Díaz-Álvarez gave the first one from the Na-

tional Institute of Agricultural and Food Research and Technology (INIA), who spoke about the recent advances and future trends in molecularly imprinted polymers-based sample preparation. Dr. Alberto Escarpa, from the University of Alcalá, was responsible for the second keynote lecture. He showed us how micromotors in action can be used as environmental micro-cleaners and their future perspectives. There were 6 oral and 8 young oral communications during the afternoon session. The gala dinner was held at the Cleopatra Swimming Pool Terrace to close the day. Here we share with our colleagues and speakers. The organizing committee surprised us with a unique award ceremony in which the last registered attendant and the first abstract sent were awarded, among others. Finally, we danced to the rhythm of the typical batucada of the Tenerife Carnival.

The last session of the ExTech 2023 Symposium started at 9:00 a.m. on Friday, July 21st. In the Auditorium, the topic of the session was food analysis, and the keynote lecture was in charge of Ana María García-Campaña, which talked about sustainable analytical strategies for multiresidue/multiclass monitoring of emerging risks in complex samples. Following the lecture, five interesting communications took place, covering emerging topics such as sample preparation for GC×GC food analysis, extraction strategies for the extraction of bioactive compounds from food residues and green vegetables, or analysis of dairy products with GC-MS. The session closed with a keynote Lecture entitled "Challenges in extraction of aroma compounds from food matrices", by Henryk H. Jeleń. At the same time, a session focused on bioanalysis took place in the Atenas Room. To open the session, Barbara Bojko talked about Solid-phase microextraction and thin-film microextraction for direct and indirect characterization of organs' quality. After five short communications, the session ends with the intervention of Prof. Schmidt, talking about sample pretreatment for microplastic analysis. After a coffee break and the interesting Plenary Lecture on the role of (bio)polymeric phases in extending the sustainability of sample preparation, by Prof. Soledad Cárdenas, the Closing Ceremony handed out awards for best oral and posters communications, and introduced next ExTech Symposium, which will be celebrated next year in Bucaramanga (Colombia). This Tenerife adventure was finalized with a fantastic trip to Mountain Teide, organized by the Symposium. I hope to see you all in the next ExTech Symposium!

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9th International Conference on Food Chemistry & Technology (FCT 2023 – Paris, France)

The 9th International Conference on Food Chemistry & Technology took place from November 27th to 29th, 2023, in Paris, France. A total of 176 presenters from 36 countries participated in the event, including 124 oral communications and 52 posters.

The opening ceremony was inaugurated on Monday and presented by Prof. Antonio Derossi, from University of Foggia, at 8:50 a.m. in "Le Grand Salon" hall and then, the plenary morning began, with Mario Jekle (University of Hohenheim) as chair. The opening session started with two plenary lectures: I) "High Pressure Processing - Principles and Recent Applications" by Prof. Hosahalli Ramaswamy (McGill University), a renowned scientist who has stablished a state of the art and already founded a high pressure tech-

nology pilot plant facility leading Canada in this area and II) "Developments of Hyperspectral Imaging Technology for Novel Food Quality and Safety Detection and Control" by Prof. Da-Wen Sun (University College Dublin), a global authority in food engineering research and education. After this time, the congress was divided into 3 parallel sessions related to different topics including food chemistry, food and health, physical properties of food and sustainability and climate neutral food sector.

The session dedicated to "Food Chemistry" featured 21 oral communications and a plenary session about Mathematical Modelling for Novel and Innovative Sustainable Food Processing presented by Dr. Ferruh Erdogdu (Ankara University). The morning sessions were mostly based on the obtention of bioactive compounds using different green methodologies (advanced extraction techniques, alternative solvents, etc.) and the presentation of novel food ingredients. During the coffee break, all the posters were exhibited and presented simultaneously up to the end of second day. In this part, Ignacio Jiménez Amezcua and Inmaculada Luque Jurado presented their posters "Optimization of ultrasound-assisted extraction to obtain multifunctional extracts from garlic (Allium sativum L.) byproducts" and "Effect of Storage Conditions on Volatile Composition of Functional Bergamot (Citrus bergamia) Extravirgin Olive Oil", respectively.

After lunch, some oral communications continued to explore the Food Chemistry topic deeply, including the presentation of Inmaculada Luque Jurado on the development of a multi-analytical strategy for the detection of different frauds in raspberry ketone food supplements, with a successful day, plenty of question and the presentation of Ilaria Benucci about the sustainable recovery of chlorophyll-based colorant and its application to a real food, among others.

On Tuesday morning, the meeting started with the plenary lecture "Design of Food Products based on Model Systems" by Mario Jekle (University of Hohenheim) and was followed by 2 parallel sessions covering different topics related to emerging technologies in the future food, food chemistry and traditional food processing. The topic of 3D-printing of food was very interesting and a lot of communications discussed its advantages, difficulties and possibilities in the market (hospitals, military meal, etc). As a personal opinion, there was an interesting oral communication by Anne Louise Dannesboe (Danish Technological Institute) about 3-D printing of food for personalised nutrition which offered the future possibilities and the immediate implementation in hospitals.

After the coffee break, talks about traditional food processing (e.g. B2 vitamin, Indian cultured butter or cookable milk gels production) were remarkable. Once the last talk finished, a session of Networking was carried out, were all the participant took the opportunity of discussing some questions about the talks or poster presented.

On Wednesday, the last day of the congress, the morning started with an interesting plenary session on multisensory flavour perception by Prof. Charles Spence (Oxford University), in which the application of cognitive neuroscience and the study of multisensory integration of the senses was discussed to understand many of the key factors in the multisensory perception of taste. This was followed by a session about sustainability and climate neutral food sector with Javier Martinez Monzo (Valencia Polytechnic University) as a chair. Joana Martin's talk entitled "Combined chitosan-fish oil-green tea extract as a potential active coating for fresh Atlantic bonito fillet preservation" was of special interest due to it was presented a novel and sustainable method that delays the process of lipid oxidation and colour changes and improves the preservation of fish.

After the coffee break, session on food sensory, modelling, contact materials and detection & control began with a keynote presented by Elliot Woolley (University of Loughborough) about sustainable ways to ensure clean surfaces and recommendations to achieve it and continued with six additional speakers. In parallel, the session entitled "Recent advances in plasma processing in the food sector", was presented with five communications developing this topic.

At 13:25, the scientific committee awarded the best oral and poster presentation, followed by lunch, thus bringing the 9th International Conference on Food Chemistry and Technology to a close.

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29th International Symposium on Electro- and Liquid-Phase Separation Techniques (ITP 2023)

The International Symposium on Electro- and Liquid-Phase Separation Techniques (ITP) is one of the most recognized conferences on separation science due to its long history. This year the 29th edition (ITP 2023) took place in San Felice Circeo (Italy) from 13th to 17th September 2023 and was chaired by Prof. Alessandra Gentili. The symposium covered the latest advances in capillary and microchip electrophoresis, electrokinetic chromatography and electrochromatography, two-dimensional electrophoresis, high-performance liquid chromatography (HPLC), ultra-high pressure liquid chromatography (UHPLC), micro- and nanoscale HPLC and their principal applications in different fields such as pharmaceutical, clinical, food and environmental analysis. The event included 3 plenary lectures, 38 keynote lectures, 31 oral communications and 54 posters and about 124 participants attended from more than 20 countries all over the world.

On Wednesday 13th afternoon, after the Opening Ceremony a Plenary Lecture entitled "Microplastics determination and analysis: an analytical challenge" was exposed by Prof. Javier Hernández-Borges. This talk provided an overview of the problems that microplastics are causing in the environment and the different analytical methodologies developed for their analysis and determination. After that, Prof. Bezhan Chankvetadze presented a keynote focused on the potential of capillary electrophoresis for understanding the chiral recognition mechanisms of cyclodextrins. The session concluded with a Welcome Cocktail with musical accompaniment by musicians from the prestigious Accademia di Santa Cecilia.

On Thursday 14th the meeting lasted from 9.00 a.m. to 18.00 p.m. A total of 15 keynotes and 9 oral communications were presented by PhD, PhD students and full professors. The presentations were divided into 2 parallel sessions which were mainly focused on fundamentals and different applications of capillary electrophoresis and HPLC. After lunch, the first poster session dedicated to the young scientists took place. In this session, 27 posters were held, and an exchange of ideas and interesting discussions were established between the different attendees. During the day two industrial seminars were carried out by Perkin Elmer and Sciex. Finally, the meeting finished with a guided visit to the historic center of San Felice Circeo.

On Friday 15th the session lasted from 9.00 a.m. to 18.20 p.m. During this session, a total of 14 keynotes, 6 oral presentations, and 6 young session poster presentations were presented. Regarding the keynotes and oral sessions, different topics were discussed including capillary electrophoresis and its applications, the use of computation in chiral chromatography, the chemometric analysis of chromatographic data, liquid-phase enantioseparation, drug monitoring, sustainable cellulose-based sorbents or metabolomics, to name a few. After the coffee break, the second plenary lecture of the conference was presented by professor Verónica Pino Estévez, titled "Metal-organic frameworks: from tailored structures to analytical performance in different microextraction strategies and sustainability". Additionally, during the day, 2 industrial seminars were held by Agilent Technologies and Shimadzu, focused on automatic reinjection for additional confirmation in suspect screening and the advantages of SFC-MS over LC-MS in food safety analysis, respectively. Finally, at evening, the meeting was closed with a street food event on the beach, in which local products were displayed, and a "pasta alla carbonara" workshop was carried out.

On Saturday 16th the session lasted from 9.00 a.m. to 14.00 p.m. In this session, 4 plenary lectures and 16 young oral presentations were presented in two parallel sessions. Keynotes discussed various topics mainly focusing on capillary electrophoresis and its application on metabolomics, chiral planar derivatives, and microscale bioseparations, as well as the quality control in drug analysis. Young oral sessions, on the other hand, discussed a wide range of themes including enantioselective degradation of chiral pesticides, the use of bimetallic ionic liquids for the analysis of human urine, the enantioselective determination of chiral agrochemicals in urine, the analysis of emerging organic pollutants in microplastics, the development of green deep eutectic solvents for the analysis of phenolic compounds, etc. After dinner, attendees were taken in a half-day tour to The Ninfa Gardens and the Priverno-Fossanova Abbey, where they enjoyed the natural beauty and the magnificent architecture of the region. Finally, the night was closed with a symposium dinner by the pool in which delicious food and music livened up a beautiful night.

On Sunday 17th, the last and final session of the congress took place, lasting from 9.00 a.m. to 12.00 p.m. During the session, 3 keynotes were presented discussing miniaturized techniques for the separation of chiral compounds, the use of complexations for capillary electrophoresis chiral separations, and LC-MS/MS glycomics and glycoproteomics methods for the characterization of biomarkers of neurodegenerative diseases. Moreover, the third and last plenary lecture of the congress was presented by Professor Federico Marini, titled "Single- and multi-block chemometric strategies for metabolomics and systems biology". After this communication, the symposium closing took place and the young scientist and young posters awards were given. Finally, a farewell drink was held in the beach, and the congress was officially ended.

As a conclusion, ITP 2023 was a standout conference, featuring enriching plenary lectures and very interesting keynotes that provided deep insights in a wide range of research fields. The oral sessions fostered in-depth discussions and idea exchange, and, notably, the young oral presentations demonstrated promising emerging talent. Additionally, the poster sessions also showcased diverse, high-quality research Overall, ITP 2023 exemplified excellence across all aspects of its program.

Acknowledgements

We would like to express our sincere appreciation to SECyTA for their generous financial support, which has facilitated our attendance at the 29th International Symposium on Electro- and Liquid-Phase Separation Techniques (ITP 2023), as well as, for their unwavering dedication to fostering the development of young researchers and promoting knowledge dissemination.

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51st International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2023)

The 51st International Symposium on High Performance Liquid Phase Separations and Related Techniques was held from Sunday 18 to Thursday 22 June 2023 in Düsseldorf, Germany. This year, the symposium was chaired by Professor Michael Lämmerhofer from the University of Tübingen, and Professor Oliver J. Schmitz from the University of Duisburg-Essen.

The HPLC symposium is globally recognized as the premier conference dedicated to liquid phase separations and associated technologies. This edition featured a comprehensive program with over 1300 participants from around the world. It encompassed all facets of separation sciences in both liquid and supercritical fluid phases, with a special emphasis on integrating cutting-edge detection technologies such as mass spectrometry. The symposium's agenda spanned from fundamental principles and theories of chromatographic separations and detection methods to the latest advances in methodologies, technologies, column materials, and instruments. Moreover, it showcased applications in diverse fields and emphasized considerations of quality assurance.

Attendees had the opportunity to benefit from various activities, including short courses, workshops, tutorials, and enlightening plenary and keynote lectures delivered by esteemed scientists in the field. However, most presentations were selected from submitted abstracts, fostering active participation, and enabling participants to share and discuss their latest research findings with the audience. This edition also encouraged younger scientists to actively participate in the conference through the 'Separation Science SLAM', in which young scientists bringing their own research closer to the HPLC audience in an entertaining and understandable way in only 5 minutes; and the 'HPLC Tube', which awarded the best self-produced video on

HPLC research. Similarly, among around 300 poster contributions, one of them was awarded with the Best Poster Award. Additionally, the HPLC 2023 also featured a large exhibition and vendor seminars, allowing attendees to explore the latest innovations and services from leading vendors in the industry.

The congress commenced on Sunday morning with a short course program led by renowned international industrial and academic experts. The courses covered various fields such as two-dimensional HPLC, supercritical fluid chromatography, miniaturized sample preparation, mass spectrometry, ion-mobility mass spectrometry, metabolomics and lipidomics, data processing, 3D-printing in separation science, chiral separation, and biopharmaceutical analysis. The opening ceremony took place on Sunday afternoon at the Düsseldorf Congress Center, where the chairs warmly welcomed the attendees. The ceremony included the presentation of awards and fellowships followed by the first plenary lectures entitled 'Molecular phenomics in systems, synthetic, and chemical biology' by Prof. John A. McLean, director of the Center for Innovative Technologies at Vanderbilt University (USA), and 'Molecular phenomics in personalized and public healthcare in a changing world: Lessons from understanding COVID-19' by Prof. Jeremy K. Nicholson, director of the Australian National Phenome Centre in Perth (Australia). The opening concluded with a welcome standing cocktail which was accompanied by a small but lively brass band.

The following days, four parallel sessions were run in different halls covering different topics: biochromatography, sample preparation, omics, industry, stationary phase, pharmaceutical analysis, multidimensional LC, fundamentals, forensic analysis, chiral separation, capillary electrophoresis, column technologies, instrumentation, untargeted analysis, data analysis, ion mobility spectrometry, food analysis, etc. Vendor seminars took place during lunch times while exhibitions and poster sessions were held during lunches and coffee breaks.

Monday was a full day of enlightening sessions, starting with two plenary lectures entitled 'Separations sciences coupled to mass spectrometry for multimodal analysis: challenges and opportunities'" by Gérard Hopfgartner, Prof. in the department of Analytical and Inorganic Chemistry at the University of Geneva, and 'Digital transformation of the analytical laboratory – big bang or evolution?' by Joachim Richert, Vice President of Analytical Science BASF in Ludwigshafen (Germany). Throughout the day, a series of keynotes presentations and oral sessions by senior and young scientist took place in different halls. Poster session 1 held during three coffee break sessions provided attendees with networking opportunities and informal discussions on various topics. The day also included a discussion on sustainability and green laboratory practices, facilitated through a mobile application that allowed attendees to follow the agenda and interact with other participants.

The schedule was similar on Tuesday, beginning with a plenary session focused on the structural characterization of biopolymers, composed of two plenary lectures given by the distinguished scientists Valérie Gabelica, professor at the University of Namur (Belgium), who presented the talk entitled 'Ion mobility mass spectrometry to infer biopolymer folding and interactions' and Christian G. Huber, professor at the University of Salzburg (Austria), who delivered the speech entitled 'Structural analysis of highly complex protein therapeutics by HPLC-MS: lessons that we have learned from an analytical chemistry perspective'. After an additional poster session 1, poster session 2 was held for four sessions from Tuesday to Wednesday during coffee breaks, covering different topics such as environmental analysis, fundamentals, ion-mobility mass spectrometry, HPLC in chemical industry, new instrumentation and mass spectrometric, LC-MS, SFC-MS and CE-MS, materials and 3D-printing or multidimensional separations, among others. A total of eleven disciplines (i.e., biochromatography, fundamentals, omics, chiral separations, multidimensional LC, column technologies, capillary electrophoresis and microfluidics, instrumentation, biopharmaceuticals, untargeted analysis, and data analysis) were discussed in scientific sessions made up of both keynote lectures and oral presentations. Tutorials on ion mobility-mass spectrometry and chromatographic methods for analysis of therapeutic oligonucleotides and mRNA were also performed. The 'HPLC Tube' competition, which took place in the evening, served the participants to exhibit their research results through 3-minute videos on the topic 'How is your chromatography making a difference in the world'. Moreover, a job speed dating was arranged to give scientists and companies the opportunity to introduce themselves and exchange contact information for future job offers.

The Wednesday program started with a plenary session on high-throughput analysis, carried out by Jennifer Van Eyk, professor of Medicine at Cedars-Sinai Medical Center in Los Angeles (USA) and Petra

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Dittrich, professor of Bioanalytics and Head of the Laboratory of Bioanalytical Chemistry at the Department of Chemistry and Applied Biosciences (D-CHAB) at ETH Zurich in Switzerland, presenting the talks entitled 'High Throughput Single Cell Proteomics of Organ-derived Cell Populations by Nanoflow Dual Trap Single Column Liquid Chromatography' and 'High density droplet arrays for high throughput analysis', respectively. Following, scientific sessions regarding several topics (i.e., ion mobility spectrometry, food analysis, drug discovery pharmacokinetics, hyphenated technologies, new technologies, and bioanalysis) took place. A total of four tutorials were conducted by experts of different fields. The exclusive conference dinner was held at The Classic Remise in Düsseldorf, where attendees enjoyed the exhibition of classic and collector vehicles.

On Thursday morning, scientific sessions on multidimensional LC, bioseparation, bioanalysis, preparative LC and process analysis, and materials and 3D-printing, were delivered through keynote lectures and oral presentations. The participants nominated for the best poster award made flash oral presentations to apply for it. The last tutorial, which was about miniaturization of sampling and sample preparation devices, was presented by renowned Prof. Janusz Pawliszyn from University of Waterloo (Canada), who developed a solid-phase microextraction procedure. The closing plenary session 'Future of HPLC' was conducted by three plenary lectures 'Fundamental Studies of Enhanced-Fluidity Liquid Chromatography – Electrospray Ionization Mass Spectrometry of Complex Biological Systems', 'New Methods Contributing to Metabolomics Analyses of Single Cells' and 'A Journey Through the Chromatographic Universe Using Kinetic Plots' given by future chairs Susan Olesik from the Ohio State University (USA), Guowang Xu from the Dalian Institute of Chemical Physics (China) and Gert Desmet from the Vrije Universiteit Brussel (Belgium), respectively, took place at Auditorium of Congress Center Düsseldorf, followed by Csaba Horváth Young Scientist Award and Best Poster Awards ceremony, and the invitations to future HPLC congresses, two editions that will take place in 2024 in the cities of Denver (USA) and Dalian (China), as well as the 54th edition in 2025 in Bruges (Belgium). Finally, a farewell drink event was celebrated to spend one last pleasant moment before returning home.

The HPLC 2023 conference provided a valuable platform to discuss and explore the latest chromatographic advances, as well as sharing not only ideas, but also experiences between experts and beginners in the field of High Performance Liquid Phase Separations and Related Techniques fields.

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NOTA DE REDACCIÓN

Desde el Comité Editorial os animamos a que nos enviéis toda aquella información que consideréis de interés (premios, jubilaciones, etc.) para su difusión entre los lectores del boletín.

SECyTA EN *INNOVAFORUM*

Como anunciamos en el último boletín (*CTA*, **44**_1, 2023), la SECyTA fue invitada a participar en INNOVAFORUM, evento englobado dentro de FARMAFORUM, como resultado de la propuesta realizada por Bernabé Bodas (SCIEX) relativa a la participación de varias sociedades científicas relacionadas con la química analítica y la instrumentación, como son la SEEM, SEQA, SEProt y SESMet.

El evento tuvo finalmente lugar los días 20 y 21 de septiembre en el recinto de IFEMA en Madrid. Asistieron distintos presidentes, vicepresidentes y secretarios de las distintas sociedades que dispusieron de un tiempo de 10 minutos para dar a conocer los objetivos de cada sociedad, así como sus principales líneas de interés. Además, se cedió un espacio para exponer pósteres o alguna información propia de cada Sociedad.

La Presidenta, Ana M.ª García-Campaña, presentó a la SECyTA y en su exposición dejó patente la diversidad de campos en los que trabajan sus socios, como muestra de una gran versatilidad. La primera jornada tuvo un apretado y denso contenido en la que también participaron otros miembros de cada sociedad que quisieron dar a conocer sus líneas de trabajo con el fin de poder interactuar y colaborar con empresas dentro del mundo farmacéutico, nutracéutico o cosmético.

Concluido el evento, hay que hacer una evaluación reposada del resultado de INNOVAFORUM. Si bien se han visto aspectos positivos, como son la posibilidad de establecer contactos con empresas de los sectores mencionados interesadas en el trabajo de los distintos socios de SECyTA que pudieran traducirse en futuras colaboraciones, así como con empresas del sector de la instrumentación, también se han apreciado otros aspectos a mejorar. Entre ellos, el tipo de sala en el que tuvieron lugar las presentaciones, el uso de auriculares que dificultaba la comunicación y la aparente escasez de contactos finalmente llevados a cabo.

