UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Química

TESIS DOCTORAL

DESARROLLO DE METODOLOGÍAS DE EXTRACCIÓN Y MICROEXTRACCIÓN COMBINADAS CON SISTEMAS CROMATOGRÁFICOS PARA LA DETERMINACIÓN DE FÁRMACOS Y PRODUCTOS DE CUIDADO PERSONAL EN MUESTRAS AMBIENTALES

Development of extraction and microextraction methodologies combined with chromatographic systems for the determination of pharmaceuticals and personal care products in environmental samples

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Agradecimientos

Una vez concluidos estos años de trabajo y esta Tesis Doctoral, no puedo sino acordarme de tantas personas que me han acompañado en el camino, ya que sin ellos el resultado habría sido distinto. Gracias a todos.

Por supuesto, a mi familia, por animarme y ayudarme siempre a seguir adelante,

A mi madre, porque eres la persona que mejor me conoce y me comprende, por escucharme cada día, y por saber siempre qué hacer para ayudarme a tomar las decisiones correctas. Por ser mí mejor amiga además de mi madre.

A mi padre, simplemente por ser el mejor padre posible. Por tu entusiasmo y tu implicación, por tantas revistas y periódicos que me has guardado, noticias que me has contado y por ponerle más ilusión que nadie, incluso más que yo.

A mi hermana, porque cada día cuando llegas del cole me sonríes como sólo tú sabes y haces que me olvide de mis agobios y preocupaciones.

A mi abuela, por estar a mi lado a cada paso, desde el día que nací hasta el día de hoy. Por compartir conmigo tantas cosas y sobre todo por quererme tanto.

Al grupo de investigación, que después de tanto años es una segunda familia,

A mis directores de Tesis, por darme la oportunidad de conocer y aprender tantas cosas. Zoraida, gracias por tu dedicación, por tu confianza, por tu paciencia, por tu apoyo, por escuchar todas mis dudas y preguntas sin perder la sonrisa y sobretodo por tener siempre tiempo para mí. José Juan, gracias por invitarme a entrar en el grupo y por apostar por mí todo este tiempo, desde el primer día hasta hoy, por saber animarme y a la vez exigirme un poquito más cada día, por convencerme de que podía lograr cosas que no imaginaba.

A Miriam, que decirte a tí que tú no sepas ya... Todo lo que tengo que agradecerte no cabe en estas líneas. Sólo pedirte perdón por la lata que te he dado (especialmente el primer año) y darte infinitas gracias por toda la ayuda que me has brindado, casi siempre antes de que te la pidiera. Gracias por ser más una amiga que una jefa desde el primer día.

A Cristina Mahugo por aportar siempre un punto de vista distinto y por tantos buenos consejos, laborales y no laborales.

A Álvaro, gracias por ayudarme a dar los primeros pasos en el laboratorio y… ¡por enseñarme a "usar" el condenado Matlab!

A Cris y a Rayco, además de por la portada y la encuadernación… gracias por ser tan buenos compañeros de trabajo y además amigos, por tantos y tantos momentos compartidos dentro y fuera del laboratorio ¡Vuelvo a tener los domingos libres para fotos acuáticas y granizadas!

A los que ya no están… Borja, Carlos, Jana, Adrián, por todos los buenos ratos.

Al Director del Departamento de Química, Miguel Suárez de Tangil, por acogerme con tanto cariño desde el primer día y por tu ayuda e implicación cuando lo he necesitado.

Al grupo de Química Orgánica por ser tan buenos vecinos.

A los profesores que me han enseñado cosas importantes que no aparecían en los libros ni estaban en el temario, Pascual Caballero Ortega, Nicolás Guerra Aguiar…

A Dagmar Solichová y Pter Solich, y a todos los compañeros de Hradec Králové, (Jirka, Masha, Barça, Lenka, Carolina, Burkhard y Eva). Gracias por su hospitalidad y su ayuda durante la estancia.

A los amigos, por aguantarme tantas veces hablar sobre la Tesis y además ¡poner interés!. Perdónenme por todas las veces que les he dicho "que va… no puedo, tengo que terminar de escribir…",

A las "Chicas del Mar". Mery, tu fuiste la primera persona que conocí en esta facultad y aquí sigues a mi lado, siempre aportando tu positividad y tu confianza en mí, gracias por tantas risas y tantos recuerdos. Séfora, tú además de amiga eres compañera de fatigas, así nadie mejor que tú para comprender todo lo vivido, especialmente estos últimos meses, ¡ya sabes lo que nos merecemos ahora!

A mis niñas del gym, Noa, Noe, Neli, Myuriel, Tati e Irene, porque nunca creí que entre una sentadilla y un "chassé" se podría encontrar amigas tan verdaderas. Gracias por alegrarme esas tardes en las que llegaba de mal humor y sobre todo por animarme tanto y estar tan cerca cuando estuve tan lejos.

A los amigos "de siempre", Sara, Himar, Azahara, Iván, la otra Sara, Belinda… por seguir ahí a pesar de todo el tiempo transcurrido, compartiendo también esta nueva etapa de mi vida.

Y por supuesto a Tana. Te he dejado para el final no porque seas el menos importante, todo lo contrario, sino porque no sabía si nombrarte en compañero, en amigo o en familia. Has participado en esta aventura conmigo desde el primer día y en primera persona, así que no ha habido nadie mejor que tú para ayudarme, comprenderme, enseñarme, calmarme y llevarme de la mano durante el camino, también en éste camino. Gracias también a tu familia por su cariño y por aguantar una segunda Tesis!.

A la Agencia Canaria de Investigación, Innovación y Sociedad de la Información por la beca de Formación de Personal Investigador cofinanciada en un 85% por el F.S.E, que me ha permitido desarrollar esta Tesis Doctoral así como realizar una estancia en el extranjero.

Resumen

A lo largo de la historia, la Química Analítica se ha dedicado al perfeccionamiento de técnicas de extracción, separación y detección, que han logrado la determinación de compuestos de diversa naturaleza en matrices cada vez más complejas y en concentraciones cada vez más bajas.

En las últimas décadas, las tendencias en este campo se han centrado en el desarrollo de técnicas de extracción y preconcentración que, además de ser eficientes y robustas, sean progresivamente más sencillas, baratas y requieran menor volumen tanto de muestra como de disolvente orgánico. Entre las estrategias empleadas para este fin, destacan la miniaturización y la automatización de las técnicas de extracción convencionales, y la sustitución de los disolventes orgánicos por otras sustancias menos tóxicas y contaminantes, como por ejemplo los surfactantes.

En este contexto, en esta Tesis Doctoral se han desarrollado metodologías de extracción que van en consonancia con estas nuevas tendencias. Así, se han

optimizado técnicas de extracción miniaturizadas, como la microextracción en fase sólida y la extracción con barras agitadoras, y técnicas automatizadas como la extracción en fase sólida en línea con un sistema cromatográfico. Además, se ha implementado el uso de medios micelares como extractantes en diferentes técnicas de extracción para evitar los disolventes orgánicos usados normalmente.

Para la separación de los analitos se ha empleado la cromatografía líquida de alta resolución y la cromatografía líquida de ultra resolución, pudiendo constatarse las ventajas aportadas por esta última en términos de rapidez y ahorro de fases móviles. A continuación, se ha utilizado la detección por fluorescencia y por espectrometría de masas. La espectrometría de masas resulta más útil a la hora de determinar concentraciones a nivel de trazas en matrices muy complejas; no obstante, la detección por fluorescencia también resulta adecuada cuando se emplean medios micelares ya que estos son capaces de realzar la señal de las moléculas fluorescentes.

Las metodologías desarrolladas en los distintos trabajos incluidos en esta Tesis Doctoral han sido aplicadas a la determinación de compuestos farmacéuticos y de cuidado personal en distintas muestras liquidas y sólidas tomadas en la isla de Gran Canaria (agua de mar, aguas residuales, sedimentos marinos y lodos de depuradora).

Se han analizado antibióticos de la familia de las fluoroquinolonas, cuyo uso está muy extendido debido a su contundente acción contra multitud de enfermedades infecciosas. La mayor fuente de contaminación medioambiental por estos compuestos son las estaciones depuradoras de aguas residuales, que no poseen mecanismos para eliminarlos completamente, y por tanto los liberan al medio ya sea a través de

emisarios submarinos, aguas de regadío o abonos para la agricultura. El mayor riesgo de la presencia de estos antibióticos en el medio ambiente es el desarrollo de cepas de bacterias resistentes a los mismos.

Asimismo, hemos aplicado las metodologías analíticas desarrolladas a los benzotriazoles estabilizadores de luz UV, una familia de compuestos empleados en gran variedad de cosméticos y otros productos de cuidado personal. En este caso, además de la contaminación derivada de los residuos generados por las depuradoras, puede darse una contaminación directa del medio por la utilización de cremas de protección solar en zonas de baño con poca circulación. Una vez llegan al medio, estos compuestos tienen propiedades tóxicas y mutagénicas sobre las comunidades acuáticas.

El desarrollo durante esta Tesis Doctoral de metodologías de extracción y preconcentración selectivas y eficaces, combinadas con sistemas de detección muy sensibles, ha permitido detectar y determinar ambas familias de compuestos en concentraciones muy bajas y en matrices de diversa naturaleza en muestras tomadas en la isla de Gran Canaria.

Abstract

Throughout history, Analytical Chemistry has been devoted to improving of extraction, separation and detection techniques, which have allowed the determination of compounds of different nature in increasingly complex matrices and increasingly lower concentrations.

In the last decades, the trends in this field have been focused in the development of efficient and robust extraction and preconcentration techniques. Moreover, these techniques must be more and more simple and cheap and it must have lower requirements of sample and organic solvent. Among the strategies used for this purpose, we can find the miniaturization and the automatization of the conventional extraction techniques, and the replacement of the organic solvents by other substances less toxic and contaminant, for example the surfactants.

In this context, in this Doctoral Thesis, different extraction methodologies have been developed according to news trends. Miniaturized techniques, such as solid

phase microextraction and stir bar sorptive extraction, and automated techniques such as on-line solid phase extraction coupled with chromatographic systems have been optimized. Moreover, the use of micellar media as extractans in different techniques has been implemented in order to avoid the use of organic solvents.

For the separation of the analytes, high performance liquid chromatography and ultra-high performance liquid chromatography have been employed, verifying the advantages provided by the latter in terms of speed and saving mobile phase. For the detection, fluorescence and mass spectrometry have been used. Mass spectrometry is more useful to detect trace concentrations in very complex matrices. Nevertheless, fluorescence detection also is adequate when micellar media are employed, because they are be able to enhance the signal of the fluorescent molecules.

The developed methodologies in the different works included in this Doctoral Thesis have been applied to the determination of pharmaceutical and personal care products in several liquid and solid samples collected in Gran Canaria island (seawater, wastewater, marine sediments and sewage sludges).

Antibiotics belonging to the fluoroquinolone family, widely used due to their strong activity against a lot of infectious diseases, have been analysed. The main source of contamination of these compounds is the wastewater treatment plants (WWTPs), which are not have mechanism to remove it completely. Fluoroquinolones reaches the environment through of the marine outfalls, the irrigation waters or the fertilizers used in the agriculture. The higher risk of the presence of the antibiotics in the natural media is the apparition of resistant bacteria.

We have also applied the developed analytical methodologies to benzotriazole UV stabilisers (BUVSs), a family of compounds used in a wide variety of cosmetics and other personal care products. In this case, besides the contamination generated by the WWTPs, a direct contamination can occur by the employment of sunscreens in closed bathing areas. Afterward, these compounds present toxic and mutagenic properties over the aquatic communities.

The development of selective and effective extraction and preconcentration methodologies combined or coupled to very sensitive detection systems allowed the detection and determination of both compound families in very low concentrations and different matrices in samples from Gran Canaria island.

I. Introducción

La etapa más crítica de un procedimiento analítico es la aplicación de una técnica de preparación de muestra para extraer y preconcentrar los analitos desde la matriz antes de su determinación instrumental. La adecuada elección de una metodología que permita una efectiva separación y purificación de los compuestos a estudiar, y la correcta optimización de todos los factores que afectan a la misma, tendrán una profunda influencia tanto en la calidad de los resultados obtenidos como en el tiempo total del análisis.

Sin embargo, hasta hace relativamente pocos años este paso no había alcanzado el lugar de importancia que tiene dentro de un proceso analítico general. La necesidad de desarrollar metodologías analíticas que permitan la determinación a nivel de trazas de distintos compuestos en muestras complejas ha sido un estímulo para muchos de los progresos en esta área.