Ahora, toca que la SECyTA, junto con las otras sociedades, hagan una evaluación propia y una puesta en común de sus conclusiones de cara a una futura edición de este evento.

CALENDARIO DE ACTIVIDADES

1. **34th Meeting of SETAC Europe**
   5-9 de mayo de 2024. Sevilla (España)

   Chairs: **J. J. Ortega-Calvo y J. Blasco**
   https://www.setac.org/discover-events/global-meetings/setac-europe-34th-annual-meeting.html

2. **MSB 2024: International Symposium on Microscale Separations and Bioanalysis**
   19-22 de mayo de 2024. Brno (Rep. Checa)

   Chair: **F. Svec**
   https://www.msb2024.org/

3. **HTC-18: 18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology**
   28-31 de mayo de 2024. Leuven (Bélgica)

   Chairs: **D. Cabooter, J. Vercammen y F. Lynen**
   https://htc-18.com/
   Este congreso está patrocinado por SECyTA.

4. **PREP2024: 36th International Symposium on Preparative and Process Chromatography**
   28-31 de mayo de 2024. Filadelfia (EE.UU.)

   Co-chairs: **O. Dapremont, S. Menegatti y N. Vechiarello**
   https://www.prep2024.org/

5. **2nd International Conference of the Spanish Metabolomics Society**
   3-5 de junio de 2024. Sevilla (España)

   Chair: **A. García**
   https://congreso2024sevilla.sesmet.org/

6. **9th EuChemS Chemistry Congress (ECC9)**
   7-11 de julio de 2024. Dublín (Irlanda)

   Chairs: **D. A. Leigh y P. Guiry**
   https://euchems2024.org/

7. **HPLC 2024. 50th International Symposium on High Performance Liquid Phase Separations and Related Techniques**
   20-25 de julio de 2024. Denver, Colorado (EE.UU.)

   Chair: **S. Olesik**
   https://2024.hplcsymposium.org/

8. **XI NyNA 2024: International Congress on Analytical Nanoscience and Nanotechnology**
   3-6 de septiembre de 2024. Santiago de Compostela (España)

   Chair: **P. Bermejo Barrera**
   https://nyna2024.com/

9. **PBA 2024: 34th International Symposium on Pharmaceutical and Biomedical Analysis**
   9-12 de septiembre de 2024. Ginebra (Suiza)

   Chair: **S. Rudaz.**
   https://pba2024.org/

10. **3rd European Sample Preparation Conference (EuSP2024) and 2nd Green and Sustainable Analytical Chemistry Conference (GSAC2024)**
    15-18 de septiembre de 2024. Creta (Grecia)

    Chair: **E. Psillakis**
    https://www.eusp-gsac2024.tuc.gr/en/home

11. **ISC 2024: 34th International Symposium on Chromatography**
    6-10 de octubre de 2024. Liverpool (Reino Unido)

    Chair: **T. Edge**
    https://isc2024.org/

12. **XXIII Reunión Científica de la SECyTA (51.ª Reunión del Científica del GCTA)**
    23-25 de octubre de 2024. Pamplona (Navarra)

    Chairs: **Elena González-Peñas y Elena Lizárraga**

NUEVAS TESIS DOCTORALES

"Enantioseparations with polysaccharide-based chiral stationary phases in HPLC. Application to the enantioselective evaluation of the biodegradability of chiral drugs in activated sludge from a Valencian waste water treatment plant"

Autor: **Mireia Pérez Baeza**
Directores: María José Medina Hernández y Laura Escuder Gilabert
Grupo de investigación: Análisis multivariante y multicomponente (GAMM), Departamento de Química Analítica, Facultad de Química, Universitat de València.
Día y lugar de defensa: 24 de julio de 2023. Universitat de València

Resumen:

La comunidad científica lleva más de un siglo estudiando las implicaciones de la quiralidad. Hoy en día, sigue siendo un área activa de investigación y debate debido al gran número de moléculas quirales que forman parte de los organismos vivos y de nuestra vida cotidiana. En este contexto, las metodologías analíticas para la separación de los enantiómeros de moléculas quirales desempeñan un papel crucial. El uso de fases estacionarias quirales (CSPs) en cromatografía líquida de alta resolución (HPLC) es la opción preferida para la separación de enantiómeros.

La búsqueda del sistema cromatográfico (CSP/fase móvil) más adecuado para una enantioseparación determinada se lleva a cabo mediante procedimientos de ensayo y error, lo que se traduce en un enorme coste y esfuerzo experimental. Por tanto, la predicción de la capacidad de un sistema cromatográfico dado, para saber si es posible una separación quiral, sería de gran utilidad.

Esta Tesis tiene dos objetivos principales:

(i) Contribuir al conocimiento de la HPLC quiral con CSPs de polisacáridos (tres derivados de amilosa y cinco de celulosa) y fases móviles hidroorgánicas (disoluciones acuosas de acetonitrilo o metanol, compatibles con matrices acuosas y detección con espectrometría de masas). Para ello, se fijaron los siguientes objetivos específicos: (a) contribuir a una selección racional del sistema cromatográfico para separar los enantiómeros de un compuesto dado. Se compara la retención y enantio-resolución de aproximadamente 60 compuestos quirales estructuralmente no relacionados en los sistemas cromatográficos indicados. Además, se desarrollan relaciones cuantitativas estructura-propiedad para parámetros relacionados con la enantio-resolución en algunos de los sistemas cromatográficos estudiados. (b) Explorar el uso de la deconvolución de picos solapados para alcanzar la resolución matemática completa cuando no puede alcanzarse experimentalmente.

(ii) Contribuir a la evaluación de los riesgos y peligros de los contaminantes quirales. Para ello, se realizan ensayos de biodegradabilidad siguiendo las guías de la OCDE utilizando lodos activados de una estación depuradora de aguas residuales valenciana para algunos fármacos quirales de uso extendido. La separación y determinación de los enantiómeros del compuesto intacto se realiza mediante métodos de HPLC quirales (con CSPs de polisacáridos y fases móviles hidroorgánicas) desarrollados para tal fin.

"Development and application of metal-organic frameworks and other materials in microextraction/chromatographic techniques and sensing"

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Día y lugar defensa: 7 de septiembre de 2023. Univ. de Valencia

Resumen:

La importancia del tratamiento de muestra cada vez es más notoria en la química analítica actual debido a la alta complejidad de las muestras, los bajos niveles de concentración de analitos o la incompatibilidad de la matriz con el sistema de detección, entre otras. Aunque, si bien es cierto que en ocasiones este proceso es necesario, el desarrollo de métodos de detección temprana, simples y no invasivos, son también de vital importancia. La presente Tesis Doctoral gira en torno a ambas premisas. Concretamente, se han abordado dos grandes líneas: i) la síntesis de nuevos materiales para el aislamiento de analitos de interés en muestras reales complejas; y ii) el diseño de dispositivos analíticos basados en papel con fines de detección rápida.

En el objetivo i) se desarrollaron métodos utilizando diferentes materiales como fases extractivas (p. ej., redes metal-orgánicas (MOFs), polímeros orgánicos, etc.). Los sorbentes se han aplicado a varias muestras reales, tanto de origen ambiental, alimenticio como biológico, con el fin de retener una amplia gama de analitos, destacando pesticidas, vitaminas, cannabinoides, antibióticos, etc. Los formatos utilizados son muy diversos, incluyendo cartuchos convencionales de extracción en fase sólida, imanes, papeles de filtro 1 cm^2, microcartuchos, entre otros. En algunos casos, se evaluó la hibridación de los materiales individuales, lo que mostró efectos sinérgicos y características mejoradas en el material híbrido final. Todos los materiales fueron caracterizados en profundidad y los métodos fueron rigurosamente validados y comparados, en la mayoría de los casos, con materiales de referencia, comerciales o análogos.

En el objetivo ii) se han estudiado diferentes dispositivos analíticos de papel microfluídicos (μPADs) para el análisis en el punto de atención (POC). Con este propósito, se diseñaron varias alternativas. Primero, dispositivos portátiles y ligeros de flujo vertical con materiales de papelería accesibles. Esta metodología se aplicó a la especiación Fe (II)/Fe (III) y la cuantificación de compuesto fenólicos en muestras de vino y frutas. Los trabajos desarrollados son fruto de una estancia breve de investigación de 3 meses en Oporto, Portugal. Con el segundo diseño basado en impresión con cera, se desarrollaron aplicaciones de biosensado utilizando enzimas bioluminiscentes (luciferasas). En este caso, se realizó una primera contribución estudiando la mutagénesis del ADN complementario (cADN). A continuación, se unió ZIF-8 a dicha luciferasa y el biocompuesto resultante se aplicó exitosamente como sonda bioluminiscente, mejorando las características de las enzimas de origen. Finalmente, se desarrolló un sensor μPAD con esta biosonda híbrida para la determinación de ATP en orina y, consecuentemente, para su posterior aplicación al análisis rápido de infección del tracto urinario en el punto de atención. Estas últimas contribuciones son el resultado de una estancia de investigación de 6 meses en Bolonia, Italia.

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Agilent

Trusted Answers

QUANTITATION OF OVER 1,000 PESTICIDE RESIDUES IN TOMATO ACCORDING TO SANTE 11312/2021 GUIDELINE.

Using LC/MS/MS and GC/MS/MS detection

Peter Kornas and Teresa Klink Agilent Technologies, Inc.

Abstract

A comprehensive multiresidue workflow was developed and validated for the simultaneous quantitation of over 1,000 pesticide residues in tomato to accelerate and simplify routine laboratory food testing. The workflow analyzes a wide range of pesticide residues simultaneously in 20 minutes and uses a single sample preparation method for both LC/MS/MS and GC/MS/MS analyses, leading to increased turnaround time, simplified analysis, and lower laboratory costs.

The workflow includes sample preparation, chromatographic separation, mass spectrometric (MS) detection, data analysis, and data interpretation using Agilent LC/MS/MS and GC/MS/MS systems. For sample preparation, the Agilent QuEChERS extraction kit was used without further cleanup. Compound transitions and associated optimized parameters were developed based on the Agilent pesticide MRM databases for both LC/MS and GC/MS workflows.

Workflow performance was evaluated and verified according to the SANTE 11312/2021 guideline based on instrument limit of detection (LOD), calibration curve linearity, recovery, and precision using matrix-matched calibration standards from 0.5 to 100 µg/L. Over 98% of analytes demonstrated linearity with R2 ≥ 0.99. Method precision was assessed using recovery repeatability (RSDr). At t he 10 µg/kg level, RSDr values of 98% of compounds were within the limit of 20%. The mean recoveries of the six technical replicates were within the limits of 40 to 120% for 98% of target analytes.

Introduction

Pesticides play an important role in the agriculture and food industries to improve crop yield and food production. Residues of pesticides remaining in or on commodities such as fruits, vegetables, or cereals can cause adverse health effects as well as environmental concerns. Regulatory agencies have set maximum residue levels (MRLs) for hundreds of pesticides and their metabolites. Most MRLs are set at low parts per billion (ppb) levels, which poses significant challenges, especially if hundreds of analytes are screened and quantified simultaneously in complex food matrices. In Europe, pesticide testing laboratories adhere to the SANTE 11312/2021 guideline.[1] This guideline ensures a consistent approach for controlling MRLs that are legally permitted in food or animal feed. Due to the vast number of pesticides, the analysis is very elaborate, often requiring multiple analytical approaches and laboratory-intensive workflows, resulting in high operating costs and slow turnaround times.

In this study, an accurate and reliable analysis of over 1,000 pesticide residues in tomato was developed using a single QuEChERS extraction for sample preparation. As shown in the Venn diagram (Figure 1), 764 analytes were analyzed by LC/MS/MS and 341 analytes were analyzed by GC/MS/MS. The GC/MS/MS analysis included 84 analytes that can also be determined using LC/MS/MS; thus, this workflow covers a total of 1,021 unique substances.

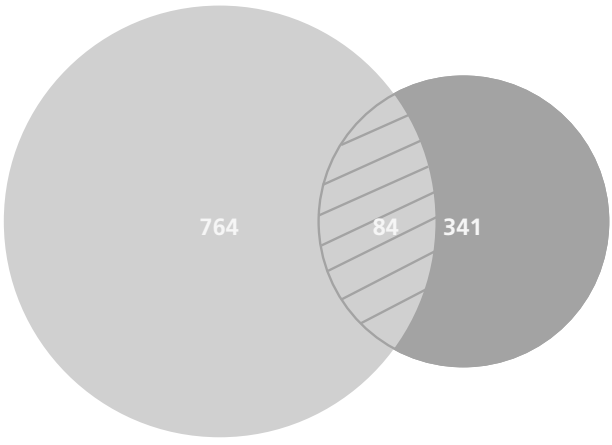

Figure 1. Venn diagram of compounds analyzed using LC/MS/MS (blue) and GC/MS/MS (orange).

This workflow, including sample preparation, chromatographic separation, MS detection, targeted quantitation, and results interpretation, helps streamline routine pesticide analysis and therefore accelerates lab throughput and productivity. Details of sample preparation procedures, instrumentation setup, and data analysis parameters are discussed, enabling the quantification and confirmation of pesticide residues.

Experimental

Chemicals and reagents

Agilent LC/MS-grade acetonitrile (ACN), methanol (MeOH), water, and ammonium formate were used in the study. LC/MS-grade formic acid was purchased from VWR. All other solvents used were HPLC grade and from VWR and Merck.

Standards and solutions

The following ready-to-use and custom premixed pesticide standards were acquired:

- Agilent LC/MS pesticide comprehensive test mix (part number 5190-0551)
- Agilent custom pesticide test mix (part numbers CUS-00000635 to CUS-00000643)
- Agilent custom organic standard (part number CUS-00004663)
- AccuStandard custom pesticide standard (part numbers S-96086-01 to S-96086-10), amchro GmbH, Hattersheim, Germany
- Agilent GC pesticide standard 1 to 10, and 12 (part numbers PSM-100-A to -J, and -L)
- Agilent GC pesticide standard no. 1 and 2 (part numbers PSM-105-A and -B)

Other single standards, either as standard solution or powders, were purchased from AccuStandard (amchro GmbH, Hattersheim, Germany) and LGC (LGC Standards GmbH, Wesel, Germany).

When single standards were purchased as powders, single stock solutions with a concentration of 1,000 mg/L were prepared in acetone and stored at –20 °C.

Intermediate standard mixes were prepared from stock solutions and used for preparation of prespiked quality control (QC) samples, solvent calibration standards, and matrix-matched calibration. Calibration standards were prepared freshly and stored in a refrigerator at 4 °C if not used immediately.

Sample preparation

Pesticide-free and organic-labeled tomatoes were obtained from local grocery stores. The tomatoes were homogenized using a domestic blender and stored in the refrigerator at 4 °C before analysis.

The following products and equipment were used for sample preparation:

- Agilent Bond Elut QuEChERS EN extraction kit (part number 5982-5650CH)
- Vortex mixer (VWR International GmbH, Darmstadt, Germany)
- Centrifuge UNIVERSAL 320 R (Andreas Hettich GmbH, Tuttlingen, Germany)

Samples of 10 ± 0.1 g of homogenized tomato were weighed into a 50 mL tube. Prespiked QC samples were fortified by spiking 200 µL of working standards (500 µg/L) to give a final concentration of 10 µg/kg. After spiking, the samples were capped tightly, vortexed, and equilibrated for 15 to 20 minutes. QuEChERS extraction was then performed and the samples were centrifuged. An aliquot of this extract was directly used for LC/MS/MS analysis. Before GC/MS/MS analysis, an aliquot of the extract was diluted by a factor of 5 with ACN. The preparation procedure is illustrated in Figure 2.

Preparation of matrix-matched calibration standards

Matrix-matched calibration standards (postspiked standards) were used and prepared for the assessment of workflow performance. A matrix blank was prepared using an unfortified, blank sample of tomato. Preparation of matrix-matched calibration levels was performed by mixing intermediate standard solutions with matrix blank extract. These solutions were used for LC/MS/MS analysis directly and diluted by a factor of 5 before GC/MS/MS analysis. The matrix-matched standard at 10 ppb was used to evaluate the matrix effect (ME) by comparing responses with the corresponding solvent standard.[1]

Instrumentation

The LC/MS/MS study was performed using an Agilent 1290 Infinity II LC system coupled to an Agilent 6470B

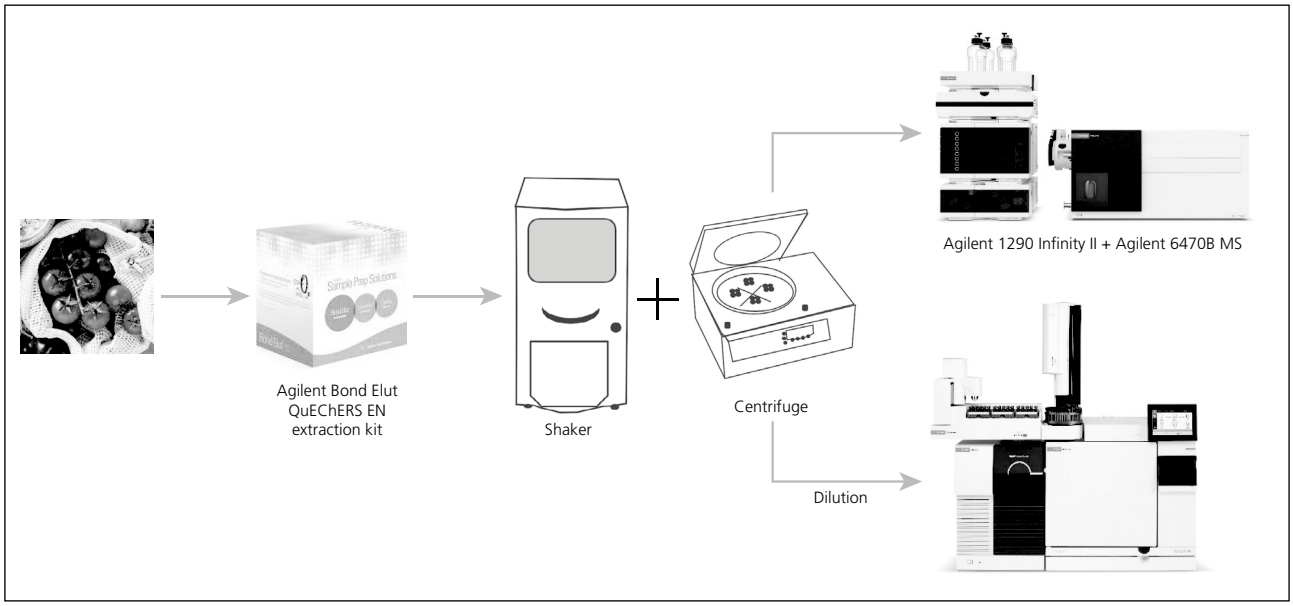

Figure 2. Sample preparation procedure using the Agilent Bond Elut QuEChERS EN extraction kit for sample cleanup before analysis.

triple quadrupole LC/MS. The modules of the LC/MS system included:

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II autosampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 6470B triple quadrupole LC/MS (G6470B)
- Agilent pesticide dynamic MRM database (G1733CA)
- Agilent MassHunter software (version 10.1)

The coupled 6470 triple quadrupole LC/MS was equipped with an Agilent Jet Stream (AJS) electrospray ion source and was operated in dynamic MRM (dMRM) mode.

The main LC and MS parameters are listed in Table 1. Please refer to the Agilent application note by Kornas for the detailed LC/TQ configuration.[2]

The GC/MS/MS study was performed using an Agilent 8890 GC and Agilent 7010C triple quadrupole GC/MS system. The modules of the GC/MS system included:

- Agilent 8890 GC (G3540A)
- Agilent 7693A automatic liquid sampler (G4513A and GG4520A)
- Agilent 7010C triple quadrupole GC/MS (G7012C)
- Agilent MassHunter pesticide & environmental pollutant (P&EP) MRM database 4.0 (G9250AA)[4]
- Agilent MassHunter software (MassHunter acquisition version 10.2 and MassHunter Quantitative Analysis version 12.0)

The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI). Chromatographic separation was performed using the conventional 15 m × 15 m midcolumn backflush configuration described in the P&EP database.

Therefore, two Agilent HP-5ms Ultra Inert (UI) GC columns (part number 19091S-431UI) were used, and midcolumn backflush capability was provided by the Agilent Purged Ultimate Union (PUU) installed between the two identical 15 m columns, and the pneumatic switching device (PSD) module on the 8890 GC. The acquisition method was retention time locked to match the retention times in the MassHunter P&EP 4.0.

The main GC and MS parameters are listed in Table 2. Please refer to the Agilent application note by Klink for the detailed GC/TQ configuration.[3] All data were acquired in dynamic MRM (dMRM) mode.

Table 1. LC and MS conditions.

Parameter	Value
LC	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column Temperature	40 °C
Injection Volume	2 µL
Autosampler Temperature	6 °C
Mobile Phase A	5 mM ammonium formate in water with 0.1% formic acid
Mobile Phase B	5 mM ammonium formate in methanol with 0.1% formic acid
Flow Rate	0.4 mL/min
Gradient	Time (min) A(%) B(%) 0 95 5 3 70 30 17 0 100 20 0 100
Postrun Time	3 min
Needle Wash	Multiwash
MSD	
Ionization Mode	Simultaneous positive/negative ESI with Agilent Jet Stream (AJS)
Scan Type	Dynamic MRM (dMRM)
Gas Temperature	200 °C
Gas Flow	9 L/min
Nebulizer	35 psi
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2,500 V (+)/3,000 V (–)
Nozzle Voltage	0 V
Total MRMs	1,590
Min/Max Dwell Time	0.52 ms/242.30 ms

Table 2. GC and MS conditions.