A pesar del esfuerzo llevado a cabo en las últimas dos o tres décadas para mejorar las técnicas usadas para la preparación de la muestra, éstas incluyen generalmente múltiples etapas de manipulación de la misma y son bastante tediosas. Los diferentes procedimientos de extracción y purificación de los analitos afectan al rendimiento total del análisis y también al coste, tiempo y consumo de disolventes. Además, frecuentemente pueden ocasionar la contaminación o degradación de los analitos. Todo esto explica el por qué se estima que la preparación de la muestra ocupa un tiempo importante dentro del tiempo total del análisis y que es una fuente de error en el procedimiento analítico general.

En definitiva, la adecuada selección y optimización en la preparación de la muestra es un aspecto clave del proceso analítico que puede afectar a la exactitud y precisión del resultado final del análisis. Las tendencias más recientes van encaminadas hacia:

1. La automatización, a través del acoplamiento entre la unidad de preparación de muestra y los sistemas de detección
2. La utilización de nuevos materiales adsorbentes más avanzados
3. La aplicación de técnicas miniaturizadas que impliquen una reducción en el volumen de muestra y disolvente empleados

En este contexto, haremos un breve repaso a las ventajas logradas en el campo de la Química Analítica con los nuevos procedimientos de extracción y

preconcentración desarrollados en los últimos años, en comparación con los convencionales, y se expondrán de manera más extensa las características de las técnicas estudiadas y desarrolladas en esta Tesis Doctoral.

I.1. Técnicas de extracción y preconcentración

I.1.1 Muestras acuosas

A pesar de que en las últimas décadas se han desarrollado potentes sistemas de detección muy sensibles y selectivos, el análisis de contaminantes en muestras medioambientales sigue estando caracterizado por la dificultad de determinar muy bajas concentraciones en muestras muy complejas. Entre las muestras acuosas, son especialmente problemáticas las aguas procedentes de estaciones depuradoras de aguas residuales (EDARs) y las muestras de agua de mar, ambas objeto de análisis en esta Tesis Doctoral. Las primeras son aguas muy sucias donde un proceso de eliminación de interferencias es vital, mientras que en las segundas, la preconcentración de las muestras resulta imprescindible para poder determinar los analitos después de haber experimentado un proceso de dilución muy grande al llegar al medio marino. Por tanto, la metodología de preparación de muestra a aplicar en cada caso debe ser escogida en función de las características de la matriz.

La **extracción liquido-liquido** (**LLE, *Liquid-Liquid Extraction***) es una técnica convencional empleada durante muchos años para la extracción de analitos desde matrices acuosas. Se basa en hacerlos pasar desde la fase acuosa a otra fase orgánica mediante varias etapas de agitación de la mezcla. Aunque se trata de una metodología sencilla y que proporciona buenas recuperaciones, presenta importantes inconvenientes, como el uso de un elevado volumen de disolventes orgánicos, normalmente caros y frecuentemente tóxicos, y la consecuente necesidad de

introducir una etapa de evaporación para la concentración de los extractos. Además es un proceso bastante largo donde se pueden introducir errores debido a la alta manipulación de la muestra, y en ocasiones surgen también problemas de tipo práctico como la formación de emulsiones. Por todo ello, en las últimas décadas ha existido una tendencia a reemplazar la extracción líquido-líquido por la extracción en fase sólida [1].

La **extracción en fase sólida (SPE, *Solid Phase Extraction*)** fue desarrollada a finales de la década de los años 70 [2] y rápidamente se erigió como una potente herramienta para la extracción y purificación de compuestos desde matrices medioambientales. Consiste en hacer pasar la muestra a través de un adsorbente sólido que retiene los analitos selectivamente según su afinidad por el mismo. Posteriormente se realiza la elución de esos analitos con un volumen mucho menor de un disolvente adecuado, resultando un extracto purificado y más concentrado. Todas las etapas del procedimiento se muestran más detalladamente en la Figura 1.

Esta técnica reduce significativamente el consumo de disolvente orgánico y el tiempo de operación respecto a la extracción líquido-líquido ya que no requiere de extracciones sucesivas. Además del enriquecimiento y purificación de las muestras, la extracción en fase sólida permite el fraccionamiento de distintos analitos eluyendo las distintas fracciones con diferentes disolventes, el almacenamiento de compuestos inestables o volátiles y la derivatización de los analitos mediante reacción con el adsorbente.

Una limitada eficiencia en la extracción puede ser causada por una insuficiente retención, por lo que la elección de un adsorbente afín a los analitos en estudio es un paso vital en la optimización del proceso.

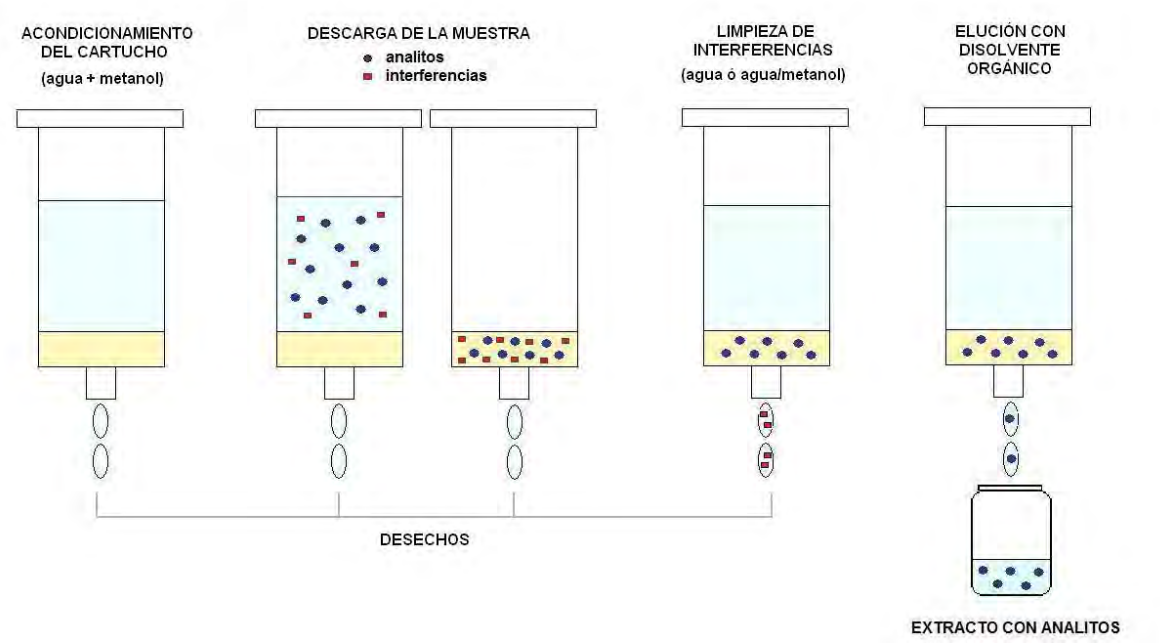

Figura 1. Etapas de la extracción en fase sólida.

Los primeros adsorbentes desarrollados en SPE fueron los de sílice enlazada, carbón grafitizado y polímeros porosos [3]. Los de sílice enlazada pueden ser de fase normal, de fase reversa o de intercambio iónico, análogamente a los rellenos de las columnas cromatográficos y presentan la desventaja de que los residuos de silanol pueden interferir en la determinación de los analitos de interés. Los de carbón grafitizado son adecuados tanto para analitos polares como no polares, pero a menudo la retención es tan grande que estos no pueden volver a recuperarse. Por su parte los poliméricos son estables en un amplio rango de pH, no generan residuos de

silanol y ofrecen mayor eficiencia para compuestos polares. Uno de los materiales poliméricos más empleados para la extracción desde muestras medioambientales complejas es el del balance hidrofílico-hidrofóbico, comercialmente denominado Oasis HLB (Waters). Es apropiado para la extracción de compuestos de cualquier polaridad y está formado de una parte hidrofílica (N-vinilpirrolidina) que aumenta la humectabilidad del polímero, y otra parte hidrofóbica (divinilbenceno) que favorece la retención del analito [4].

Actualmente existen otros materiales más específicos, como los inmunoadsorbentes, los materiales de acceso restringido (RAM, restricted access materials) y los polímeros de impronta molecular (MIPs, moleculary imprinted polymers) [5]. Los inmunoadsorbentes están formados por anticuerpos inmovilizados sobre sílice, vidrio, agarosa u otros geles y extraen selectivamente a los analitos (antígenos) mediante un proceso de reconocimiento molecular. Aunque tiene una amplia aplicabilidad en el área medioambiental y biológica, presentan como limitaciones su alto coste y su escasa posibilidad de reutilización. Los RAM están específicamente diseñados para evitar las macromoléculas basándose en mecanismos de exclusión por tamaño, de manera que sólo las moléculas pequeñas son capaces de entrar en contacto con la fase estacionaria. Son especialmente útiles para evitar proteínas y lípidos en las muestras biológicas [4]. Por último los MIPs se obtienen mediante el ensamblaje de una matriz polimérica alrededor de un analito o molécula diana, de manera que se crea una huella del analito. Una vez obtenido el polímero es posible extraer el compuesto quedando así huecos libres con “memoria” selectiva que reconocerán de forma específica nuevas moléculas del contaminante molde. Entre las

ventajas de estos materiales destacan la sencillez y rapidez de preparación, la elevada estabilidad química, física y térmica y el bajo coste. Presentan el inconveniente de que la incompleta eliminación de las moléculas molde origina problemas de contaminación.

Para el correcto empleo de la SPE deben optimizarse todos aquellos parámetros que afectan al proceso, como son el volumen de muestra, el pH y la fuerza iónica de la misma, así como el volumen y la naturaleza del eluyente.

Además, es una técnica adecuada para ser automatizada, denominándose entonces **extracción en fase sólida "en línea" (On-line SPE).** Se acopla a sistemas cromatográficos permitiendo reducir el tiempo de análisis [6] y eliminar los posibles errores manuales [7]. El volumen de muestra necesario es menor en la técnica "en línea" y a diferencia de la SPE convencional, donde sólo se inyecta una porción del extracto, aquí la elución se realiza con la propia fase móvil y por tanto toda la masa de analito retenida en el adsorbente es inyectada. Así, los factores de preconcentracion y los límites de detección logrados son mejores.

La limpieza de las columnas después de una extracción también se realiza de manera automática, y en condiciones de alta presión en lugar de en condiciones de vacío como en la SPE convencional, por lo que la posibilidad de la aparición de problemas debido a contaminación entre muestras o "efecto memoria" también es minimizada.

En esta Tesis Doctoral se ha aplicado la extracción en fase sólida tanto en forma convencional como en modo "en línea", y se ha realizado además una comparación

entre ambas técnicas para la extracción de una misma familia de compuestos. Estos trabajos serán descritos posteriormente en los epígrafes III.1.3., III.2.1. y III.2.2.

Otra tendencia dentro de la investigación en el campo de la Química Analítica es el desarrollo de nuevas técnicas de extracción con menores requerimientos de volúmenes de muestra y de disolvente orgánico. La miniaturización ha sido un factor clave en el logro de estos objetivos, dando lugar a la aparición de las técnicas de microextracción. Estas técnicas proporcionan en muchos casos altos factores de enriquecimiento y minimizan el consumo de disolventes.

Una de las metodologías miniaturizadas más empleadas es la **microextracción en fase sólida (SPME, *Solid Phase Microextraction*)** introducida por Arthur y Pawliszyn en 1990 [8]. En esta técnica se emplea como fase extractante una fibra de sílice fundida con un diámetro interno normalmente de 150 µm, cubierta de una capa de material adsorbente/absorbente de entre 5 y 100 µm de grosor [9]. El pequeño tamaño de la fibra y su geometría cilíndrica permiten incorporarla a una jeringa, facilitando así su manipulación. Existe en el mercado una amplia gama de fibras con diferentes recubrimientos, algunas de una sola fase y otras combinando dos materiales extractantes, de manera que es posible encontrar una fibra adecuada para casi cualquier analito en un amplio rango de polaridad. Los recubrimientos más empleados son polidimetilsiloxano (PDMS), poliacrilato (PA), carbowax (CW) y divinilbenceno (DVB). En la mayoría de los casos la extracción de los analitos se produce por adsorción sobre la fibra, pero existen casos donde el fenómeno que ocurre de manera prioritaria es la absorción (en PDMS y PA).