Parameter	Value
GC	
Columns	Agilent HP-5ms, 15 m × 0.25 mm, 0.25 µm film thickness (two) (p/n 19091-431UI)
Carrier	Helium
Column 1 Flow	0.94 mL/min
Column 2 Flow	1.14 mL/min
Injection Volume	1 µL, solvent vent
Inlet Liner	Agilent Ultra Inert dimpled liner (p/n 5190-2297)
MMI Temperature Program	60 °C for 0.06 min, 720 °C/min to 280 °C and hold
Oven Temperature Program	60 °C for 1 min, 40 °C/min to 170 °C, 10 °C/min to 310 °C and hold for 3 minutes
Run Time	20.75 minutes
Transfer Line Temperature	280 °C
Backflush Conditions	1.5 min postrun, 310 °C oven temperature
MSD	
Source	High-efficiency source (HES)
Vacuum Pump	Performance turbo
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM
EM Voltage Gain Mode	10
Total MRMs (dMRM Mode)	2,093
Min/Max Dwell Time	1.2 ms/100.2 ms

Results and discussion

Developcment of multicompound methods

A major part of this study was the development of dMRM transitions for all pesticides from the Agilent databases. For LC/MS/MS, the Agilent pesticide dynamic MRM database was used. MRM transitions as well as fragmentor voltages, collision energies, and ionization polarity were optimized using the Agilent MassHunter Optimizer software by flow injection. Approximately 1,600 MRM transitions from 764 pesticides were stored in the final dMRM method. Typical chromatographic peak widths were between 8 to 12 seconds. The selected cycle time of 490 ms ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and confirmation of results.

For GC/MS/MS, most of the compounds were already listed in the MassHunter P&EP database.4 Compounds whose MRM transitions were not listed in this database were developed using the MassHunter Optimizer for GC/TQ. Starting with a GC method that provides good chromatographic compound separation, the MassHunter Optimizer first identifies precursor ions and product ions, then optimizes collision energies for each promising precursor-product combi-

nation to identify the best MRM parameters. Around 2,100 MRM transitions from 341 pesticides were stored in the final dMRM method. The selected cycle time of 300 ms ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and confirmation of results. The GC acquisition method was retention time locked to match the retention times in the Agilent P&EP database, which was used to seamlessly create the MS method. The use of P&EP increased the ease and speed of setting up a targeted dMRM method. Retention time locking allows a new column or instrument to have retention times that match the MRM database or an existing method exactly, allowing methods to be easily ported from one instrument to another and across instruments globally. This simplifies method maintenance and system setup.

Two or three target specific MRM transitions were selected per pesticide in each method to satisfy the regulatory requirements for identification and confirmation by LC/MS/MS and GC/MS/MS, respectively.[1]

Data were acquired in dynamic MRM (dMRM) mode, which enables the capability for large multi-analyte assays and to accurately quantitate narrow peaks by an automated and most-efficient dwell time distribution. Furthermore, dMRM enables the analyst to add and remove additional analytes with ease.

Matrix effect assessment

Effects caused by the sample matrix are frequent and cause suppression or enhancement of the MS detection system response.[1] ME was assessed by the ratio of target response in matrix-matched standards to that in corresponding solvent standards. Typically, there is no strict requirement on acceptance ME criteria, because ME can be corrected by the matrix-matched calibration curve. However, ME is an important parameter for method sensitivity and reliability assessment, and less than 20% signal suppression or enhancement is usually considered as insignificant ME.1 In this study, ME was investigated using a 10 µg/L standard in tomato extract (post-spiked standard) and the response was compared to the corresponding solvent standard. The 10 µg/L standard was chosen, as this is the lowest MRL for pesticides and their metabolites.

More than 45% of the 1,021 targets in tomato showed significant ME at 10 µg/L.

Based on the results of the ME assessment, matrix-matched calibration standards were used to compensate MEs in this study.

Verification of workflow performance

The workflow performance criteria were verified based on linearity, method sensitivity, recovery, and precision. The batch included solvent blank, matrix-matched calibration standards, matrix blank, and prespiked QCs. Six technical replicates were prepared for the prespiked QCs.

Linearity

Calibration curves were generated for all compounds using matrix-matched standards ranging from 0.5 to 100 µg/L, and eight calibration points. Linear or quadratic regression with 1/x weight and unspecified origin were used for calibration curve generation. The calibration range was determined based on LOQ sensitivity and selectivity requirements. Results in Figure 3A show that more than 98% of the targets met the calibration curve linearity requirement of $R^2 \geq 0.99$.[1] Only some compounds showed a modified calibration range due to either lack of sensitivity at low calibration levels or detector saturation at high concentration levels.

Instrument limit of detection (LOD)

A sensitive workflow for pesticide residue analysis is beneficial for users to perform routine operations following various regulatory guidelines. Instrument LODs were used to evaluate method sensitivity. Instrument LOD was established based on matrix-matched calibration standards for signal-to-noise ratio (S/N) of 10 and up. The S/N was defined using the peak height and peak-to-peak algorithm embedded in MassHunter Quantitative Analysis software. The noise region was manually chosen and had a minimum length of 0.1 minutes.

More than 97% of target compounds showed an instrument LOD of ≤ 10 µg/L, and, even at a concentration level of 1 µg/L, more than 88% of compounds had an S/N of 10 and up (Figure 3B). These results demonstrate the high sensitivity of both systems, the 6470 triple quadrupole LC/MS and the 7010 triple quadrupole GC/MS, against a complex matrix such as a tomato QuEChERS raw extract.

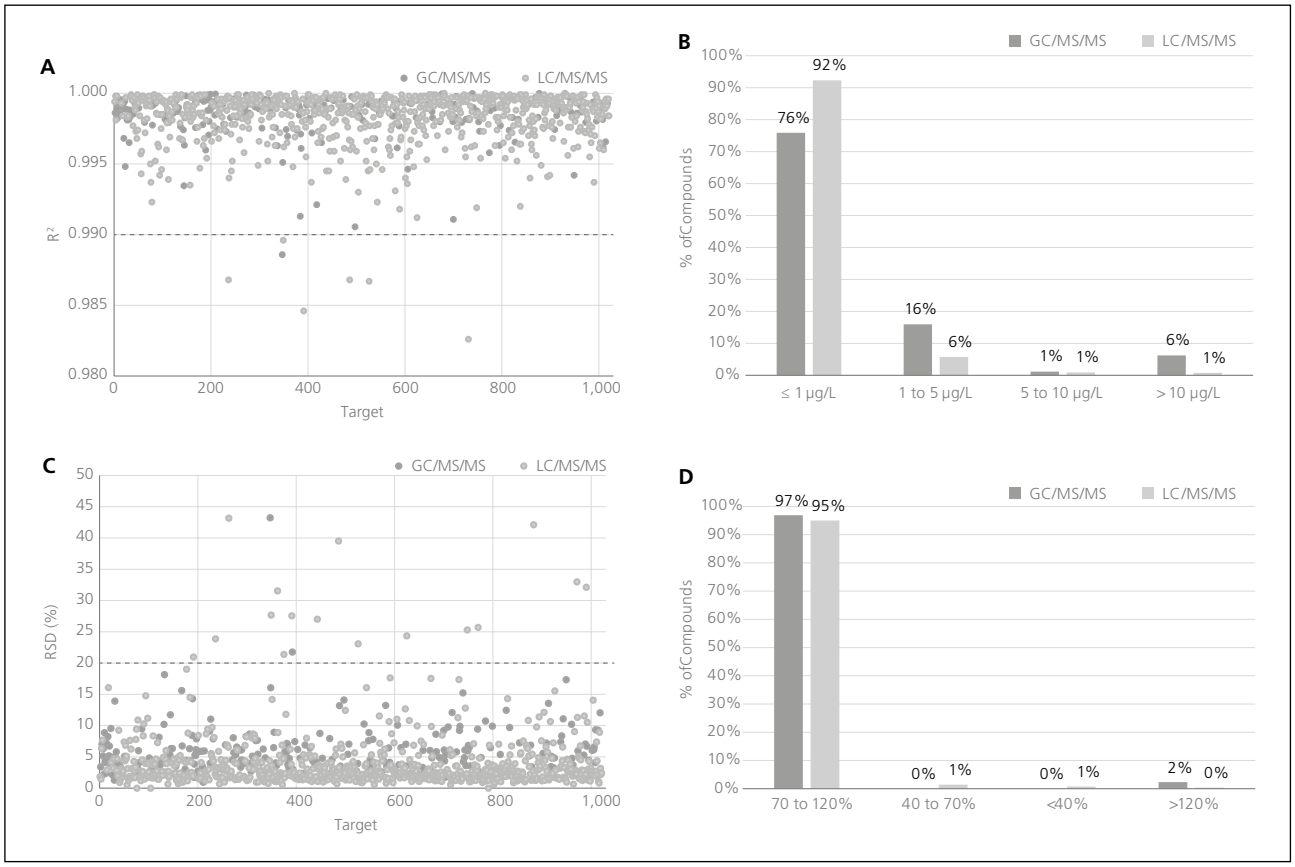

Figure 3. (A) R^2 distribution of linearity curves for 1,021 pesticides, compounds below $R^2 = 0.98$ are not shown (9 in total); (B) Instrument LOD in tomato QuEChERS raw extract; (C) RSDr of recovery rates at 10 µg/kg in QuEChERS tomato raw extract; (D) Recovery rates in tomato QuEChERS raw extract (RSDr ≤ 20%).

Method precision and recovery

Method precision was estimated using recovery repeatability (RSDr) based on the variation of recovery values from technical replicates of prespiked QC samples that were spiked at 10 µg/kg. The RSDr was determined by calculating percent relative standard deviation (%RSD) of recovery using these six technical preparations. Typically, the acceptable RSDr is 20% or less. The RSDr values of 98% of all targets were within 20%, demonstrating consistent behavior with each technical preparation. These results confirmed the high repeatability of this workflow. Figure 3C shows that the vast majority of compounds had RSD of recovery rates below 20%.

Recovery was used in this experiment to evaluate the capability of a quantitative analytical workflow for over 1,000 pesticides. Recovery was calculated based on analyte response ratios between prespiked QCs and corresponding matrix-matched calibration levels. Mean recovery at 10 µg/kg level was obtained for six technical replicates. According to SANTE 11312/2021, mean recoveries are acceptable within the range of 40 to 120% if they are consistent (RSDr ≤ 20%). Based on these criteria, the mean recovery results for more than 97% of targets in tomato QuEChERS raw extract at 10 µg/kg met the acceptance criteria. The vast majority of compounds (975) were within the recovery range of 70% to 120% and only 26 compounds (3%) were below 70% or above 120%, respectively (Figure 3D).

Combination of methods

The combination of LC/MS/MS and GC/MS/MS allows users to cover the widest range of pesticides and metabolites occurring in food. Due to the molecular structure of this huge class of compounds, it is impossible to analyze various pesticides solely by GC or LC techniques. Exploiting both techniques makes it possible to get a wide coverage of these residues that can potentially endanger human health.

The presented workflow used both techniques and covered in total 764 pesticides analyzed by LC/MS/MS and 341 compounds analyzed by GC/MS/MS. All detailed results can be found in references 2 and 3. Furthermore, the analyses covered pesticide residues (84) that can be analyzed by either technique. This gives a clear benefit when, for example, positive results must be confirmed or higher sensitivity is needed.

In Figure 4, the chromatograms of silafluofen in a spiked matrix sample at 10 µg/kg are shown. The left chromatogram shows that sensitivity using LC/MS/MS was not good enough to get reliable results at MRL of 10 µg/kg. The full Agilent solution allows analysis of this compound using GC/MS/MS, resulting in much better sensitivity (right chromatogram). The use of the other technique for confirmatory analysis can be demonstrated for bifenthrin. This compound can be reliably quantified using both techniques. The chromatograms in Figure 5 clearly demonstrate that sensitivity is high enough to determine and confirm positive results by either LC or GC technique.

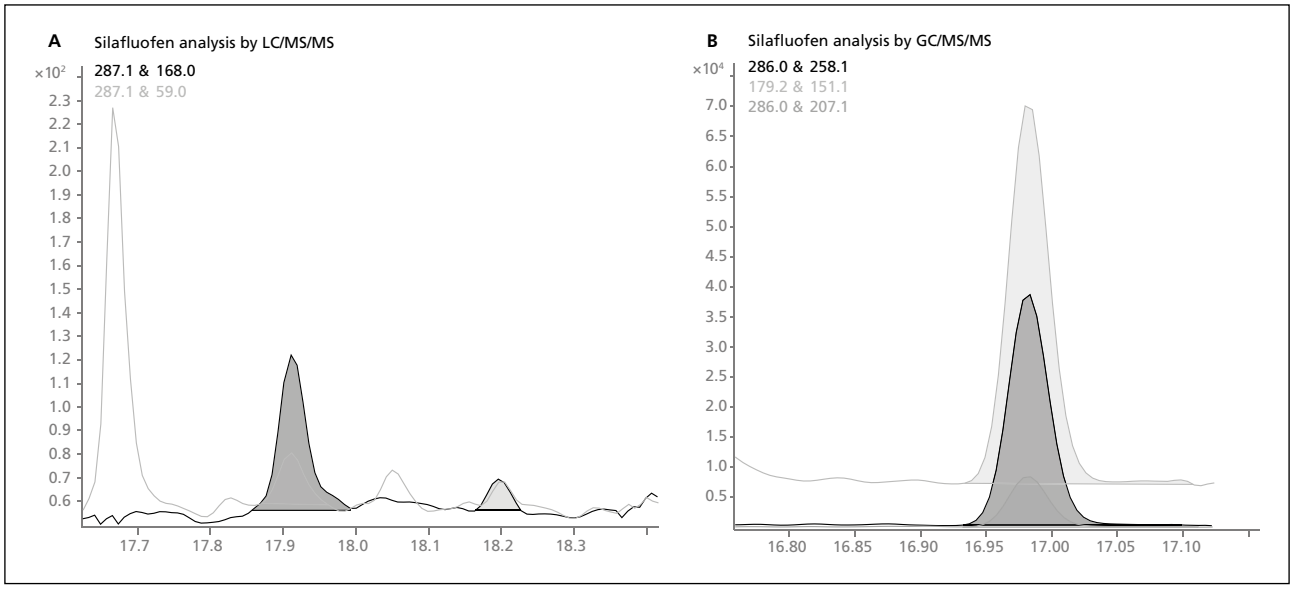

Figure 4. Analysis of silafluofen by LC/MS/MS (A) and GC/MS/MS (B).

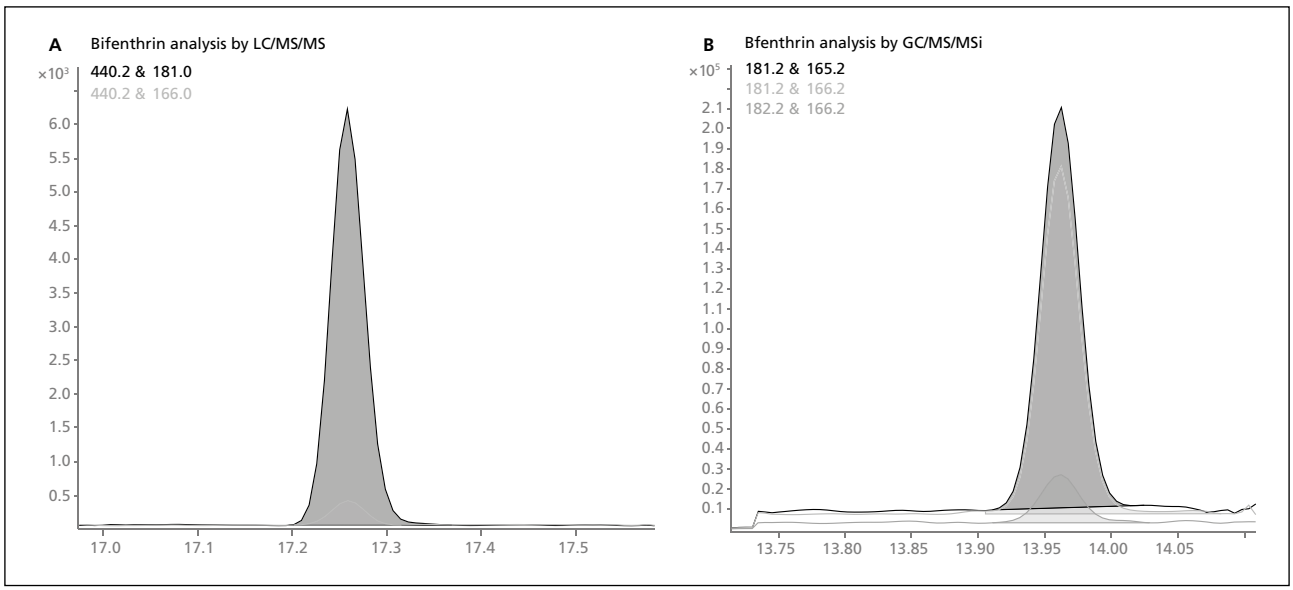

Figure 5. Analysis of bifenthrin by LC/MS/MS (A) and GC/MS/MS (B).

Conclusion

This application note demonstrates the applicability of a sensitive and reproducible workflow for fast and reliable quantitation of more than 1,000 pesticide residues in tomato QuEChERS raw extract conforming to the SANTE 11312/2021 guideline. The simple sample preparation protocol uses the Agilent Bond Elut QuEChERS EN extraction kit for facile extraction without requiring further sample cleanup. A single sample preparation procedure can be used and then split into two aliquots for subsequent analysis by LC/MS/MS and GC/MS/MS.

An Agilent 1290 Infinity II LC system coupled to an Agilent 6470 triple quadrupole LC/MS was used to quantify 764 pesticides, and an Agilent 8890 GC coupled to an Agilent 7010C triple quadrupole GC/MS was used to quantify 341 pesticide residues with matrix-matched calibration. Both methods had 20-minute run times, and column setups offered good chromatographic separation and even retention time distribution of all targets.

To achieve the most efficient use of instrument cycle time, all data were acquired in dMRM mode. The dMRM methods were created and developed based on the Agilent pesticide MRM databases.

The overall workflow performance was assessed for linearity, instrument LOD, recovery, and precision, demonstrating its suitability for the quantitation of over 1,000 pesticide residues in the same QuEChERS raw extract.

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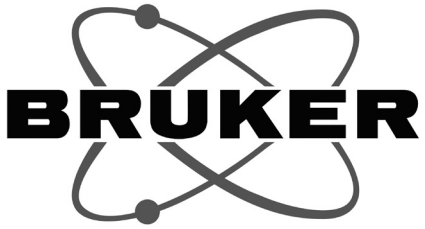


MALDI IMAGING: PAST, PRESENT AND FUTURE
Bruker is leading this exciting field

Introduction

The development of new drugs and therapeutic treatments is dependent on understanding complex biochemical pathways of disease development. To elucidate these pathways, researchers often use tools for visualizing the distribution of various biopolymers in specific tissues, at the molecular level, leading to the discovery of new biochemical activity. Critically then, it is the ability to monitor molecular level changes in organisms that promises better understanding of disease and subsequent development of effective new treatments (1).

Advances in analytical technology, with improved sensitivity and higher throughput, have enabled biomedical research to keep pace with the continuing demand for novel therapies. Mass spectrometry (MS), in particular Matrix-Assisted Laser Desorption/Ionization (MALDI) technology, has proven instrumental for mapping, identifying, characterizing and quantifying the relative expression of thousands of proteins, peptides, lipids, metabolites and small molecules in tissue. Also this is not only relating biological tissues, but other materials and matrix which may beneficiate of the spatial molecular distribution in a surface.

Known as MALDI Imaging, this method was first used to map the spatial distribution of proteins, by using images to gain insights into the distribution of single proteins and any post-translational modifications (PTMs) or degradation. Its application in proteomics and biomarker research has been met with an array of other applications, such as the ability to image molecules in tissue like metabolites and drugs.

For example, MALDI Imaging can differentiate between parent drugs and metabolites within tissue, contributing to the growing knowledge of tissue distribution and its application to pharmacology and pharmacokinetics. Such information can be used to develop treatments that depend on the active drug reaching a specific target site, or for cancer therapeutics with specific tumor tissue targets (2). As a highly sensitive technique capable of detecting a wide range of compounds simultaneously, regardless of their nature and mass, MALDI Imaging has helped redefine the way biological sample testing is carried out since its introduction in the late 1990s.

What is MALDI Imaging?

MALDI Imaging is a powerful tool for investigating the distribution of proteins, lipids, metabolites and small molecules in the body through analyzing intact tissue sections in a label-free manner. It has been shown to be versatile in its many applications in the analysis of biological samples, especially peptides and proteins, and developments in laser technology as well as standardization of sample preparation have furthered the adoption of MALDI technology.

How does it work?

To perform MALDI Imaging, sections of biological tissues are prepared and introduced into a MALDI molecular imaging instrument, where a pulsed laser generates a molecular fingerprint at every image pixel. From this array of molecular fingerprints, hundreds of distribution maps or images can be generated from the molecule detected.

MALDI Imaging utilizes a matrix, usually a small acidic aromatic molecule that absorbs energy at the wavelength of the irradiating laser. Analyte molecules are extracted from the biological matrix by aerosol application of a solution of matrix and allowed to dry. During the drying process, matrix-analyte co-crystals form. These crystals are then sampled at the selected pixel resolution by the laser inside the MALDI Imaging instrument.

Imaging the component distributions across cohorts of tissues can be used to track distribution changes in many classes of endogenous compounds relative to disease state, or to map drug/tissue interactions. Histological features within the sample can be correlated with molecular species without the need for target-specific reagents such as antibodies.

The early years of MALDI Imaging

The first applications for MALDI Imaging to tissue sections are largely attributed to Professor Richard Caprioli, who helped secure its place as an "essential technology in the process of unraveling the complexities of molecular interactions in living cells".

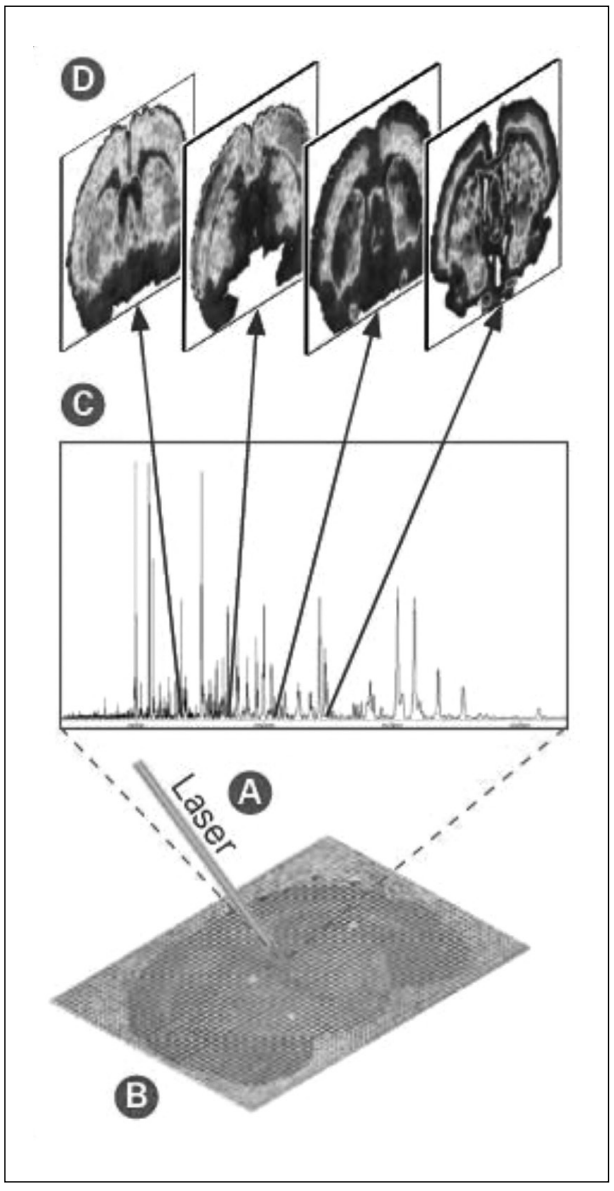


Figure 1. MALDI Imaging provides spatial mapping of compounds in tissue. (A) Spatially resolved mass spectra are recorded by rastering the laser across the sample surface. (B) Prepared sample on plate inside instrument. (C) Each peak represents a molecule present at a specific location. (D) Molecular images show the distribution on any measured peak as function of its location.

In his 1997 study, MALDI MS was shown to be "versatile in application in the analysis of biological samples, especially to peptides and protein (3). The study concluded that, "Mass spectrometry provides an excellent means for the identification of the molecular weights of molecules in biological samples. The ability to find and trace molecules in tissue and cells remains of vital importance to biological research in many different fields. In a single analysis of a small piece of tissue, the entire array of biochemical processing of peptide hormones and proteins can be displayed." This study helped pave the way for further work using MALDI Imaging to obtain higher resolution images, higher sensitivity, better sample preparation and faster data acquisition and analysis.

The laser breakthrough

Desorption and ionization techniques have been instrumental in revolutionizing biomolecule analysis, offering new levels of mass accuracy and sensitivity (4).

Traditional mass spectrometric methods, of value for measuring compounds with low molecular masses, were unable to measure those with high molecular masses. It was realized that laser desorption/ionization (LDI) held promise, particularly the new aspect of matrix-assisted LDI analysis.

The first systematic attempts to generate ions of organic molecules with lasers date back to the early 1970s.

It was Nobel Prize for Chemistry (2002) winner Tanaka who was the first to demonstrate the applicability of laser technology to biological macromolecules (5). This principle is fundamental for many of today's powerful laser desorption methods, particularly MALDI.

At a symposium in 1987, Tanaka showed that large protein molecules could be ionized from a droplet of liquid glycerol doped with ultrafine cobalt powder using soft laser desorption. The ions released as intact hovering molecule ions with low charge are then accelerated by an electrical field and detected by recording their time of flight (6).

Later, a study by Karas and Hillenkamp (1988) showed that laser beams could produce ions of large proteins from solid crystals of nicotinic acid (7). Solid crystalline matrices couple more easily with the vacu-

um conditions of mass spectrometers and their use has become the standard approach to MALDI Imaging.

MALDI Imaging through the 2000s

Introducing standardized sample preparation

Before the launch of the first dedicated sample preparation device in 2006, sample preparation was more of an art than a science. Scientists would develop essentially home-made sample preparation devices, which had an obvious impact on the reproducibility of results, particularly between operators.

MALDI Imaging was brought to more mainstream use through the introduction of two new features for applying matrix to tissue sections, the software-controlled spray generator for even matrix application, and the automated matrix sensor, which determines the depth of the matrix and fine-tunes its insertion into the instrument.

The automated ImagePrep station from Bruker was the first commercial device to bring a degree of standardization to the industry – which also began the process of transitioning MALDI Imaging to researchers who were not trained mass spectrometrists. For the first time, trained lab technicians could use ImagePrep to prepare samples by applying the matrix reproducibly. This advance delivered multiple benefits that paved the way for widespread acceptance of the technology, including a more rapid process, less likelihood of human error and, most significantly, more reproducible results that could be shared and repeated across different laboratories.

Standardizing on the same device across different geographies opened the door to improved collaboration and exchange of ideas. The ability to recreate and repeat the exact research conditions enabled researchers to develop new methods that built on previously published studies.

Data-rich and information-rich

At the same time as sample preparation developments were launched, software was introduced that not only brought speed and standardization to the testing stage, but also to data analysis.

The introduction of new software tools such as Bruker flexImaging, launched in 2004, was a turning point for the industry. Providing an easy-to-use tool for acquiring and visualizing two-dimensional imaging of cells or tissue sections, subsequent integration with Bruker ClinProTools software provided the capacity to extract regionally specific molecular fingerprints from MALDI Imaging data and perform statistical analysis to determine molecular changes. Together these two packages moved the laboratory from having a plethora of data but little meaningful information to a position of being both data-rich and information-rich. For the first time, information was both available and able to be interpreted in a consistent and therefore meaningful way.

The ability to apply statistics to thousands of spectra allows for the identification and analysis of data patterns. This level of data modeling represented the first step in standardizing on a platform, and changed the way the industry worked to share information.

Familiarity with the new process grew among the scientific community, cemen- ting Bruker's place as the industry standard in MALDI Imaging technology.

Laser improvements

Throughout the 2000s, faster and more reliable laser technology continued to drive MALDI development. Bruker's introduction of the modulated beam profile offered a new parameter for optimizing the MALDI process.

Given the throughput and life span limitations of the commonly used nitrogen lasers, diode-pumped solid-state lasers emerged as a viable alternative. A groundbreaking paper published in 2006 showed for the first time that a spatially structured laser beam profile, instead of using a Gaussian profile, was of striking importance because of its applicability to a broader range of matrix compounds than an unmodulated beam (8). This result enabled the design of smartbeam II lasers that on various critical applications showed equal or superior performance for MALDI as the nitrogen lasers.

MALDI Imaging to locate diagnostic proteins

The application of MALDI protein profiling and imaging to cancer research has become a natural fit. The many tissue samples available to laboratories, such as

biopsies and tumors, as well as the need to understand the molecular complexities that exist as cells transform from normal to malignant, make MALDI Imaging ideal for this type of study.

In a study by Caldwell and Caprioli in 2005, MALDI Imaging analysis of cancerous and non-cancerous human prostate cells from laser capture microdissection revealed overexpression of a protein in the cancerous tissue (9). The study concluded that tissue profiling and MALDI Imaging provide unique information to greatly facilitate understanding of normal biological and pathological processes, particularly at the high-throughput level typical of this technology.

MALDI has a unique ability to provide information on relative protein abundances while maintaining information on their spatial location. A study by Reyzer and Caprioli in 2007 concluded that "There is a tremendous amount of potential for MALDI Imaging based imaging applications. MALDIImaging is a simple, quick and molecularly specific analytical platform that can distinguish hundreds of signals corresponding to proteins on tissue sections (10).

The role of MALDI in clinical and biomedical research

There are two distinct research tracks supported by MALDI Imaging, one which considers the advancement of science behind disease development and the other which takes a more holistic, patient-oriented view:

Mechanistic-driven

- Researchers seek to understand the biochemical pathways and signaling as a disease develops to understand the cause and identify the key molecular players in its transformation.

Diagnostic-driven

- Researchers look to diagnose and treat the disease more effectively by understanding which treatment to prescribe. This is determined by looking at its molecular signature and making predictions, such as disease stage or drug uptake.

Bringing tissue analysis to clinical relevance

Early driving forces set out to bring the technique of tissue analysis to significant clinical relevance, improving the situation in terms of time to results for medical decision making and the patient outcome and follow-up.

In the early days of MALDI Imaging the limitations were clear. Commercial MALDI instruments had never been designed for imaging and simply did not have the required speed or spatial resolution for the level of imaging technology needed. Then, 100 µm pixel size was the smallest possible and measurements would typically run over a weekend. Today, MALDI Imaging measurements that previously required 48 hours to perform can be achieved in as few as fifteen minutes. Modern sample preparation devices continue to improve reproducibility and user experience. The need for speed as well as the link to microscopy rapidly became obvious.

Commensurate with improved design of imaging systems, data complexity increased. Early software tools have been replaced by sophisticated statistical and modeling tools. Bruker's SCiLS Lab has become industry standard software for analyzing MALDI Imaging data. SciLS Lab can combine data from multi-sample cohorts and perform unsupervised analyses to discover molecular changes between cohort classes, while supervised analysis tools provide for class modeling and prediction. Most importantly, all of these calculations are made with full consideration of the spatial relationship of the molecular content within the specimen.

Compound identification

With the market receptive to new developments in instrumentation that delivered increased throughput and sensitivity, industry leader Bruker pioneered new technology.

In small molecule imaging and drug metabolism, it is critical that the drug can reach the site of action to help researchers assess its potential value as a pharmaceutical product and its toxicological outcomes (4). In contrast to the standard method of whole-body autoradiography (WBA), MALDI Imaging does not require radiolabeleddrugs – the preparation of which is laborious and expensive. MALDI Imaging can also differentiate between the drug and its metabolites (hav-

ing different m/z), whereas in WBA, the detected radioactivity may not differentiate a drug or a metabolite.

MALDI tandem time-of-flight (MALDI-TOF/TOF) MS enables the selection of specific ions for fragmentation and analysis in a second mass analyzer. Bruker's leading FLEX technology platform offers gold standard solutions for MALDI Imaging, providing quick and reliable results from small sample volumes. Furthermore, MALDI magnetic resonance mass spectrometry (MRMS) combines the power of MALDI Imaging with MRMS. MALDI MRMS enables straightforward identification of compounds from tissue directly and, compared with traditional Magnetic Resonance mass spectrometry (MRMS), MRMS provides unmatched resolution and mass accuracy and enables routine isotopic fine structure (IFS) analysis for a broad mass range, resulting in unmatched confidence for compound identification. IFS is the unique mass spectral signature arising from naturally occurring isotopes within the molecule being measured, creating an exact fingerprint specific to a molecular formula.

Traditional workflows that sample small tissue sections (<100 µM) provide deep insights into the molecular details of a variety of chemical classes including RNA/DNA, proteins, glycans, lipids and metabolites. However, processing times can be long and tissue excise locations are determined with optical images that lack molecular signal information. Combining MALDI with electrospray ionization (ESI) in a single dual source instrument has allowed Bruker to bring spatially-resolved OMICS – SpatialOMx® – to life sciences and translational MALDI Imaging with the timsTOF fleX.

Since ions developed by MALDI and ESI travel the same path from the source to the detector, MALDI workflows can exploit the most advanced features found in Bruker's timsTOF Pro, such as TIMS based on the collisional cross section (CCS) of detected molecules and high-speed high-sensitivity parallel accumulation – serial fragmentation (PASEF) fragmentation. Modification and calibration can be carried out in ESI mode and used for the MALDI experiment for further ease of optimization.

Built on the timsTOF Pro platform, the timsTOF fleX adds a high spatial resolution MALDI Imaging source specifically designed for resolving molecular distributions and bringing a spatial dimension to OMICS analyses.

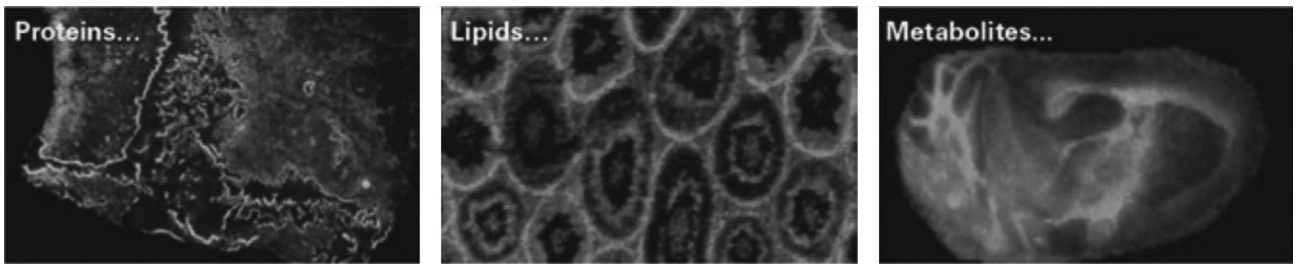


Figure 2. MALDI Imagin can simultaneously map hundreds of different compounds within a small region or across large cohorts of samples.

Future perspectives

It is increasingly being recognized that there are more degrees of freedom for separating molecules other than by molecular weight. Molecules exist that have the same molecular weight but different structures – which therefore can never be separated using MS.

Looking to the future, the advantage will be gained by adding those additional degrees of freedom. One way is to integrate different techniques.

Ion mobility, supported by instrumentation such as the timsTOF fleX, to separate compounds by size and molecular weight, can now be integrated with MALDI ionization, meaning this separation can be conducted on an imaging scale. There is more information contained in the data than the instruments are capable of extracting. For example, the most sophisticated instruments generate data from a sample that shows several thousand peaks, whereas in common use, the data typically shows around one hundred.

Given that the goal is always to extract as much information as possible, integrating other techniques with MALDI will enable higher degrees of analysis. Such integrations have successfully created new paradigms for models that pharma can use in the future,

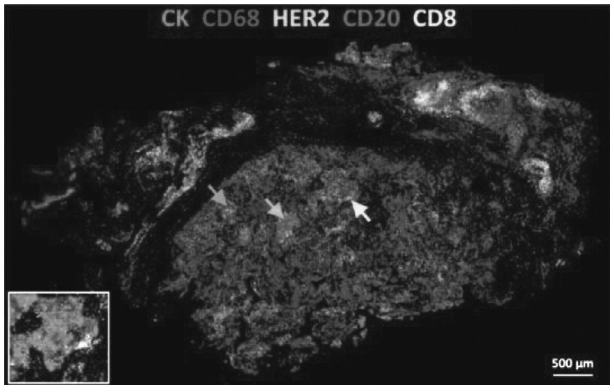
based on what is available now and what predictions can be made for future translations.

SpatialOMx on the timsTOF fleX is a revolutionary new way of analyzing tissue that identifies and enables capture of a high-definition, label-free molecular overview for specific cell phenotypes. Utilizing the capabilities only found in timsTOF fleX, analysis of the tissue by MALDI Imaging segments tissue micro environment by molecular phenotype, allowing one to identify and locate with high precision the cell subpopulation of interest. Subsequent microextraction of these targeted subpopulations by PASEF-enabled 4D-Omics yields molecular information with highest specificity for targeted cell phenotypes.

While operating, software activation of the SmartBeam 3D laser is the only alteration in the source region, maintaining productivity and the ability to transition smoothly from OMICS identification and quantification workflows to the development of high-definition tissue section molecular maps.

Many researchers are striving towards single cell imaging, to capture each cell's molecular signature. Although two cells may look the same when analyzed by the more traditional process of histology, they may have different molecular signatures. For example, one cell may be in the process of transforming, and another in a state that may not transform for three months. Differentiation at the single cell level could be a critical step forward in identifying and, ultimately, treating disease.

Latest developments like very recent MALDI HiPLEX-IHC, as a novel tissue imaging tool set to revolutionize spatial multiomics. This new technology based on novel photocleavable mass-tags (PC-MTs), which enables highly multiplexed IHC based on MALDI Imaging – termed MALDI HiPLEX-IHC.



It explores the capabilities of this innovation to provide more powerful methods for researchers to explore the spatial distribution of biomolecules in tissues at the cellular level in various areas of research, including proteomics, tissue pathology, tissue diagnostics, therapeutics, and precision medicine.

Conclusion

Significant developmental improvements in MALDI Imaging technology have made it a valuable method in many bioscience fields, and direct tissue analysis by MALDI Imaging is now an important technology for revealing the underlying molecular signatures indicative of disease.

As MALDI becomes increasingly commercially available – with new benchtop models set to drive further developments throughout different industry sectors – its potential will continue to be explored.

Adapted from originally report by authors: Shannon Cornett, Applications Development Manager and Mike Easterling, VP, Bruker Daltonics.

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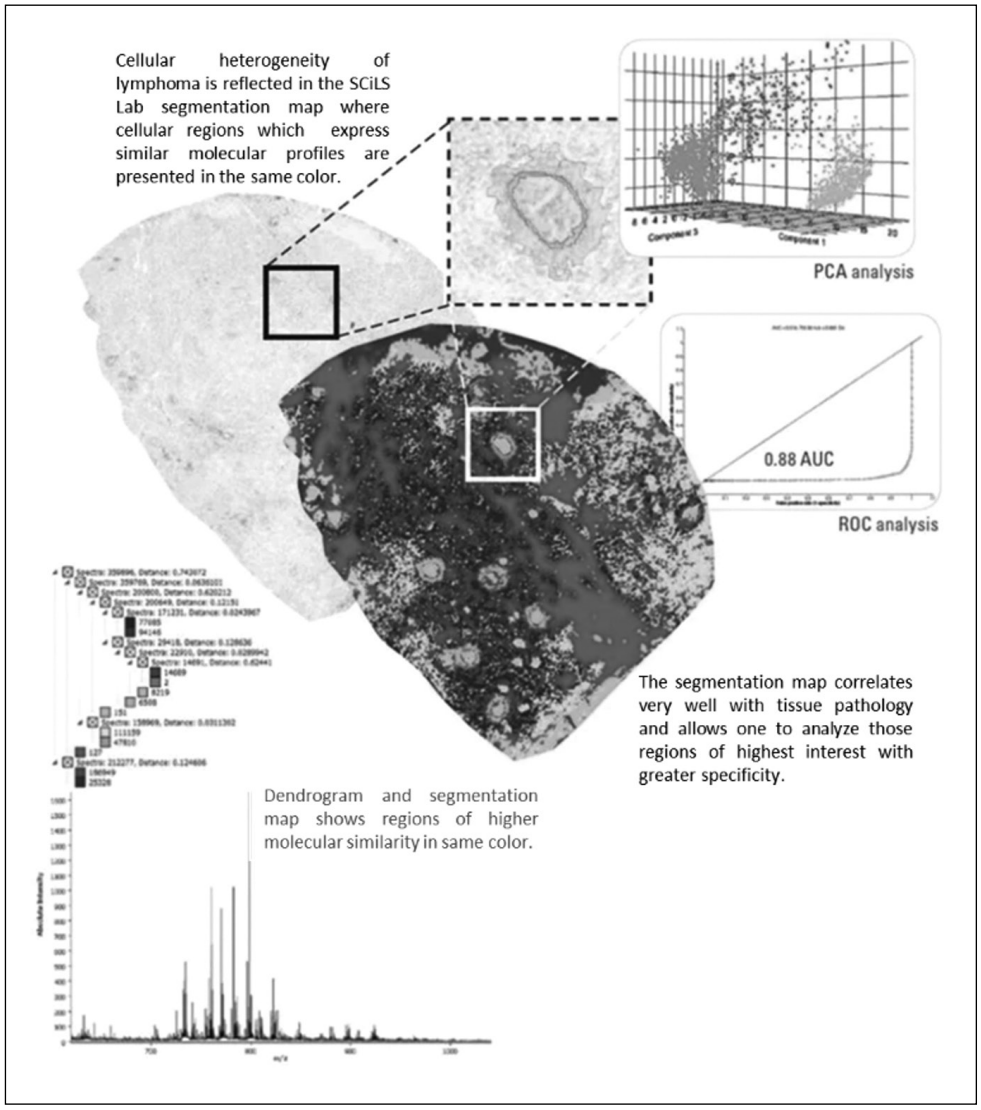

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DETERMINATION OF ALL PFAS RELEVANT IN THE DRINKING WATER GUIDELINE USING DIRECT INJECTION

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

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Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are heat-resistant, oil- and water repellant as well as chemical and thermally stable, resistant to UV light and weathering compounds. Due to these properties, PFAS are widely used e.g. as fire retardants, food packaging materials or coatings for textiles and housewares. Because of their anthropogenic origin, PFAS cannot be degraded, and hence they accumulate and can now be detected ubiquitously in the environment. In addition, these substances are harmful to health and are also suspected of being carcinogenic. Since drinking water is considered to be an important source of human PFAS intake, testing drinking water for PFAS levels has been essential for several years now. The following application note demonstrates the determination of all PFAS requested in the EU directive 2020/2184 on the quality of water intended for human consumption in an appropriate concentration range by using direct injection LC-MS.