La fibra puede ponerse en contacto con la muestra por inmersión directa, utilizando el modo de espacio en cabeza o con protección de membrana. El modo de espacio en cabeza permite evitar la extracción de interferencias no volátiles y proporciona una vida más larga de la fibra, mientras que la protección con una membrana semipermeable impide el acceso a compuestos de elevado peso molecular.

Después de la extracción, puede emplearse desorción térmica en un cromatógrafo de gases o usar un disolvente orgánico si se va a realizar la inyección en un cromatógrafo líquido. En este último caso puede utilizarse una interfase para llevar a cabo la desorción "en línea" en modo dinámico (empleando la propia fase móvil que desorbe los analitos al pasar por la fibra) o en modo estático (llenando la cámara con el disolvente). Si no se dispone de una interfase adecuada, existe también la posibilidad de realizar la desorción fuera de línea en un vial que contenga el disolvente y luego inyectar ese extracto. Esta forma de desorber los analitos desde la fibra de SPME fue la empleada en esta Tesis Doctoral, tal como se detalla en los epígrafes III.1.1. y III.1.2. de la sección experimental. La Figura 2 muestra el procedimiento de SPME usando extracción y desorción por inmersión directa de la fibra.

El desarrollo de la técnica requiere la optimización de todas las variables que afectan tanto a la extracción como a la desorción para alcanzar las condiciones de equilibrio para cada compuesto.

La SPME presenta ciertas ventajas frente a otras técnicas, tales como la utilización de volúmenes de disolvente orgánico mucho menores, la facilidad de automatización y la posibilidad de realizar muestreos *in situ* ya que es fácilmente

transportable. A pesar de que esta técnica puede integrar muestreo, extracción, concentración e introducción de la muestra en un único proceso [10], también presenta algunas desventajas, como por ejemplo la fragilidad de las fibras y su coste relativamente alto. También pueden darse problemas de contaminación por efecto memoria, sobre todo con compuestos de alto peso molecular que pueden quedar retenidos irreversiblemente sobre la fibra, y esto a su vez generar problemas de repetibilidad y linealidad [11].

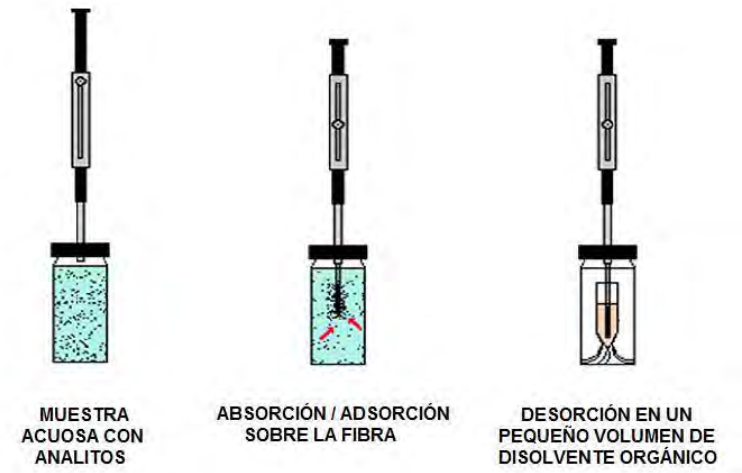

Figura 2. Etapas de la extracción en fase sólida con extracción por inmersión directa y desorción liquida.

Aunque la SPME es considerada como una miniaturización de SPE, debe tenerse en cuenta que la SPE es una técnica exhaustiva, es decir, en condiciones optimas de extracción se pretende recuperar el 100 % de los analitos presentes en la matriz, mientras que la SPME es una técnica no exhaustiva o de equilibrio, donde la

cantidad de contaminante extraída será proporcional a la concentración total [12]. El pequeño volumen de la fase adsorbente/absorbente supone una limitación para la masa de analito que puede ser extraída, lo cual significa que en ocasiones no se logran límites de detección lo suficientemente bajos para el análisis de trazas en muestras medioambientales [13].

Otra técnica de extracción miniaturizada, que resuelve algunas de las limitaciones de la SPME es la **extracción por absorción con barras agitadoras (SBSE, *Stir Bar Sorptive Extraction*),** desarrollada por Baltussen y colaboradores en 1999 [14]. En este caso la extracción de los analitos se realiza a través de una barra magnética agitadora recubierta casi siempre por PDMS. Recientemente han aparecido en el mercado barras con nuevos recubrimientos para compuestos polares, ya que el PDMS limita su aplicación a especies poco polares [15].

Al igual que en SPME, utilizando SBSE la desorción de los analitos se puede realizar térmicamente con cromatografía de gases, con una interfase acoplada a cromatografía liquida, ó por desorción liquida por inmersión de la barra en un disolvente orgánico [9]. La desorción térmica es la más apropiada en términos de sensibilidad ya que permite introducir todo el extracto en el sistema cromatográfico [16]. Sin embargo, la desorción líquida evita las posibles interferencias que pueden surgir de la degradación del PDMS por las altas temperaturas y además es compatible con la cromatografía liquida sin necesidad de invertir en costosas unidades de desorción [17].

SBSE y SPME son técnicas basadas en los mismos principios, pero el volumen de absorbente en SBSE es entre 50 y 250 veces mayor [18], lo que resulta en una mayor relación de fases que en la SPME y una mayor capacidad de extracción [19]. Además, las barras agitadoras son más robustas y menos costosas que las fibras empleadas en SPME [20].

En la actualidad los esfuerzos para la mejora de esta técnica se centran en el desarrollo de nuevos recubrimientos que combinen el PDMS con otros absorbentes para aumentar la selectividad y la eficiencia en el proceso de extracción.

Al igual que en SPME, durante la optimización de ésta técnica debemos considerar diferentes parámetros tales como volumen de muestra, pH y fuerza iónica de la misma, velocidad de agitación y naturaleza y volumen del disolvente empleado para la desorción. En este caso, el tipo de material extractante no es una variable a optimizar ya que no existe un amplio abanico de adsorbentes/absorbentes para analitos de distintas características como ocurre en SPME, sino que, como ya se comentó anteriormente, sólo existe un recubrimiento de PDMS, adecuado para compuestos poco polares y otro más novedoso para compuestos polares pero cuyo uso aún no se ha extendido. En esta Tesis Doctoral se aplicó esta técnica usando barras de PDMS aprovechando que una de las familias de compuestos estudiados durante la misma tiene una naturaleza muy afín por este material no polar. Dicho trabajo se expone en el apartado III.2.3.