Materials and Methods

A fast, sensitive and robust LC-MS/MS system is crucial for routine analysis in drinking water laboratories. For following application note a Shimadzu LCMS-8060NX triple- quadrupole mass spectrometer was used, coupled with a Nexera X3 UHPLC system (Figure 1). Due to the various properties of the short- and long-chain PFAS, two different methods were used for the analysis. The differences are described in the following section.

PFAS standards, which includes all 20 relevant compounds in the EU directive 2020/2184, and one suited mixture of internal standards (ISO 21675-LSS) were purchased (Wellington Laboratories).

Evian mineral water was used as the drinking water matrix for the calibration samples. The PFAS samples were diluted with methanol and combined to a single standard mixture with a final a concentration of 1 ng/µL for each compound to prepare stock solutions. Furthermore, dilutions of this mixture were produced and spiked into the Evian water to get calibration samples in the concentration range from 0.1 ng/L to 50 ng/L. For each calibration point, there was a multiple determination of three injections.

Table 1 shows the LC-MS/MS method parameters. There are slightly different LC gradient settings regarding starting conditions of the solvent between method 1 and 2.

Table 1. Analytical conditions.

Nexera X3	
Flow:	0.8 ml/min
Eluent A:	Water + 2 mM ammonium acetate
Eluent B:	Methanol + 10 mM ammonium acetate
Column Oven:	50 °C
Column:	Analytical: Shim-Pack Scepter C18-120 3 µm, 3 × 150 mm Delay: Shim-Pack GIST HP 3 µm, C18-AQ, 3 × 30 mm

LCMS-8060NX (ESI)	
Interface Voltage:	–0.5 kV
Focus Voltage	–2 kV
Desolvation Line:	150 °C
Heating Gas:	15 L/min
Interface Temp.:	200 °C
Nebulizing Gas:	3 L/min
Drying Gas:	3 L/min
Heat Block:	400 °C
Dwell Time/Pause-time	10 (3 for IS) / 1 msec
Other Parameters:	Default (analog tuning file)

Method 1:

For sample preparation, 750 µL of aqueous sample was mixed with 750 µL of methanol and acidified with 0.1% acetic acid by volume. That means, the concentrations on column are actually halved. Regarding the data analysis in this note, all concentrations refer to the water volume. A co- injection method with water was used – in total 200 µL milliQ water and 350 µL-sample. The duration of the method is 8 minutes.

Method 2:

All samples were spiked with internal standards to a final concentration of 20 ng/L. More sample preparation was not required. 500 µL of sample is injected directly for analysis. The duration of this method is 10 minutes.

Figure 1. LCMS-8060NX Triple Quadrupols Mass Spectrometer.

Results

Exemplary MRM-Chromatograms and calibration curves of different PFAS are shown in figure 2 for method 1 and figure 3 for method 2. All calibration points used for determination showed an accuracy within ± 30%.

Table 2 shows the limits of quantification (LOQ) of the analysed PFAS.

The calibration curves were calculated using linear regression. The R^2 of all relevant PFAS are at least 0.99.

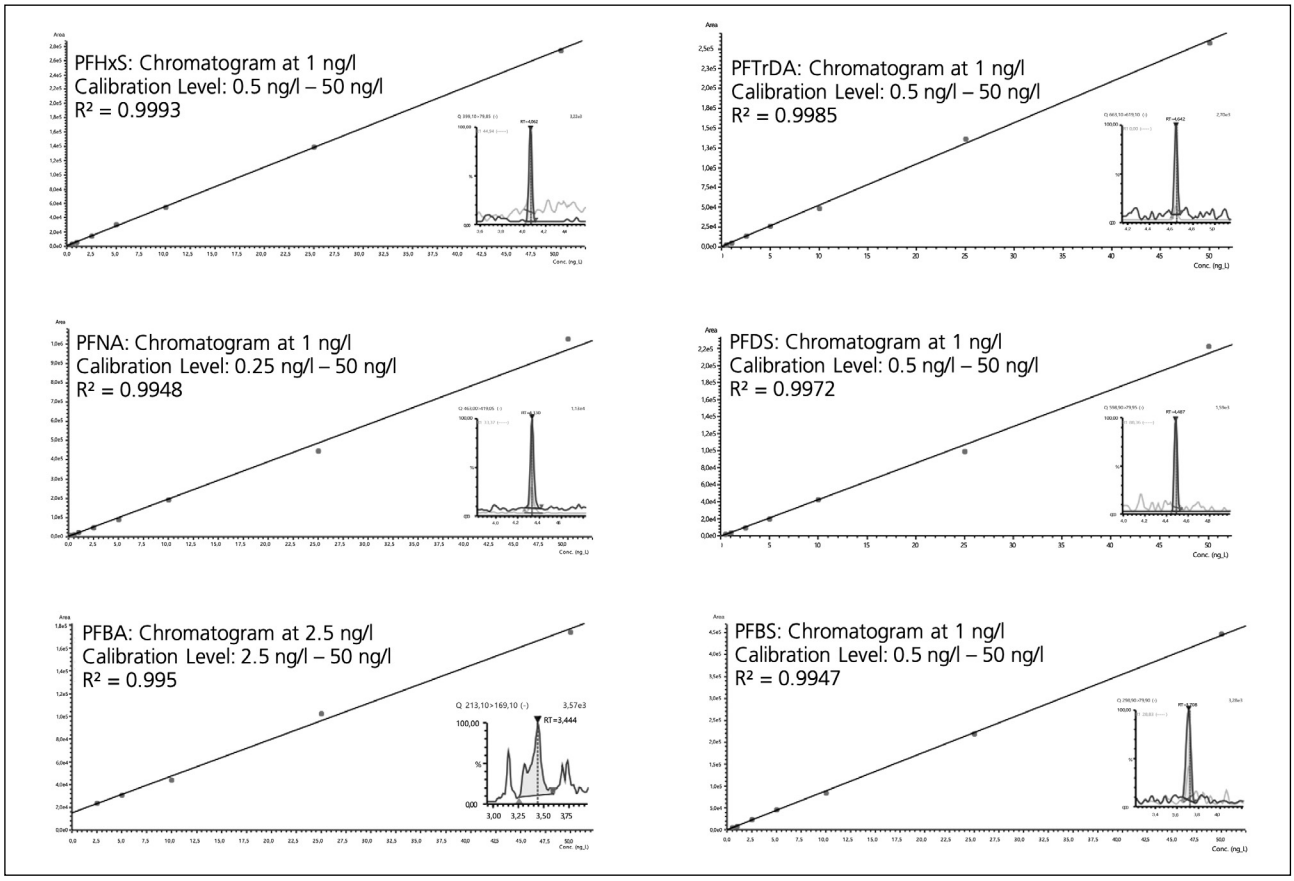


Figure 2. Method 1: Exemplary MRM-Chromatograms and Calibration Curves of different PFAS; Smoothing: standard Counts: 1 Width: 1; external calibration; Weighting: 1/C.

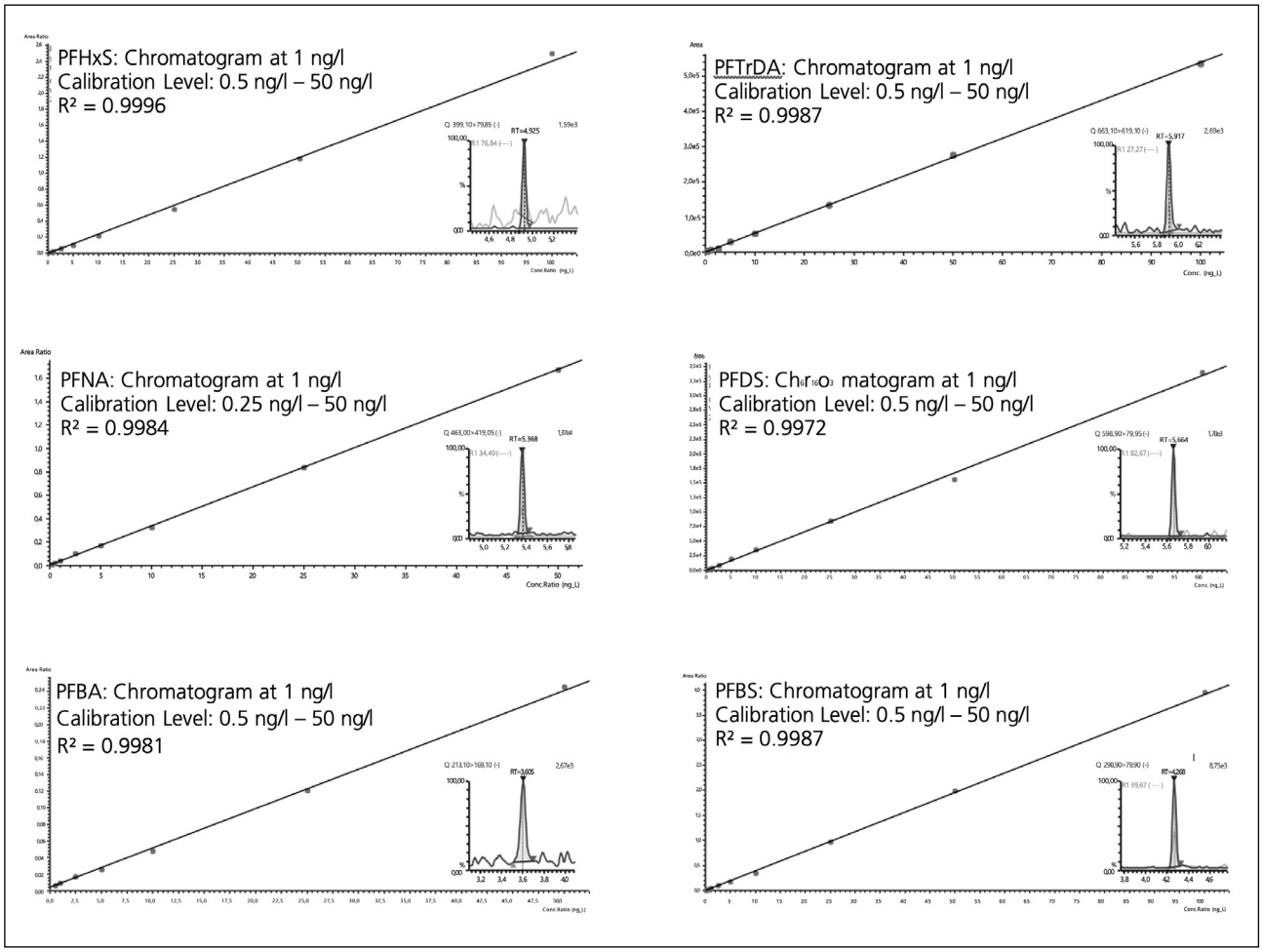


Figure 3. Method 2: Exemplary MRM-Chromatograms and Calibration Curves of different PFAS;Smoothing: standard Counts: 1 Width: 1; internal calibration; Weighting: 1/C

Table 2 LOQ of all relevant PFAS

Compound	LOQ [ng/L] method 1	LOQ [ng/L] method 2
PFBA	2.5	0.5
PFBS	0.3	0.1
PFPeA	0.5	0.1
PFPeS	0.25	0.1
PFHxA	0.25	0.1
PFHxS	0.3	0.5
PFHpA	0.5	0.3
PFHpS	0.25	0.1
PFOA	0.5	0.2
PFOS	0.2	0.25

Compound	LOQ [ng/L] method 1	LOQ [ng/L] method 2
PFNA	0.3	0.2
PFNS	0.1	0.25
PFDA	0.5	0.2
PFDS	0.25	1
PFUnDA	0.5	1
PFUnDS	0.2	0.3
PFDoDA	0.4	0.25
PFDoDS	0.25	0.3
PFTrDA	0.5	0.5
PFTrDS	0.5	1

Comparison of the stability of both methods:

Especially for long-chain PFAS, it is known that adsorption to vial materials and incomplete solubility over time has a negative impact on the analysis. To show this behavior, figure 4 shows the intensity of an internal standard of the same concentration during a batch measurement. For e.g. PFDoDA decreasing intensities over time can be easily observed. Adding methanol, the intensity respectively the area is comparable for all samples and even more precise. For PFHxA, as an example for a short-chain PFAS, the reduction of intensity over the batch is not observed for both methods. Nevertheless, the precision is better for samples added with methanol as well. The adsorbance issue for long-chain PFAS in aqueous solutions is demonstrated well.

Conclusion

This application note shows two usable LC-MS/MS method to monitor all PFAS requested in the EU directive 2020/2184. These methods are highly sensitive, fast and robust. Additionally, there is no complicated sample preparation needed.

The results clearly demonstrate the benefit of using methanol regarding the analysis of long-chain PFAS. In this case, there is no urgent need for the addition of an internal standard. Nevertheless, the use of internal standards is recommended. By using the LabSolutions Insight Software the data analysis become quick and easy for routine analysis. This method shall be verified for other drinking water types in near future.

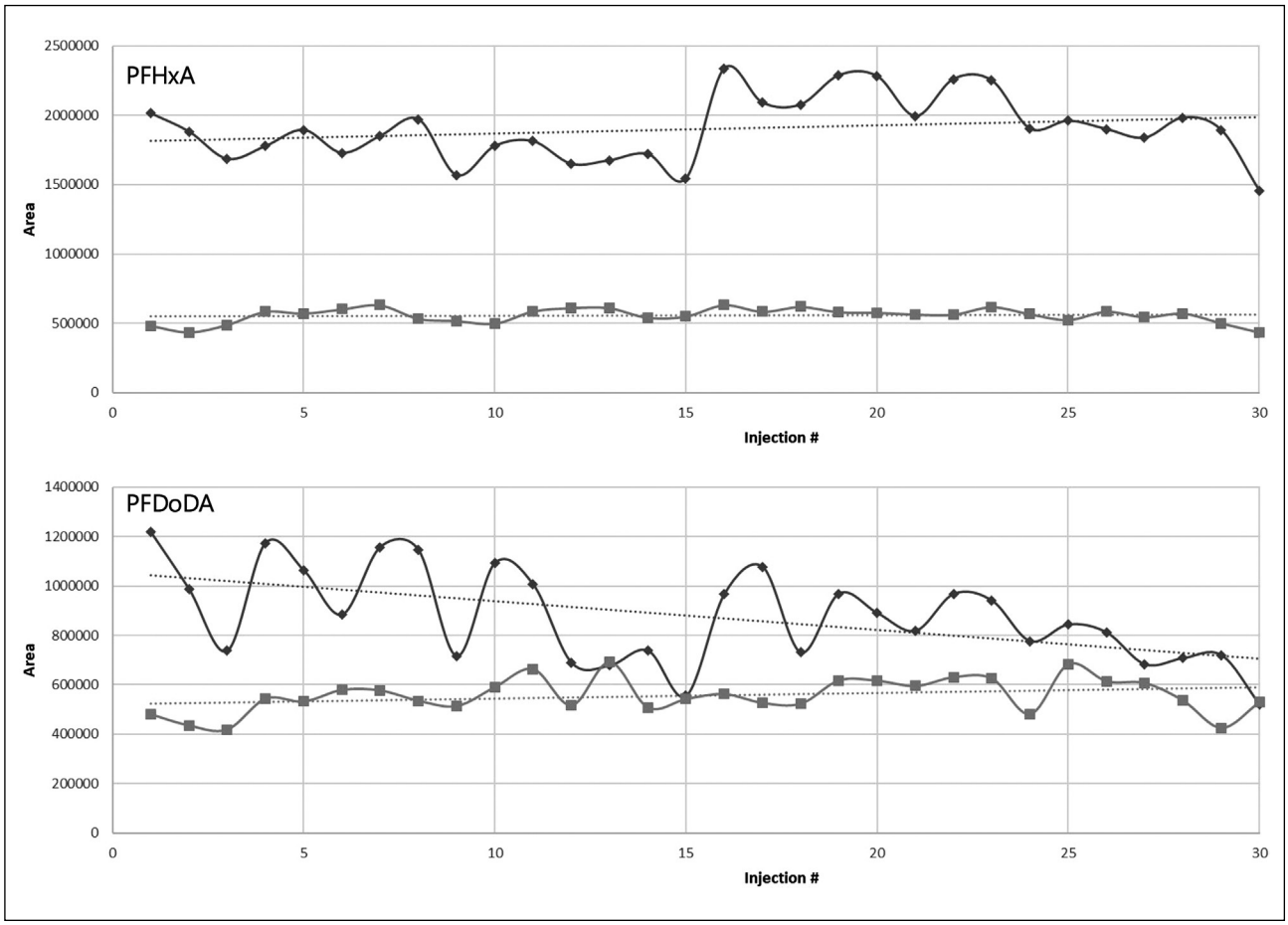


Figure 4. Stability curve over the number of injections comparing with (orange) and without (blue) adding methanol; exemplarily shown by the internal standards PFHxA and PFDoDA.

Figure 5. LCMS-8060NX coupled to a NexeraTM X3 system.

The Package

- Main Unit

  LCMS-8060NX:TQ Mass spectrometer

  Nexera X3: Liquid chromatograph
  CBM-40
  DGU-405
  2x LC-40D X3
  SIL-40C X3
  CTO-40c
  Reservoir Tray

- Accessory

  Mixer: Mir20 µL

  Loop: 500 µl

- Main Consumables:

  Shim-pack Scepter C18

  (150 mm × 3 mm I.D., 3 µm; P/N 227-31015-04)

  Shim-pack GIST HP C18-AQ

  (30 mm × 3.0 mm I.D., 3 µm; P/N 227-30766-01)

  Shimadzu LabTotal Vial for LC/LCMS

  (P/N 227-34001-01)

  PP-caps, with aluminium septa

  (P/N 961-10030-31, this part number is available in EU area only. If you are in another territory, please contact for your Shimadzu local office.)

- Software and Libraries

  LabSolutions LCMS

  LabSolutions Insight

INSTRUMENT: PEGASUS® GC-HRT 4D

CHARACTERIZATION OF EXTRACTABLES FROM COMMON PHARMACEUTICAL PACKAGING MATERIALS WITH GC×GC AND HR-TOFMS

Key Words: Extractable and Leachable, E&L, Pharmaceutical Materials, GC×GC, HR-TOFMS

Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) and high-resolution time-of-flight mass spectrometry (HR-TOFMS) were used to characterize extracts from pharmaceutically relevant materials. Butyl rubber stoppers and plastic syringes were extracted with methylene chloride and subsequently analyzed with the Pegasus® GC×GC-HRT+4D, equipped with a Multi-Mode Ion Source™ (MMS™) (LECO Corporation, St. Joseph, MI, USA). The use of thermal modulation in combination with both a nonpolar and polar column, significantly increased separation selectivity and peak capacity, providing cleaner spectra for interpretation. HR-MS with both electron ionization and chemical ionization (EI and CI) provided spectra for commercial library searching and accurate mass data for formulae determinations and/or to support fragments and molecular ions. Chromatographic elution order in both dimensions —first dimension retention index (RI) and structured GC×GC chromatograms— was also used to support analyte identifications. Several representative materials were evaluated, and several representative analytes are highlighted.

Introduction

The characterization of extractable and leachable components in different types of materials and products is an important area of research. These studies enable insights into which chemicals (e.g., impurities, breakdown products, etc.) could be released from packaging or production materials (extractables) and what chemicals are released into products, such as pharmaceuticals or other consumer goods, which may have toxicological implications or concerns (leachables). Information about extractable and leachable components from packaging materials, delivery devices, and manufacturing equipment for pharmaceutical products is a particular area of growing interest and focus. Analytical testing to determine this information can also be part of submission requirements to the FDA or other regulatory bodies.

These analyses can be challenging due to the complexity of the samples and because there is the potential for many unknown features amongst the observed analytes. A variety of analytical methods can be used, and many approaches are compliant with testing guidance.[1] Generally, chromatographic separations coupled to mass spectrometry are needed to address both sample complexity and to determine tentative identifications.

In this work, we demonstrate the use of GC×GC and HR-TOFMS, with multiple ionization modes, to characterize the complex extracts. GC×GC enhances the chromatographic performance by separating the complex extracts with two stationary phases of contrasting polarities in a single analysis. This leads to an improved peak capacity, enhanced chromatographic separations, cleaner spectra, and provides additional context for identifications from the elution order. High-resolution MS detection, with EI and CI modes, enhances identification confidence by providing library searchable spectra, accurate mass, and molecular ion information for formulae support and/or determinations. Here, we highlight benefits and capabilities of this analytical approach, and show the analysis of representative samples and observed analytes.

Experimental

Extracts were prepared from various materials commonly used in packages and closures for pharmaceutical products. Butyl rubber stoppers and plastic syringes (with and without rubber components) were extracted with methylene chloride at room temperature for 72 hours. The extracts were then analyzed by GC×GC-HR-TOFMS (Pegasus HRT+ 4D, LECO Corporation, St. Joseph, MI, USA), as described in Table I. Each sample was analyzed with both EI and CI (meth-

Table I. Instrument (Pegasus GC-HRT+ 4D) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	1 µL
Gas Chromatograph	**LECO GC×GC QuadJet™ Thermal Modulator**
Inlet	280 °C, splitless
Carrier Gas	He @ 1.4 mL/min, constant flow
Column	Primary: Rxi-5ms, 30 m x 0.25 mm i.d. × 0.25 µm coating Secondary: Rxi-17Sil MS, 0.9 m × 0.25 mm i.d. × 0.25 µm coating
Temperature Program	50 °C (hold 2 min), ramp 8 °C/min to 340 °C (hold 5 min) Secondary Oven: +20 °C
Modulation	3 s with temperature maintained +15 °C relative to secondary oven
Transfer Line	350 °C
Mass Spectrometer	**LECO Pegasus HRT**
Ion Source	LECO MMS - EI and CI (methane)
Ion Source Temperature	250 °C (EI) and 165 °C (CI)
Mass Range	35-900 m/z (EI) and 60-900 m/z (CI)
Acquisition Rate	125 spectra/s

ane) using a *Multi-Mode Ionization Source (MMS)*. An alkane standard was also analyzed for retention index (RI) calculations.

Results and Discussion

Extracts from rubber and plastic materials associated with pharmaceutical products can be quite complex. These extracts contain a large number of analytes, including both known and unknown chemical features. Powerful analytical techniques are crucial for understanding these complex samples, and GC×GC with HR-MS is well-suited for this type of sample characterization. GC×GC chromatographically isolates more features, and HR-MS provides accurate mass data to assist with feature identification and understanding of these complex samples. Multiple complementary pieces of information are generated for each feature to support analyte identifications, or to help propose formulae and structures of complete unknowns. Individually, the enhanced chromatography and high resolution mass spectral data offer unique benefits for analysis of complex samples, and the combination of the two can take the analytical interpretation even further. An example of these capabilities is described in Figure 1.

GC×GC adds a second dimension of separation compared to single dimension GC, allowing significantly increased analyte separation to be achieved. Representative GC and GC×GC chromatograms for an extract of a plastic syringe with a rubber- tipped plunger are shown in Figure 1A. The GC data was collected with the same hardware and methods described in Table I, but with the modulator turned off. With GC×GC, the primary column effluent is modulated to a second column with a different polarity stationary phase where analytes are further separated, often allowing first dimension coelutions to be resolved. This leads to more chromatographically resolved analytes and cleaner MS spectra overall, enhancing identification ability. GC×GC also generates structured chromatograms where chemical compound classes elute in ordered bands across the GC×GC separation space, based on the difference in both analyte volatility and polarity, offering additional context to support identifications.

In some cases, the additional separation can uncover features that were obscured in the primary separation, as shown in Figure 1B, which highlights a small section of the separations shown in Figure 1A. The single GC separation determined a single analyte, tentatively identifying it as dodecyl acrylate. The GC×GC separation uncovered an additional feature, identifying this as 2,4-di-t-butyl-6-nitrophenol. The spectrum for the GC peak, shown in Figure 1C, had a good library match to dodecyl acrylate, but also contained some additional m/z that were not part of the library spectrum. The GC×GC data revealed that these additional m/z were from the second feature

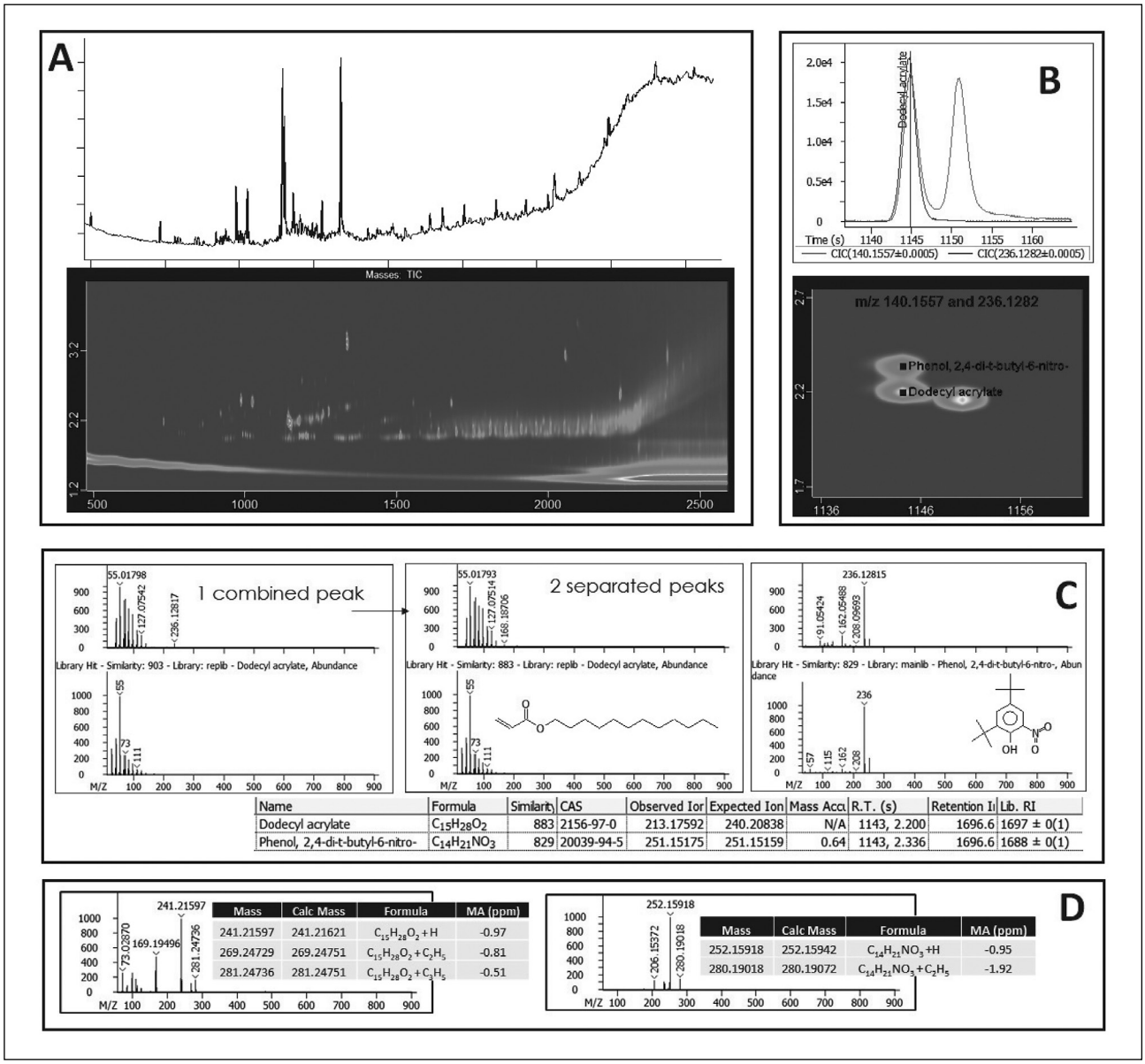


Figure 1. (A) Representative GC and GC×GC chromatograms for extracts from a plastic syringe with a rubber-tipped plunger. Analytes are separated in one dimension with GC and in two dimensions with the complementary stationary phases used for GC×GC. In some instances, GC×GC helps uncover new analytes that were hidden in the GC separation. (B) Here, a phenol compound and dodecyl acrylate completely coelute and are combined as one peak in the GC separation. Both were determined with GC×GC where the analytes were chromatographically separated in the second dimension. (C) The single peak true spectrum in the GC data is the combination of both peaks observed in the GC×GC data. Peak metrics like spectral similarity, first dimension RI, elution order in the structured GC×GC space, and mass accuracy supported these identifications. A molecular ion was observed for the phenol compound in the EI data also supporting the identification. (D) CI data added molecular ion information for dodecyl acrylate that did not have a molecular ion in the EI data and also supported the molecular ion for 2,4-di-t-butyl-6-nitrophenol. GC×GC revealed more information than could be determined with just GC.

merged together with the primary peak in the GC data. The additional peak was chromatographically separated in the second dimension using GC×GC, generating cleaner mass spectral data, and therefore enabling tentative identifications of both features to be made with greater confidence. As shown in the peak table in Figure 1C, these identifications are supported by spectral matching to the NIST library (simi-

larity scores of 883 and 829), and RI matching in the first dimension (RI observed of 1697 compared to library RI values of 1697 and 1688). The high-resolution data also supports identification further, providing the molecular ion for the phenol compound with a mass accuracy of 0.64 ppm. While a molecular ion was not observed for dodecyl acrylate in the EI data, mass fragmentation accuracies were good. With the *Multi-Mode Ion Source (MMS)*, additional CI data was readily collected and used to enhance and verify the molecular ions for both features, as shown in Figure 1D. The M+H adducts were observed with mass accuracies of -0.97 ppm and -0.95 ppm for dodecyl acrylate and the phenol compound, respectively.

As shown in Figure 1, GC×GC can uncover more features and HR-MS can then lead to tentative identifications of those features. Initial spectral matching to library databases typically provides the first proposed identification. The accurate mass data can then either support those identifications, as shown in Figure 1, or eliminate them as possibilities, as shown in Figure 2. The analyte shown in Figure 2 had a top library hit of an aromatic sulfur compound with a formula of $C_{13}H_{18}N_2S$, based on nominal mass spectral matching. However, this identification was not reasonable when comparing the observed accurate mass to the expected mass for that formula. If this were the formula for the observed molecular ion, the mass accuracy would be 182 ppm. The second library hit, with a formula of $C_{15}H_{22}O_2$, had a mass accuracy of -0.74 ppm. Switching from library hit #1 to library hit #2 provided a much-improved tentative identification, which also had support from spectral matching and RI.

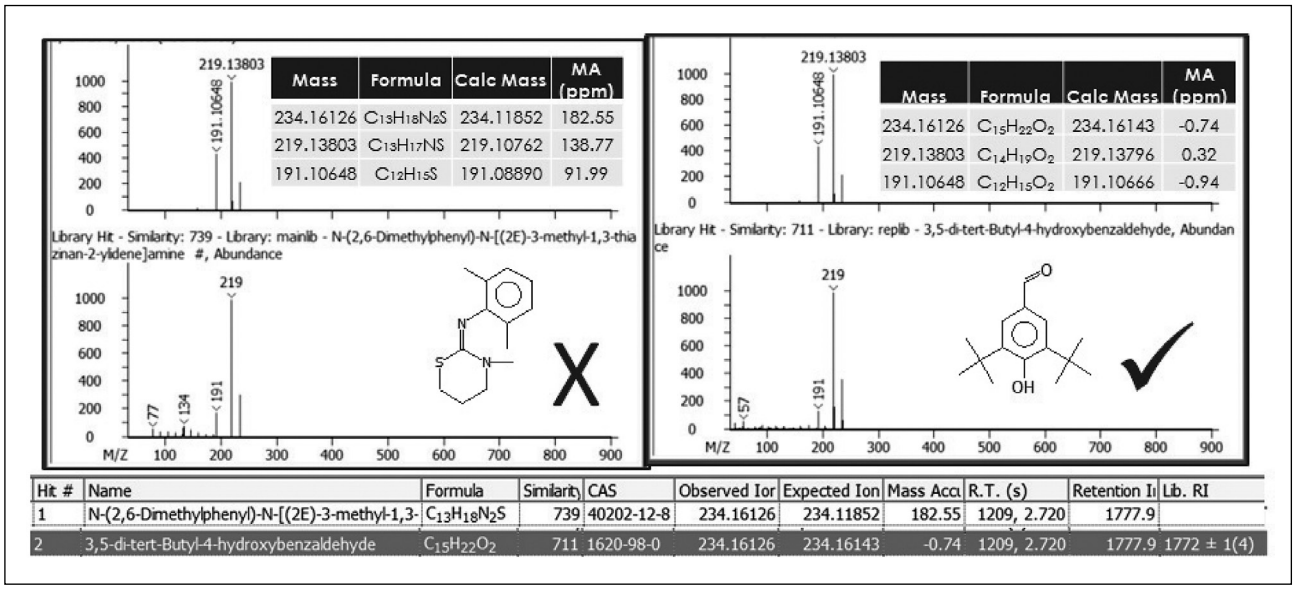


Figure 2. Accurate mass and formula determinations can also allow for selecting an improved spectral match from the library database. In this example from a plastic syringe extract, both library hit #1 and #2 were supported by nominal mass fragmentation patterns, but the formula for the first library hit was not well-supported and the second library hit was.

The information generated from this analysis can support or improve analyte identifications, as shown in Figures 1 and 2, and can also be used to better understand features when there is no preliminary library match, as shown in Figure 3. Four peaks (A, B, C, and D) from a rubber stopper extract are labeled in the chromatogram. These features did not have good spectral matches from the NIST library database. Thus, the observed accurate mass data were used to propose formulae. Feature A and B are supported with the formulae of $C_{13}H_{24}$ and $C_{21}H_{40}$ with mass accuracies of -1.13 ppm and –1.18 ppm, respectively. These are likely butyl rubber oligomers. Features C and D are likely chlorinated butyl rubber oligomers with formulae of $C_{13}H_{23}Cl$ with mass accuracies of –0.30 ppm and –0.87 ppm. In addition to the mass information, the chromatographic location of these features in the GC×GC contour plot adds support to the tentative identification. Furthermore, both butyl rubber oligomers and halogenated butyl rubber oligomers are anticipated analytes in rubber stopper extracts, which also adds support for these tentative identifications.

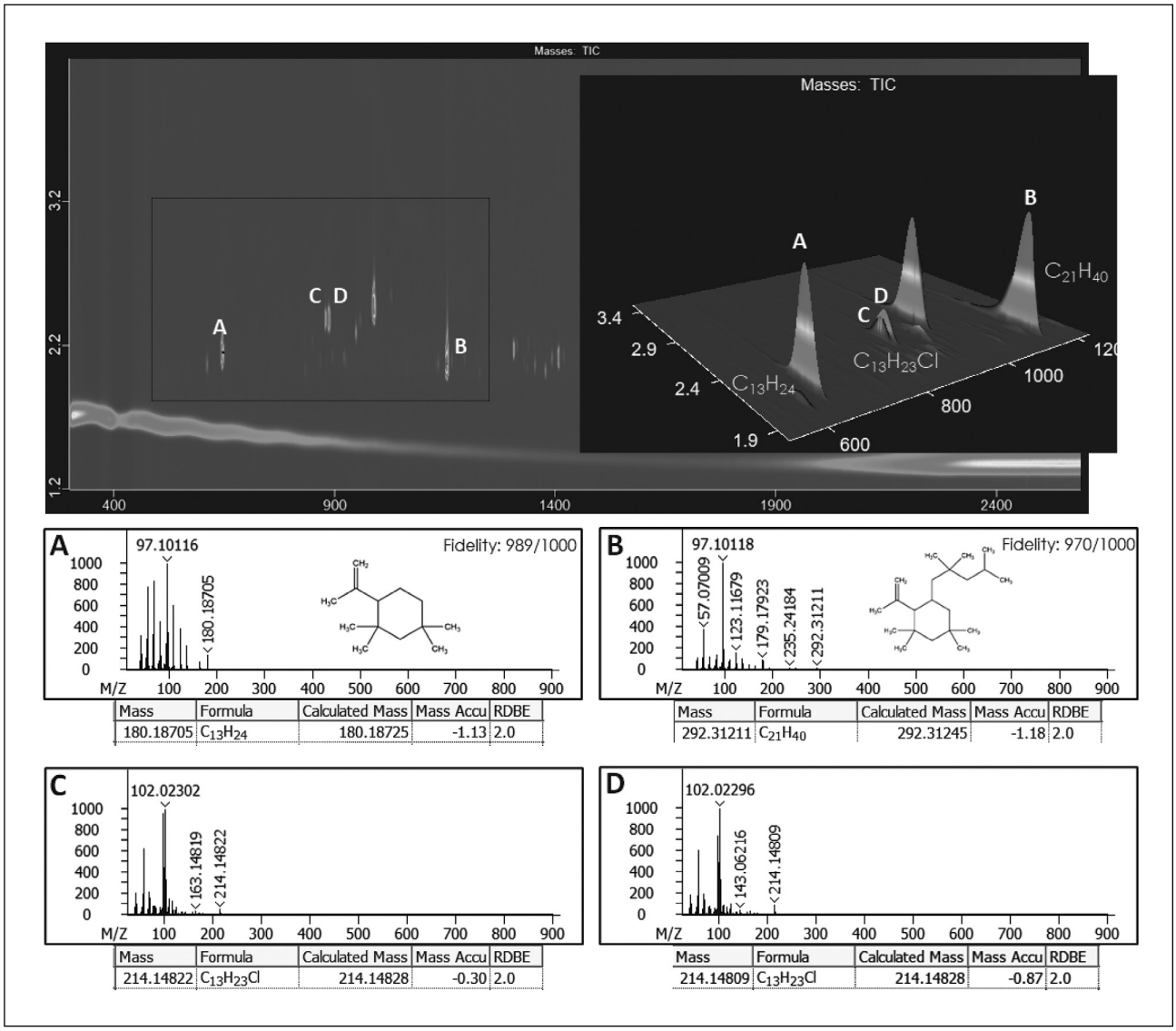


Figure 3. Accurate mass information can also help determine formulae for features that are not present in library databases. In this example, likely butyl rubber oligomers (A and B) and chlorinated butyl rubber oligomers (C and D) that were not in the NIST library database were observed in a rubber stopper extract. Accurate mass information was used to determine the formula. Elution position in the structured space and anticipated presence in these sample types also supported the tentative identifications.

The benefits and capabilities of this analytical approach allow for excellent characterization of complex samples like these extracts of pharmaceutical contact materials. As demonstrated in Figures 1-3, this analytical approach can isolate more individual analytes and provides data to support or propose identifications. A variety of sample extracts from plastic syringes, with and without rubber-tipped plungers and butyl rubber stoppers, were evaluated with GC×GC and HR-TOFMS. Representative GC×GC chromatograms are shown in Figure 4. Both the complexity and variation of the samples are apparent. GC×GC helped separate individual analytes from each other and from matrix background in the samples, and HR-MS helped to determine identifications for these features.

There are many analytes of interest in these samples and some representative examples are listed in Table II. Support for these tentative identifications is listed in the table and can include spectral matching to library databases, matching accurate mass data for

the molecular ion using EI and/or CI, library RI matching, and elution in the GC×GC structured space. The different materials had different extracts, as shown in Figure 4. Marks in columns A-E, corresponding to extracts from samples A-E, indicate whether that feature was observed in each sample.

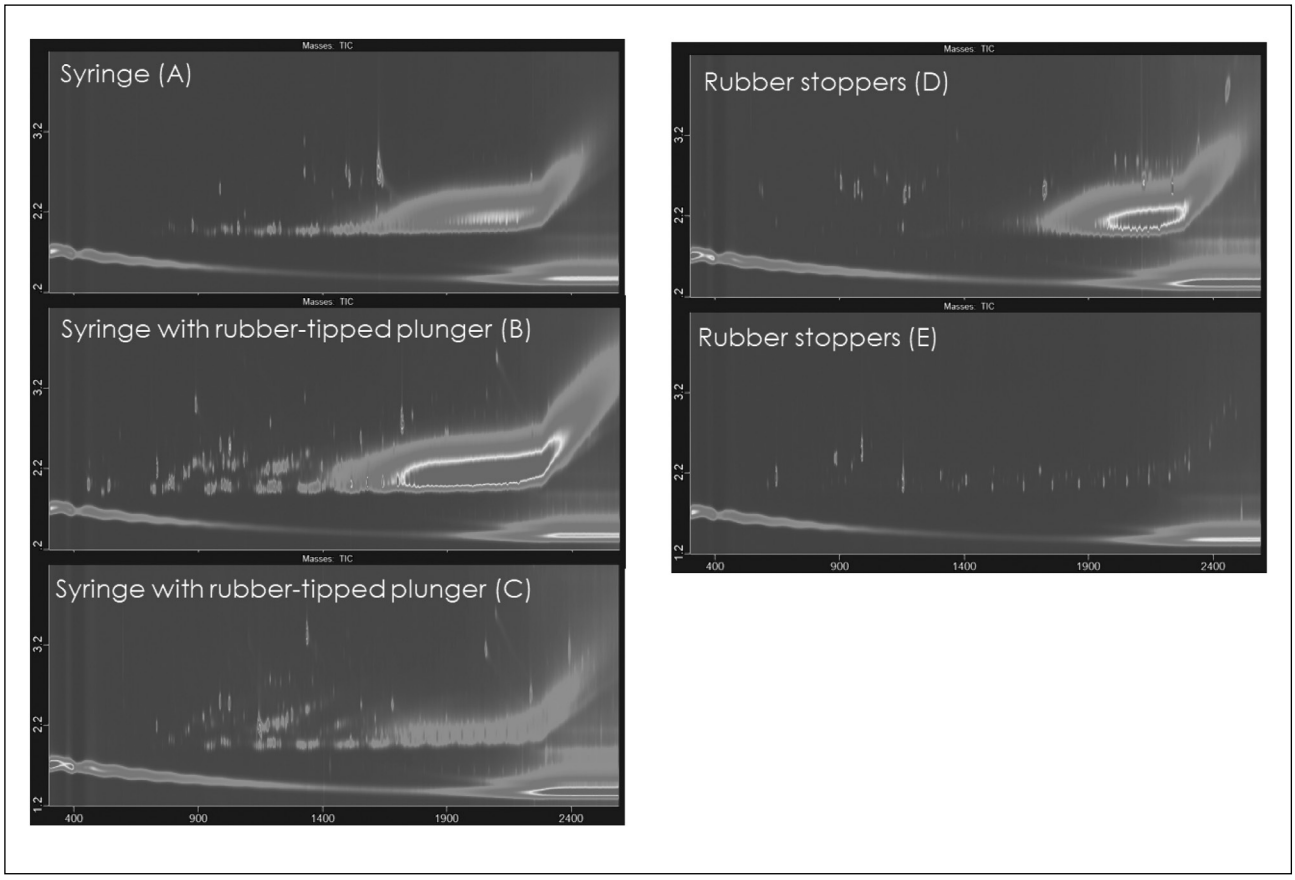


Figure 4. GC×GC-HR-TOFMS data for five different samples are shown. One plastic syringe without a rubber-tipped plunger (A), two syringes with rubber-tipped plungers (B and C), and two rubber stoppers (D and E) were evaluated. GC×GC was important for separating the components in these complex samples, and HR-TOFMS (with both EI and CI) was crucial for determining the identifications. Several analyte examples are shown in Table II.