En la misma década, y con el objetivo común de reducir los volúmenes de muestra y de disolvente orgánico, surgieron también las técnicas de microextracción en fase líquida, que en este caso podrían considerarse miniaturizaciones de la LLE. Se trata de técnicas generalmente sencillas y rápidas que eliminan una de las principales desventajas de SPME y SBSE, la dependencia de un marca comercial que proporcione los dispositivos para la extracción. Dentro de éstas técnicas miniaturizadas se han ido desarrollando diferentes modalidades, entre las que destacan la microextracción con gota suspendida, la microextracción en fase liquida con fibra hueca, la microextracción liquido-liquido dispersiva y la microextracción con gota solidificada. La versión más simple de la LLE es la **microextracción con gota suspendida (SDME, *Single Drop Microextraction*),** donde un volumen de aproximadamente 10 µL de disolvente orgánico inmiscible en agua es suspendido en la muestra en forma de gota usando una jeringa [21]. Después de realizarse la migración de los analitos desde la matriz hacia la gota, ésta se retrae dentro de la jeringa y es llevada al cromatógrafo para su inyección. Una variante mejorada de la SDME es la **microextracción en fase líquida con fibra hueca (HF-LPME, *Hollow Fiber Liquid Phase Microextraction***). En este caso el disolvente orgánico forma una fina capa sobre la pared de la fibra hueca, que es porosa y evita el paso de compuestos de alto peso molecular [22]. Otra combinación, la **microextracción liquido-liquido dispersiva (DLLME, *Dispersive Liquid-Liquid Microextraction*),** no emplea uno sino dos disolventes orgánicos, uno extractante y otro dispersivo, siendo éste último miscible tanto en la fase acuosa de la muestra como en el extractante [23]. Se basa en introducir rápidamente en la muestra una mezcla de los dos disolventes, de manera que se produce una turbulencia que genera

la formación de pequeñas gotas dentro de la fase acuosa. Este aumento del área superficial de contacto entre muestra y extractante hace que se alcance el equilibrio más rápidamente y que mejore la eficacia de la extracción. Por último, en la **microextracción con gota solidificada (SDLPME, *Solid Drop Liquid Phase Microextraction*),** se añade un disolvente orgánico, con un punto de fusión entre 10 y 30ºC, en la superficie de la muestra acuosa, se agita y se coloca en un baño de hielo, de manera que la fase orgánica se solidifica y puede separarse fácilmente con una espátula. A continuación en contacto con la temperatura ambiente vuelve a estado líquido y puede inyectarse en un cromatógrafo [24].

I.1.2 Muestras sólidas

La extracción de compuestos desde muestras sólidas generalmente es más problemática que desde muestras líquidas ya que la interacción matriz-analito es más intensa. Generalmente el contenido en materia orgánica dificulta la extracción debido a la fuerte unión con los analitos. Además, si los contaminantes han estado en contacto con la matriz durante mucho tiempo, la interacción no será debida sólo a procesos de absorción sino que será necesario revertir procesos de transporte por difusión mucho más fuertes. Todo esto hace que sea necesario aplicar condiciones más agresivas para lograr la separación, lo que se traduce normalmente en extractos finales menos purificados y con mayor número de interferencias.

Las técnicas empleadas convencionalmente para el tratamiento de matrices sólidas son el **Soxhlet** y la **extracción asistida por ultrasonidos**. Ambas tienen la desventaja de requerir un volumen bastante alto de disolvente orgánico. Además conllevan una alta manipulación de la muestra y no son fáciles de automatizar. Sin embargo siguen utilizándose en análisis de rutina y como técnicas de referencia.

El Soxhlet fue desarrollado en 1879 por F. von Soxhlet y es una técnica barata y sencilla consistente en poner en contacto repetidas veces la muestra pulverizada con porciones nuevas de disolvente orgánico a una temperatura elevada [25]. Además de necesitar mucha cantidad de disolvente también emplea mucho tiempo, entre 6 y 48 horas, lo que la convierte en una técnica lenta y tediosa. Por otro lado, requiere una etapa posterior de evaporación del disolvente para concentrar el extracto.

La **extracción asistida por ultrasonidos (UAE, *Ultrasonic Assisted Extraction*)** también es una técnica sencilla y poco costosa donde la interacción entre la muestra y el disolvente orgánico es favorecida por la energía de ultrasonidos. Aunque es una técnica más rápida que el Soxhlet [26] (entre 30 y 120 minutos) y emplea menos disolvente, no elimina el inconveniente de una etapa de evaporación antes de la inyección del extracto y además es menos reproducible [27], debido a la ausencia de de uniformidad en la distribución de la energía. Por otra parte, presenta baja selectividad y limitada capacidad de enriquecimiento de la muestra.

Al igual que en la extracción desde muestras acuosas, donde los avances en el campo de la Química Analítica se han centrado en el desarrollo de técnicas miniaturizadas, también para muestras sólidas se ha realizado un esfuerzo para conseguir técnicas de extracción con menos requerimiento de disolventes orgánicos. Además, se apuesta por procedimientos automatizados que reduzcan la manipulación de la muestra y los errores asociados a ella.

Así, en 1995 surgió como una poderosa técnica, la **extracción con líquidos presurizados (PLE, *Pressurized Liquid Extraction*)**, también llamada **extracción acelerada con disolventes (ASE, *Accelerated Solvent Extraction*)** [28]. Se trata de una técnica simple y rápida (aproximadamente 15 minutos), en la que la muestra es puesta en contacto con un volumen relativamente bajo de disolvente dentro de una cámara con alta presión y temperatura (1500-2000 psi y 50-200ºC). Estas condiciones favorecen la rotura de las interacciones analito-matriz, lográndose muy buenas

eficiencias de extracción y no requiriéndose en general una etapa posterior de limpieza [29]. El problema fundamental que presenta es el elevado coste de la instrumentación necesaria, lo que la convierte en una técnica inaccesible para laboratorios modestos.