Conclusion

In this work, GC×GC-HR-TOFMS was used to evaluate extracts from several pharmaceutically relevant packaging/delivery system materials. Two dimensions of separation helped address sample complexity and high-resolution MS helped address analyte identification requirements. GC×GC improved the peak capacity of the separation, which allowed for chromatographically isolating more analytes from each other and from interferences. This uncovered new analyte peaks, provided spectra with fewer interferences, and led to more identified analytes compared to one-dimensional GC. Incorporating EI and CI accurate m/z information from HR-TOFMS led to better identifications with more confidence. Analyte identifications were supported with spectral matching, accurate mass formulae, and GC×GC elution position. In situations without library matches, this data can help with proposing chemical formulae and structures. Several examples to highlight these benefits were shown, as well as representative samples and representative analytes.

References

[1] USP 1663 "Assessment of Extractables Associated with Pharmaceutical Packing/Delivery Systems"

Table II. Representative analytes of interest in the samples.

Analyte Name	Similarity	CAS	Formula	Obs Ion m/z	Expected m/z	MA (ppm)	R.T. (s)	RI (Obs:Lib)	Notes	A	B	C	D	E
Triacetonamine	896	826-36-8	$C_9H_{17}NO$	155.1304	155.23772	–0,42	585, 2.616	1124 : 1137	*stabilizer*		✓	✓		
Butyl Oligomer	NA		$C_{13}H_{24}$	180.18705	180.18725	–1.13	645, 2.152	1178 : NA	*butyl oligomer*		✓	✓	✓	✓
m-Di-tert-buthylbenzene	937	1014-60-4	$C_{14}H_{22}$	190.17164	190.32500	0.22	732, 2.184	1261 : 1249	*polymer linking agent*	✓	✓	✓		
Chlorinated oligomer	NA		$C_{13}H_{23}Cl$	214.14822	214.14828	–0.30	879, 2.376	1404 : NA	*halogenated oligomer*		✓	✓	✓	✓
4-tert-Pentylphenol	891	80-46-6	$C_{11}H_{16}O$	164.11948	164.24455	–0.54	882, 2.720	1408 : 1400	*antioxidant*		✓	✓	✓	
Chlorinated oligomer	NA		$C_{13}H_{23}Cl$	214.14809	214.14828	–0.87	888, 2.376	1414 : NA	*halogenated oligomer*		✓	✓	✓	✓
Diphenyl ether	942	101-84-8	$C_{12}H_{10}O$	170.07252	170.20764	–0.59	888, 2.976	1414 : 1405			✓	✓		
Phenol, 2-chloro-4-tert-pentyl-	937	5323-65-9	$C_{11}H_{15}ClO$	198.08052	198.68955	–0.38	903, 2.624	1430 : 1460	*phenol*		✓	✓	✓	✓
Phenol, 2-(1,1-dimethylethyl) - 4-ethyl	907	96-70-8	$C_{12}H_{18}O$	178.13520	178.27117	–0.12	906, 2.568	1433 : 1459	*phenol*		✓			
2,6-Di-tert-butylquinone	861	719-22-2	$C_{14}H_{20}O_2$	220.14592	220.30793	0.61	948, 2.384	1477 : 1472		✓	✓	✓	✓	✓
2,4-Di-tert-butylphenol	940	96-76-4	$C_{14}H_{22}O$	206.16645	206.32440	–0.32	987, 2.489	1519 : 1514	*antioxidant*	✓	✓	✓		
Butylated Hydroxytoluene	916	128-37-0	$C_{15}H_{24}O$	220.18214	220.35102	–0.12	990, 2.456	1522 : 1513	*antioxidant*	✓	✓	✓	✓	✓
Isopropyl laurate	904	10233-13-3	$C_{15}H_{30}O_2$ +H	203.19044	242.39807	–0.36	1086, 2.176	1630 : 1618		✓	✓	✓		
Diphenylamine	866	122-39-4	$C_{12}H_{11}N$	169.08857	169.22288	–0.17	1104, 3.229	1651 : 1622				✓		
Dodecyl acrylate	883	2156-97-0	$C_{15}H_{28}O_2$ +H	213.17592	240.38219	–0.97	1143, 2.200	1697 : 1697	*acrylate*			✓		
Phenol, 2,4-di-t-butyl-6-nitro-	829	20039-94-5	$C_{14}H_{21}NO_3$	251.15175	251.32198	0.64	1143, 2.336	1697 : 1688	*phenol*	✓	✓	✓	✓	
Butyl Oligomer	NA		$C_{21}H_{40}$	292.31211	292.31245	–1.18	1152, 2.072	1708 : NA	*butyl oligomer*				✓	✓
4-tert-Butyldiphenyl ether	890	5331-28-2	$C_{16}H_{18}O$	226.13520	226.31411	–0.05	1188, 2.768	1752 : NA	*phenol*		✓	✓		
4-Formyl-2,6-di-tert-buthylphenol	834	1620-98-0	$C_{15}H_{22}O_2$	234.16137	234.33454	–0.28	1209, 2.736	1778 : 1772	*phenol*	✓	✓	✓	✓	✓
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	925	82304-66-3	$C_{17}H_{24}O_3$	276.17210	276.37130	0.37	1326, 2.696	1933 : 1923		✓	✓	✓	✓	
9,9-Dimethylacridan	926	6267-02-3	$C_{15}H_{15}N$	209.11982	209.28685	–0.39	1335, 3.328	1945 : NA				✓		
Metilox	759	6386-38-5	$C_{18}H_{28}O_3$	292.20331	292.41380	0.05	1341, 2.568	1953 : 1943	*plasticizer*	✓				
Dibutyl phthalate	908	84-74-2	$C_{16}H_{22}O_4$ +H	224.09991	278.34409	–1.97	1359, 2.752	1977 : 1965	*plasticizer*	✓	✓	✓		
Stearyl aldehyde	862	638-66-4	$C_{18}H_{36}O$ +H	269.28377	269.28389	–0.44	1395, 2.232	2027 : 2021			✓			
Palmitoleamide	904	106010-22-4	$C_{16}H_{31}NO$	253.23985	253.42405	–0.64	1494, 2.703	2172 : 2153		✓				
Hexadecanamide	877	629-54-9	$C_{16}H_{33}NO$	255.25574	255.43993	0.28	1506, 2.592	2190 : 2184		✓	✓			
N,N-Dimethyl palmitamide	823	3886-91-7	$C_{18}H_{37}NO$	283.28650	283.49316	–1.64	1554, 2.456	2265 : 2256		✓	✓	✓		
Oleamide	911	301-02-0	$C_{18}H_{35}NO$	281.27072	281.47728	–2.13	1626, 2.648	2381 : 2386	*slip agent*	✓				
Cyanox 425	916	88-24-4	$C_{25}H_{36}O_2$	368.27109	368.55307	0.29	1719, 2.752	2541 : 2529	*rubber antioxidant*		✓			
Irgafos 168	608	31570-04-4	$C_{42}H_{63}O_3P$	646.45084	646.92315	–0.15	2160, 2.429	3438 : 3397	*processing stabilizer*	✓				
Irganox 1076	838	2082-79-3	$C_{35}H_{62}O_3$	530.46981	530.86630	0.86	2235, 2.536	3620 : 2603	*antioxidant*	✓	✓	✓	✓	
Tris(2,4-di-tert-butylphenyl) phosphate	869	95906-11-9	$C_{42}H_{63}O_4P$	662.44656	662.92256	1.08	2238, 2.600	3628 : 3582	*Irgafos transformation product*	✓	✓	✓	✓	

SCIEX

The Power of Precision

IMPROVING COMPLEX PHOSPHOPEPTIDE CHARACTERIZATION WITH HYBRID EAD/CID MS/MS FRAGMENTATION

Jeremy Potriquet[1], Patrick Pribil[2] and Daniel Winter[3]

[1] SCIEX Australia; [2] SCIEX Canada; [3] All G Foods

This technical note describes the use of combined electron activated dissociation (EAD) and collision-induced dissociation (CID) on the ZenoTOF 7600 system to enhance the fragmentation of peptides from casein tryptic digests (Figure 1).

In many organisms, proteins can exist in multiple isoforms and have diverse post-translational modifications (PTMs). Characterizing proteins invariably requires a deep understanding of the nature of these PTMs and their effects on protein structure and biological function. Mass spectrometry can be used for PTM characterization through different modes of fragmentation of modified peptides. Compared with EAD or CID alone, using a hybrid EAD/CID fragmentation approach improved the sequence coverage for casein peptides and the characterization of PTMs on these peptides. In particular, the hybrid EAD/CID approach was useful in differentiating between multi-phosphorylated peptide isomers, leading to the unambiguous assignment of phosphorylation sites in casein peptide sequences. We demonstrate that this differentiation can be achieved at high acquisition speeds, with limited sample preparation and without the need for derivatization.

Key features of the ZenoTOF 7600 system for phosphopeptide characterization

- Zeno trap pulsing allows accumulation of ions during TOF pulsing for enhanced duty cycle, generating higher quality MS/MS spectra for low abundant targets such as phosphopeptides.
- EAD fragmentation preserves phosphorylation sites and accurate positional information due to the generation of c and z+1 fragment ions.
- When EAD is combined with CID, nearly full sequence coverage can be achieved even with larger peptides with multiple modifications.
- PTM data processing can be performed using SCIEX OS software and is compatible with other processing softwares such as Skyline software, MSFragger algorithm and Peaks Studio software.

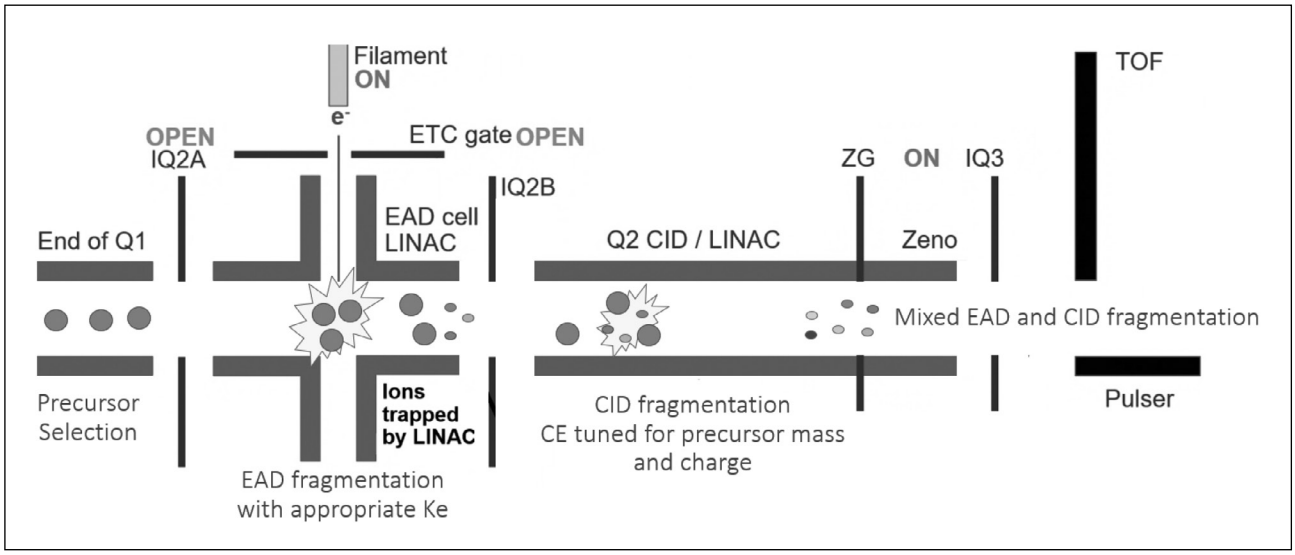


Figure 1. Concept of hybrid EAD/CID fragmentation on the ZenoTOF 7600 system.

Introduction

Caseins are an important component of milk and play a major role in diets worldwide, as they are highly nutritious and provide essential amino acids. Milk proteins include 4 caseins (αs1-casein, αs2-casein, β-casein and κ-casein) and 2 major whey proteins (α-lactalbumin and β-lactoglobulin).[1] Casein studies have shown that this group of proteins can have extremely complex PTMs with αs1-casein harboring 9-10 phosphorylations, αs2-casein harboring 10-13 phosphorylations and β-casein harboring 5 phosphorylations. In addition, glycosylations and many genetic variants in which specific amino acid replacements occur can change protein functionality.[2] Studies have shown that phosphorylation of the casein proteins plays a critical role in the formation of casein micelles and in the interaction with, and transport of, divalent cations such as calcium and zinc. These casein functions are important to facilitate the adsorption of these nutrients in the gut of the nursing infant.[3,4,5,6]

Characterizing phosphorylation sites on casein proteins is inherently difficult because casein-containing mixtures include peptides with highly variable levels of phosphorylation. Overcoming the resulting ionization suppression in positive-ion mass spectrometry (MS) often requires the derivatization of phosphoserines.[7] These analysis limitations are significant because recent improvements in recombinant DNA technology allow the possibility for large-scale production of recombinant milk proteins. With the significant economic interest and growing need for alternative sustainable food production, it is essential to accurately and rapidly monitor milk batches for genetic variants and to characterize the sites and relative ratios of phosphorylations.

Here, the ability to perform a hybrid EAD/CID fragmentation is demonstrated to provide enhanced sequence coverage and greater confidence in assigning phosphorylation sites on casein proteins with complex phosphorylation profiles.

Methods

Sample preparation: Casein proteins (native β-casein, αS1-casein and αS2-casein) were purified from cow's milk by precipitation under low-pH conditions, then solubilized in 6 M urea and further purified by ion exchange. Samples were digested with trypsin, desalted in pure water, lyophilized and resuspended in water with 0.1% formic acid to the desired concentration. Sample loadings ranged from 400 ng to 700 ng.

Chromatography: Separations were performed using a Waters ACQUITY UPLC M-Class system plumbed for microflow chromatography (7 µL/min) and operated in direct-inject mode. The analytical column was a ProteCol PEEKSIL C18G column (3 µm, 200 Å, 250 × 0.3 mm). Column temperature was controlled at 40 °C. A 25-min gradient was used for all data-de-

pendent acquisition (DDA) experiments, as shown in Table 1. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile.

Table 1. Chromatographic gradient for peptide separations.

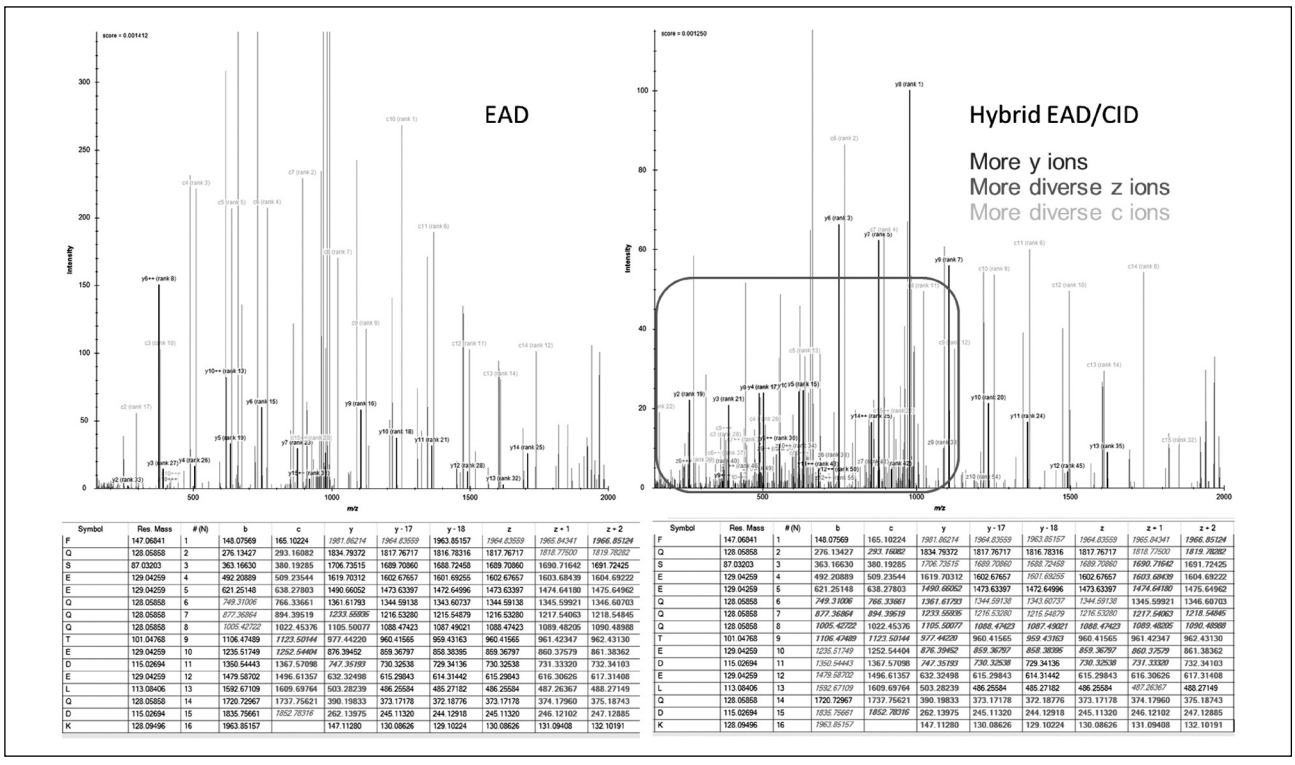
Time (min)	Mobile phase A (%)	Mobile phase B (%)
1.0	97	3
20.0	97	3
20.1	20	80
22.0	20	80
22.1	97	3
25.0	97	3

Mass spectrometry: Data were acquired using the ZenoTOF 7600 system in DDA mode. TOF MS scans were 200 ms across a mass range of 400-1750 m/z. The MS/MS scan range was 100-2000 m/z using a hybrid EAD/CID fragmentation approach with 65 ms accumulation times and 30 ms reaction times. An electron beam current setting of 3000 nA and an electron KE setting of 2 eV were used. Dynamic collision energy (CE) was applied for CID and hybrid EAD/CID. Source conditions included 20 psi for GS1, 15 psi for GS2, 35 psi for curtain gas, 5000 V for spray voltage and 150°C for source temperature. The top 15 candidates were selected for MS/MS with charge states from 2 to 4, with an exclusion of 5 s after 1 occurrence.

***Data processing*:** Data were processed with Skyline software using the DDA peptide search tool using the MSAmanda search engine with 25 ppm MS1 and MS2 mass tolerances and the MSFragger algorithm. Spectrum identifications were manually confirmed and annotated using SCIEX OS software.