Usando la **extracción por fluidos supercríticos (SFE, *Supercritical Fluid Extraction*)** también se consigue una extracción relativamente rápida además de efectiva [30]. La utilización del disolvente por encima de sus condiciones críticas de presión y temperatura (resultando un fluido con características intermedias entre un líquido y un gas) proporciona un gran poder de penetración en matrices complejas. El fluido supercrítico más utilizado es el CO_2, por ser barato y no tóxico, aunque en ocasiones su baja polaridad puede resultar un problema para la extracción de muchos analitos de interés medioambiental [18].

Otra técnica donde se reduce considerablemente el volumen de disolvente orgánico es la que se ha empleado en los apartados III.1.4., III.1.5. y III.2.2. de esta Tesis Doctoral, la **extracción asistida por microondas (MAE, *Microwave Assisted Extraction*).** Se basa en aplicar energía microondas a un vaso que contiene la muestra en contacto con el disolvente, lográndose el calentamiento de ésta sin calentar el recipiente. Una de las principales ventajas de esta técnica es la reducción del tiempo empleado, lo cual es debido principalmente a este modo de calentar la muestra. Normalmente con otras técnicas es necesario un tiempo para calentar el recipiente y que luego ese calor llegue al contenido, mientras que en este caso la radiación calienta la muestra directamente [31]. La energía microondas es de alta frecuencia (entre 300 y

300.000 MHz) y su modo de producir calor es generando movimiento molecular por la migración de iones y la rotación de dipolos.

Los primeros ensayos de ésta técnica se realizaron a finales de la década de los 80 usando un microondas doméstico [32] y en la actualidad se trata de una técnica que requiere una instrumentación relativamente barata en comparación con PLE y SFE, ya que sólo es necesario un horno microondas y un carrusel donde colocar varias muestras. Este carrusel gira durante todo el proceso, homogeneizando las condiciones de temperatura de los distintos vasos y permitiendo realizar la extracción de varias muestras (generalmente 6) de manera simultánea y con buena repetibilidad.

La extracción puede realizarse con vasos abiertos (a presión atmosférica) o cerrados (controlando la presión y la temperatura), siendo más rápido y efectivo utilizar vasos cerrados ya que puede calentarse el disolvente por encima de su punto de ebullición. En ese caso el tiempo de extracción suele ser entre 5 y 15 minutos, añadiendo luego otros 5 minutos para enfriar la muestra antes de abrir el recipiente y evitar así la posible pérdida de analitos volátiles.

Se ha demostrado que ésta técnica tiene una amplia aplicabilidad y puede competir en términos de eficiencia con la PLE ó la SFE, siendo además muy sencilla de optimizar en comparación con SFE por ejemplo, donde interviene un número mayor de variables [33]. En la MAE deben ser optimizados los siguientes parámetros: potencia, tiempo y volumen y naturaleza del disolvente.

Generalmente una alta temperatura dentro de los vasos (determinada por el tiempo y la potencia aplicados) aumenta la eficiencia de extracción por un aumento de

la difusión del disolvente hacia en interior de la matriz y por la desorción de los analitos desde los sitios activos de la misma. Sin embargo, con altos valores de temperatura también aumenta la posibilidad de que los analitos se degraden y de que se extraigan sustancias no deseadas. Así, como desventajas hay que señalar que en algunos casos ofrece menos selectividad que otras técnicas [33], la necesidad de realizar un paso de limpieza del extracto antes de su análisis y que no es una técnica adecuada para su automatización.

Por último, otra técnica que presenta la ventaja de un uso moderado de disolventes es la **dispersión de la matriz en fase sólida (MSPD, *Matrix Solid Phase Dispersion*),** introducida en 1989 por Barker y colaboradores [34] donde la muestra se mezcla en un mortero con un adsorbente adecuado también sólido. La estructura de la matriz sólida se rompe y se homogeniza alrededor de las partículas de adsorbente, incorporándose luego a un cartucho o jeringa de polipropileno que contiene un adsorbente de limpieza que retiene las interferencias extraídas. Por último, los analitos son recuperados con un pequeño volumen de disolvente orgánico. Así, una de las principales ventajas de esta técnica es la posibilidad de integrar extracción y limpieza en un solo paso [35], aunque en algunos casos sigue siendo necesario un paso adicional de limpieza para eliminar componentes de la matriz que son co-eluídos con los analitos [36]. Se trata de una técnica de rápida aplicación y bajo coste [37].

I.2 Medios micelares

El desarrollo de metodologías de extracción tanto para muestras líquidas como sólidas ha ido evolucionando hacia técnicas con un menor empleo de disolventes orgánicos. El problema que presenta el uso de estos disolventes es que son caros, muchas veces tóxicos para el analista y contaminantes para el medio ambiente.

Además de la miniaturización de las técnicas, otra alternativa al uso los disolventes orgánicos es sustituirlos por otras sustancias menos nocivas, como los medios micelares o surfactantes, también llamadas detergentes o agentes tensioactivos.

La característica principal de estas sustancias es que son anfipáticas, es decir, que presentan en su estructura una parte polar y otra no polar. Cuando se disuelven en agua, las dos partes de la molécula experimentan comportamientos opuestos: la zona polar tiende a establecer interacciones electrostáticas con las moléculas de agua, mientras que la zona no polar opta por agregarse para ofrecer la mínima superficie de contacto con ella. El resultado de estas dos tendencias contrapuestas es que las moléculas anfipáticas se asocian para constituir unas estructuras estables denominadas micelas. La concentración mínima a partir de la cual se forma la micela es propia de cada surfactante y se denomina concentración micelar crítica (CMC).

En la Figura 3 se muestra la estructura de un surfactante en medio acuoso, donde las zonas polares de la molécula se disponen hacia el exterior en contacto con el agua, mientras que las zonas no polares lo hacen hacia el interior, aisladas del contacto con

el agua y mostrando un carácter hidrofóbico. La parte no polar generalmente consiste en una larga cadena hidrocarbonada, lineal o ramificada, con un número variable de átomos de carbono.

Por otro lado, dependiendo de la naturaleza del grupo polar, los surfactantes se pueden clasificar en catiónicos (con carga positiva), aniónicos (con carga negativa), zwitteriónicos (con carga positiva y negativa) o no iónicos (sin carga).