Combining EAD and CID fragmentation to generate information-rich spectra

The ZenoTOF 7600 system allows either EAD fragmentation alone or the ability to do consecutive EAD and CID fragmentation on isolated precursors, with no difference in overall cycle times between these 2 approaches. Hybrid EAD/CID fragmentation generates richer MS/MS spectra which can increase confidence in sequence assignments, as shown in Figure 2 for the



Symbol	Res. Mass	# (N)	b	c	y	y - 17	y - 18	z	z + 1	z + 2
F	147.06841	1	148.07569	165.10224	*1981.86214*	*1964.83559*	1963.85157	*1964.83559*	*1965.84341*	*1966.85124*
Q	128.05858	2	276.13427	293.16082	1834.79372	1817.76717	1816.78316	1817.76717	*1818.77500*	*1819.78282*
S	87.03203	3	363.16630	380.19285	1706.73515	1689.70860	1688.72458	1689.70860	1690.71642	1691.72425
E	129.04259	4	492.20889	509.23544	1619.70312	1602.67657	1601.69255	1602.67657	1603.68439	1604.69222
E	129.04259	5	621.25148	638.27803	1490.66052	1473.63397	1472.64996	1473.63397	1474.64180	1475.64962
Q	128.05858	6	*749.31006*	766.33661	1361.61793	1344.59138	1343.60737	1344.59138	1345.59921	1346.60703
Q	128.05858	7	*877.36864*	894.39519	*1233.55935*	1216.53280	1215.54879	1216.53280	1217.54063	1218.54845
Q	128.05858	8	*1005.42722*	1022.45376	1105.50077	1088.47423	1087.49021	1088.47423	1089.48205	1090.48988
T	101.04768	9	1106.47489	*1123.50144*	977.44220	960.41565	959.43163	960.41565	961.42347	962.43130
E	129.04259	10	1235.51749	*1252.54404*	876.39452	859.36797	858.38395	859.36797	860.37579	861.38362
D	115.02694	11	1350.54443	1367.57098	*747.35193*	730.32538	729.34136	730.32538	731.33320	732.34103
E	129.04259	12	1479.58702	1496.61357	632.32498	615.29843	614.31442	615.29843	616.30626	617.31408
L	113.08406	13	1592.67109	1609.69764	503.28239	486.25584	485.27182	486.25584	487.26367	488.27149
Q	128.05858	14	1720.72967	1737.75621	390.19833	373.17178	372.18776	373.17178	374.17960	375.18743
D	115.02694	15	1835.75661	*1852.78316*	262.13975	245.11320	244.12918	245.11320	246.12102	247.12885
K	128.09496	16	1963.85157		147.11280	130.08626	129.10224	130.08626	131.09408	132.10191

Symbol	Res. Mass	# (N)	b	c	y	y - 17	y - 18	z	z + 1	z + 2
F	147.06841	1	148.07569	165.10224	*1981.86214*	*1964.83559*	*1963.85157*	*1964.83559*	*1965.84341*	*1966.85124*
Q	128.05858	2	*276.13427*	*293.16082*	1834.79372	1817.76717	1816.78316	1817.76717	*1818.77500*	*1819.78282*
S	87.03203	3	363.16630	380.19285	*1706.73515*	*1689.70860*	*1688.72458*	*1689.70860*	*1690.71642*	*1691.72425*
E	129.04259	4	492.20889	509.23544	1619.70312	1602.67657	*1601.69255*	1602.67657	*1603.68439*	1604.69222
E	129.04259	5	621.25148	638.27803	*1490.66052*	1473.63397	1472.64996	1473.63397	*1474.64180*	1475.64962
Q	128.05858	6	*749.31006*	*766.33661*	*1361.61793*	*1344.59138*	*1343.60737*	*1344.59138*	1345.59921	1346.60703
Q	128.05858	7	*877.36864*	*894.39519*	*1233.55935*	*1216.53280*	*1215.54879*	*1216.53280*	*1217.54063*	*1218.54845*
Q	128.05858	8	*1005.42722*	1022.45376	*1105.50077*	*1088.47423*	*1087.49021*	*1088.47423*	*1089.48205*	*1090.48988*
T	101.04768	9	*1106.47489*	*1123.50144*	*977.44220*	960.41565	*959.43163*	960.41565	961.42347	962.43130
E	129.04259	10	*1235.51749*	*1252.54404*	*876.39452*	*859.36797*	*858.38395*	*859.36797*	*860.37579*	861.38362
D	115.02694	11	*1350.54443*	*1367.57098*	*747.35193*	*730.32538*	729.34136	*730.32538*	*731.33320*	*732.34103*
E	129.04259	12	*1479.58702*	1496.61357	632.32498	615.29843	614.31442	615.29843	616.30626	617.31408
L	113.08406	13	*1592.67109*	1609.69764	503.28239	486.25584	485.27182	486.25584	*487.26367*	488.27149
Q	128.05858	14	1720.72967	1737.75621	390.19833	373.17178	372.18776	373.17178	374.17960	375.18743
D	115.02694	15	*1835.75661*	*1852.78316*	262.13975	245.11320	244.12918	245.11320	246.12102	247.12885
K	128.09496	16	*1963.85157*		147.11280	130.08626	129.10224	130.08626	131.09408	132.10191

Figure 2. Effect of hybrid EAD/CID fragmentation on the MS/MS spectra for the β-casein peptide FQSEEQQQTEDELQDK. The theoretical fragment ions for this peptide are shown in the table. The fragment ions observed in the MS/MS spectra that matched the theoretical fragment ions are highlighted in red.

example β-casein peptide FQSEEQQQTEDELQDK. While EAD fragmentation provided excellent sequence coverage with MS/MS spectra that have predominantly c, z, z+1 and z+2 fragments, using hybrid EAD/CID fragmentation generated more diverse ions, especially in the low-mass region.

Increased sequence coverage using hybrid EAD/CID for challenging larger peptides

Combining EAD and CID fragmentation is also valuable for increasing sequence coverage on larger peptides, which might be harder to fragment (Figure 3). The αS1-casein peptide QFYQLDAYPSGAWYYVPLGTQYT-

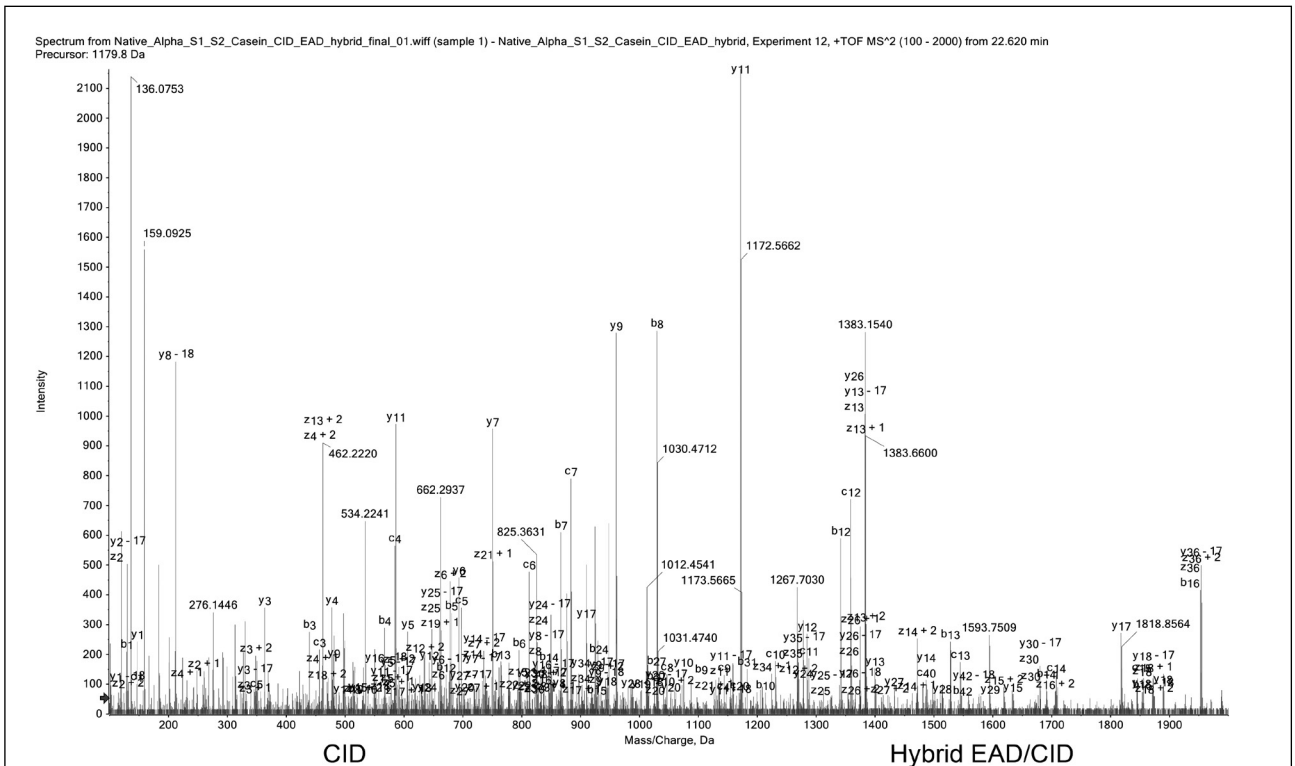
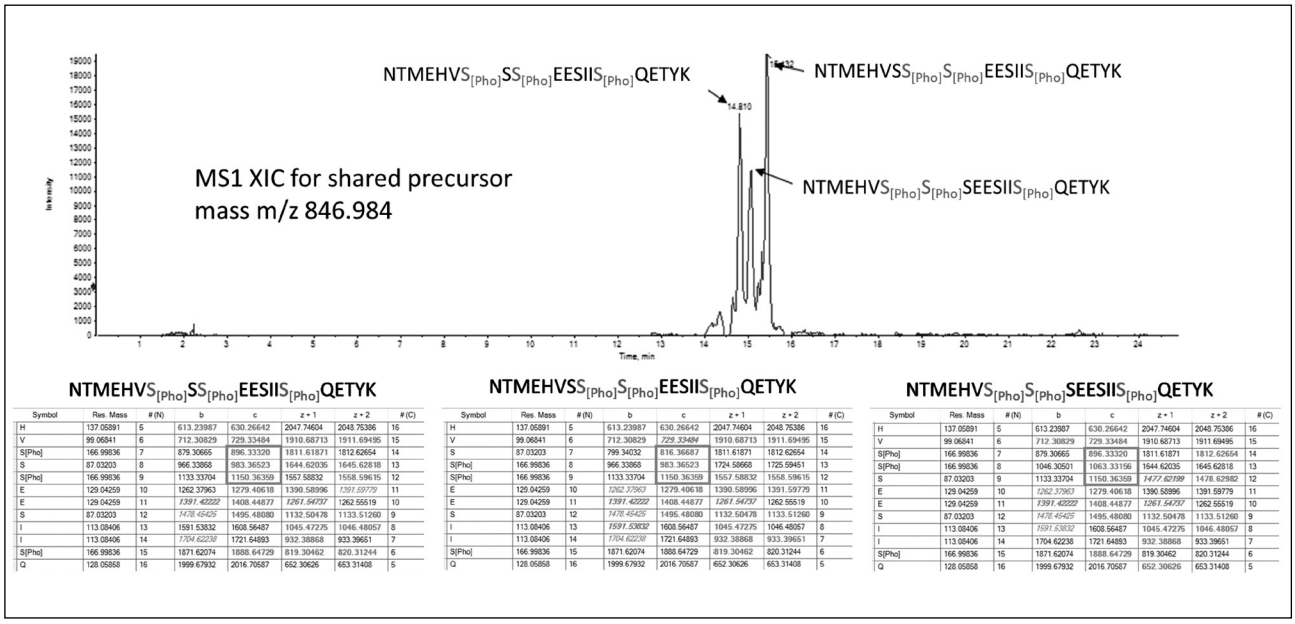


Figure 3. Comparison of MS/MS spectra and sequence coverage between the CID and hybrid EAD/CID fragmentation for the αS1-casein peptide QFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEK. The theoretical fragment ions for this peptide are shown in the table. The fragment ions observed in the MS/MS spectra that matched the theoretical fragment ions are highlighted in red.

DAPSFSDIPNPIGSENSEK contains 42 amino acids and 4 proline residues and benefits significantly from the use of hybrid EAD/CID fragmentation. The observed abundance of b, c, y, z+1 and z+2 fragment ions provides near complete sequence coverage, including for the regions of the peptide with proline residues that are typically challenging to sequence with EAD fragmentation.

Phosphorylation site differentiation and multi-phosphorylated peptide sequence coverage

When analyzing mixtures of isomeric phosphopeptides, chromatographic separation is a key method used to overcome ionization suppression of some of the phosphorylated variants. As shown in Figure 4, the 3 isomeric variants of the triple-phosphorylated αS2-casein peptide NTMEHVSSSEESIISQETYK were chromatographically resolved through separation using a relatively long gradient on a 250 mm analytical column. Using a DDA approach with hybrid EAD/CID fragmentation allowed the identification of all the different positional variants using the MSFragger algorithm. It was subsequently confirmed with Skyline software using the MSAmanda search function against a FASTA casein database. The results were annotated using SCIEX OS software as shown in Figure 4. Notably, excellent complementarity of fragment ion information was observed between b, c, y, z, z+1 and z+2 ions, with the c, z+1 and z+2 ion series contributing most significantly to the correct assignment of the modifications. No loss of fragment ions carrying labile modifications was observed when combining EAD and CID fragmentation.



Symbol	Res. Mass	# (N)	b	c	z + 1	z + 2	# (C)
H	137.05891	5	613.23987	630.26642	2047.74604	2048.75386	16
V	99.06841	6	712.30829	729.33484	1910.68713	1911.69495	15
S[Pho]	166.99836	7	879.30665	896.33320	1811.61871	1812.62654	14
S	87.03203	8	966.33868	983.36523	1644.62035	1645.62818	13
S[Pho]	166.99836	9	1133.33704	1150.36359	1557.58832	1558.59615	12
E	129.04259	10	1262.37963	1279.40618	1390.58996	*1391.59779*	11
E	129.04259	11	*1391.42222*	1408.44877	*1261.54737*	1262.55519	10
S	87.03203	12	*1478.45425*	1495.48080	1132.50478	1133.51260	9
I	113.08406	13	1591.53832	1608.56487	1045.47275	1046.48057	8
I	113.08406	14	*1704.62238*	1721.64893	932.38868	933.39651	7
S[Pho]	166.99836	15	1871.62074	1888.64729	819.30462	820.31244	6
Q	128.05858	16	1999.67932	2016.70587	652.30626	653.31408	5

Symbol	Res. Mass	# (N)	b	c	z + 1	z + 2	# (C)
H	137.05891	5	613.23987	630.26642	2047.74604	2048.75386	16
V	99.06841	6	*712.30829*	*729.33484*	1910.68713	1911.69495	15
S	87.03203	7	799.34032	816.36687	1811.61871	1812.62654	14
S[Pho]	166.99836	8	966.33868	983.36523	1724.58668	1725.59451	13
S[Pho]	166.99836	9	1133.33704	1150.36359	1557.58832	1558.59615	12
E	129.04259	10	*1262.37963*	1279.40618	1390.58996	1391.59779	11
E	129.04259	11	*1391.42222*	1408.44877	*1261.54737*	1262.55519	10
S	87.03203	12	*1478.45425*	1495.48080	1132.50478	1133.51260	9
I	113.08406	13	*1591.53832*	1608.56487	1045.47275	1046.48057	8
I	113.08406	14	*1704.62238*	1721.64893	932.38868	933.39651	7
S[Pho]	166.99836	15	1871.62074	1888.64729	819.30462	820.31244	6
Q	128.05858	16	1999.67932	2016.70587	652.30626	653.31408	5

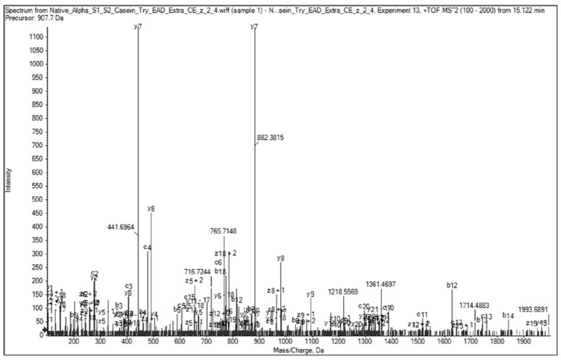
Symbol	Res. Mass	# (N)	b	c	z + 1	z + 2	# (C)
H	137.05891	5	613.23987	630.26642	2047.74604	2048.75386	16
V	99.06841	6	*712.30829*	729.33484	1910.68713	1911.69495	15
S[Pho]	166.99836	7	879.30665	896.33320	1811.61871	1812.62654	14
S[Pho]	166.99836	8	1046.30501	1063.33156	1644.62035	1645.62818	13
S	87.03203	9	1133.33704	1150.36359	*1477.62189*	1478.62982	12
E	129.04259	10	*1262.37963*	1279.40618	1390.58996	1391.59779	11
E	129.04259	11	*1391.42222*	1408.44877	*1261.54737*	1262.55519	10
S	87.03203	12	*1478.45425*	1495.48080	1132.50478	1133.51260	9
I	113.08406	13	*1591.53832*	1608.56487	1045.47275	1046.48057	8
I	113.08406	14	*1704.62238*	1721.64893	932.38868	933.39651	7
S[Pho]	166.99836	15	1871.62074	1888.64729	819.30462	820.31244	6
Q	128.05858	16	1999.67932	2016.70587	652.30626	653.31408	5

Figure 4. Extracted ion chromatogram (XIC) and MS/MS sequence coverage with phosphorylation site differentiation for the multi-phosphorylated αS2-casein peptide NTMEHVSSSEESIISQETYK. The green boxes denote the variable phosphorylated region (3 successive serine residues, of which 2 were phosphorylated for a given peptide), highlighting the distinctive c ions for a given peptide.

The same observation was made when targeting the multi-phosphorylated αS1-casein peptide QMEAESISSSEEIVPNSVEQK, which consisted of 21 amino acids, 5 phosphorylation sites and a proline residue. Figure 5 shows that 100% sequence coverage was achieved with this challenging peptide and all the correct modification sites were confirmed through the resulting c and z+1 fragment ion evidence.

Conclusions

- EAD or hybrid EAD/CID fragmentation on the ZenoTOF 7600 system can be used to elucidate complex peptide sequences and correctly assign PTMs, such as phosphorylation.
- A single DDA experiment with a 25-minute gradient was sufficient to acquire all the informa-



Symbol	Res. Mass	# (N)	b	c	y	y - 17	y - 18	z	z + 1	z + 2	# (C)
Q	128.05858	1	129.06585	146.09240	2720.91282	2703.88627	2702.90225	2703.88627	2704.89409	2705.90192	21
M	131.04049	2	260.10634	277.13289	2592.85424	2575.82769	2574.84367	2575.82769	2576.83551	2577.84334	20
E	129.04259	3	389.14893	406.17548	2461.81375	2444.78720	2443.80319	2444.78720	2445.79503	2446.80285	19
A	71.03711	4	460.18605	477.21260	2332.77116	2315.74461	2314.76059	2315.74461	2316.75243	2317.76026	18
E	129.04259	5	589.22864	606.25519	2261.73404	2244.70750	2243.72348	2244.70750	2245.71532	2246.72315	17
S[79.9663]	166.99833	6	756.22697	773.25352	2132.69145	2115.66490	2114.68089	2115.66490	2116.67273	2117.68055	16
I	113.08406	7	869.31103	886.33758	1965.69312	1948.66657	1947.68256	1948.66657	1949.67440	1950.68222	15
S[79.9663]	166.99833	8	1036.30936	1053.33591	1852.60906	1835.58251	1834.59849	1835.58251	1836.59033	1837.59816	14
S[79.9663]	166.99833	9	1203.30769	1220.33424	1685.61073	1668.58418	1667.60017	1668.58418	1669.59201	1670.59983	13
S[79.9663]	166.99833	10	1370.30602	1387.33257	1518.61240	1501.58585	1500.60184	1501.58585	1502.59368	1503.60150	12
E	129.04259	11	1499.34861	1516.37516	1351.61407	1334.58752	1333.60351	1334.58752	1335.59535	1336.60317	11
E	129.04259	12	1628.39120	1645.41775	1222.57148	1205.54493	1204.56092	1205.54493	1206.55276	1207.56058	10
I	113.08406	13	1741.47527	1758.50182	1093.52889	1076.50234	1075.51832	1076.50234	1077.51016	1078.51799	9
V	99.06841	14	1840.54368	1857.57023	980.44482	963.41827	962.43426	963.41827	964.42610	965.43392	8
P	97.05276	15	1937.59645	1954.62300	881.37641	864.34986	863.36584	864.34986	865.35769	866.36551	7
N	114.04293	16	2051.63937	2068.66592	784.32365	767.29710	766.31308	767.29710	768.30492	769.31275	6
S[79.9663]	166.99833	17	2218.63770	2235.66425	670.28072	653.25417	652.27015	653.25417	654.26199	655.26982	5
V	99.06841	18	2317.70612	2334.73267	503.28239	486.25584	485.27182	486.25584	487.26367	488.27149	4
E	129.04259	19	2446.74871	2463.77526	404.21398	387.18743	386.20341	387.18743	388.19525	389.20308	3
Q	128.05858	20	2574.80729	2591.83384	275.17138	258.14483	257.16082	258.14483	259.15266	260.16048	2
K	128.09496	21	2702.90225		147.11280	130.08626	129.10224	130.08626	131.09408	132.10191	1

Figure 5. Hybrid EAD/CID MS/MS spectrum and sequence coverage for the multi-phosphorylated αS1-casein peptide QMEAES$_{[Pho]}$IS$_{[Pho]}$S$_{[Pho]}$S$_{[Pho]}$EEIVPNS$_{[Pho]}$VEQK.

tion necessary to identify and differentiate isobaric phosphorylation permutations on peptide sequences from a casein tryptic digest.

- Combining CID and EAD fragmentation allows for the generation of information-rich MS/MS spectra with excellent sequence coverage while retaining PTM positional information
- The hybrid EAD/CID method can easily be converted into a targeted multiple reaction monitoring (MRMHR) method on the ZenoTOF 7600 system to quantify and rapidly monitor the different phosphopeptide variants in casein extracts for day-to-day quality controls.
- To date, the complete mapping of phosphorylation from casein samples has not been achieved without peptide enrichment. In this experiment, the detection and characterization of the different expected isoforms of phosphorylated caseins was possible, which allows for future development of MS-based quantitation methods for such peptides.

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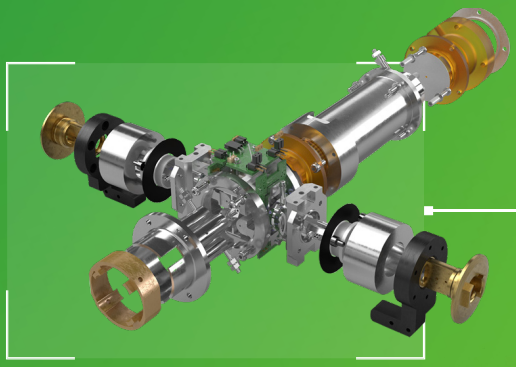

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