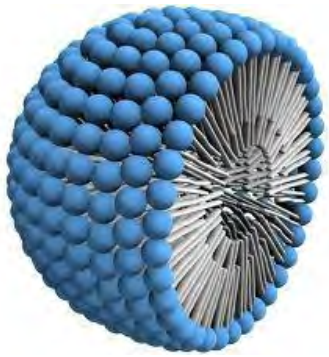

Figura 3. Organización en forma de micelas de un surfactante en medio acuoso. La zona exterior de la estructura, representada en azul, corresponde a la parte polar de la molécula en contacto con el agua, mientras que la zona no polar se dispone hacia el interior.

La estructura anfipática de los surfactantes le proporciona importantes propiedades tales como la posibilidad de reducir la tensión superficial del agua y la de adsorberse en las superficies e interfases de un sistema formado por fases inmiscibles, fenómeno responsable de la mayoría de las aplicaciones industriales de los surfactantes como detergentes y estabilizadores de emulsiones.

La característica más importante de estas sustancias desde el punto de vista de la Química Analítica, es su capacidad para solubilizar solutos de diferente naturaleza [38],

lo que permite incorporarlos como extractantes en diferentes técnicas de preparación de muestra. Así, se han publicado numerosas aplicaciones donde los medios micelares reemplazan con éxito a los disolventes orgánicos [39]. Además tienen la capacidad de realzar la señal de los analitos cuando se emplean técnicas de detección luminiscentes [40]. Los surfactantes son además compatibles con las fases móviles empleadas en cromatografía líquida, por lo que pueden ser empleados como desorbentes en la SPME (epígrafes III.1.1. y I.1.2.) como eluyentes en la SPE (epígrafe III.1.3.) y como extractantes en la MAE (epígrafes III.1.4. y III.1.5). En todos esos casos, se ha comprobado que pueden sustituir a los disolventes orgánicos en términos de eficiencia de extracción desde las distintas matrices líquidas y sólidas.

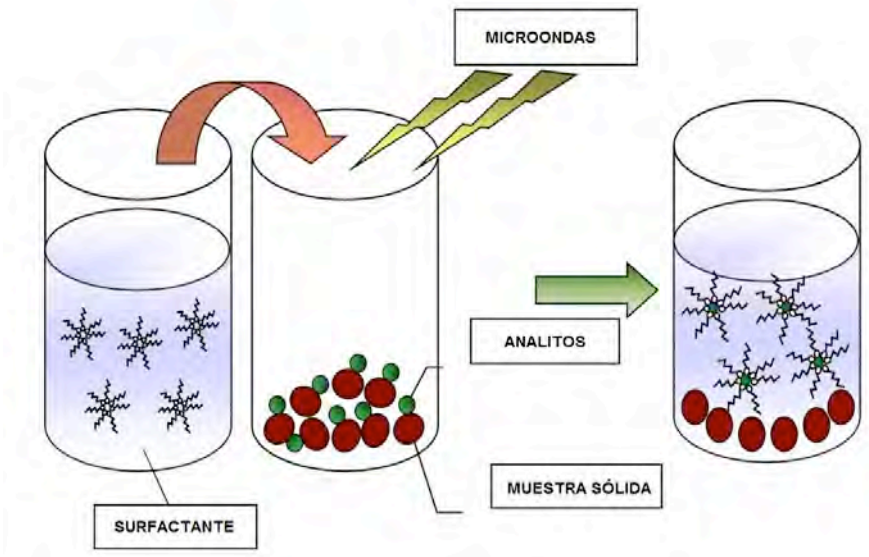

Figura 4. Solubilización de analitos desde una matriz sólida mediante el empleo de surfactantes en la MAE.

En la Figura 4 se muestra un esquema del uso de surfactantes en la MAE. En él se representa cómo al añadirlos a una muestra sólida y aplicar energía microondas, los

analitos se solubilizan desde la matriz y quedan envueltos en las micelas. Las micelas no son sistemas estáticos, sino que están, junto con los solutos que han sido solubilizados por ellas, en equilibrio dinámico con el medio. De esta forma, las moléculas de surfactante entran y salen de la micela en microsegundos.

I.3. Fármacos y productos de cuidado personal

Los fármacos y productos de cuidado personal (PPCPs, *pharmaceuticals and personal care products*) son un grupo de contaminantes emergentes muy interesantes desde el punto de vista medioambiental.

Los compuestos emergentes son contaminantes no contemplados por la legislación, cuyos efectos adversos no se conocen con exactitud y que son candidatos a ser regulados en el futuro en función de si se realizan estudios para dilucidar sus efectos sobre la salud y de si se lleva a cabo una monitorización espacial para trazar un mapa de ocurrencia [41].

Los PPCPs, cuyo uso ha aumentado muchísimo en los últimos años, llegan a las estaciones de aguas depuradas (EDARs) después de haber sido consumidos por los seres humanos. En 2010 existían aproximadamente 4000 fármacos distintos en el mercado [42], muchos de ellos administrados bajo prescripción y otros que son tomados por la población sin control médico (paracetamol, ibuprofeno, anticonceptivos, etc.). En cuanto a los productos de cuidado personal, los ingredientes que contienen son muy variados y los artículos donde podemos encontrarlos son numerosísimos (jabones, cosméticos, tintes, perfumes…).

Una vez estos compuestos salen de las EDARs sin haber sido eliminados ni controlados de ninguna forma, pueden llegar al medio ambiente a través de emisarios o mediante regadío. Además, pueden suponer una fuente directa de contaminación si se usan en acuicultura o por las propias excreciones de los animales.

Concretamente en la isla de Gran Canaria, donde se ha llevado la toma de muestras para validar las metodologías desarrolladas en esta Tesis Doctoral, la reutilización de aguas depuradas para riego es una práctica utilizada desde hace más de treinta años, dada la escasez de recursos hídricos que existe en la isla [43]. El resto de las aguas depuradas son descargadas en vertidos controlados a lo largo de la costa.

Por otro lado, una parte de los lodos que se obtienen como productos en los procesos de tratamiento en las EDARs se utilizan como abono y el resto son depositados en los vertederos. Todas estas vías generan una contaminación directa (descargas en el mar) o indirectas (contaminación de suelos y posteriormente de cultivos, y filtraciones en los niveles freáticos y en consecuencia en las aguas subterráneas). A partir de ahí los contaminantes pueden circular a través de una cadena en la que finalmente pueden volver a los humanos (Figura 5).

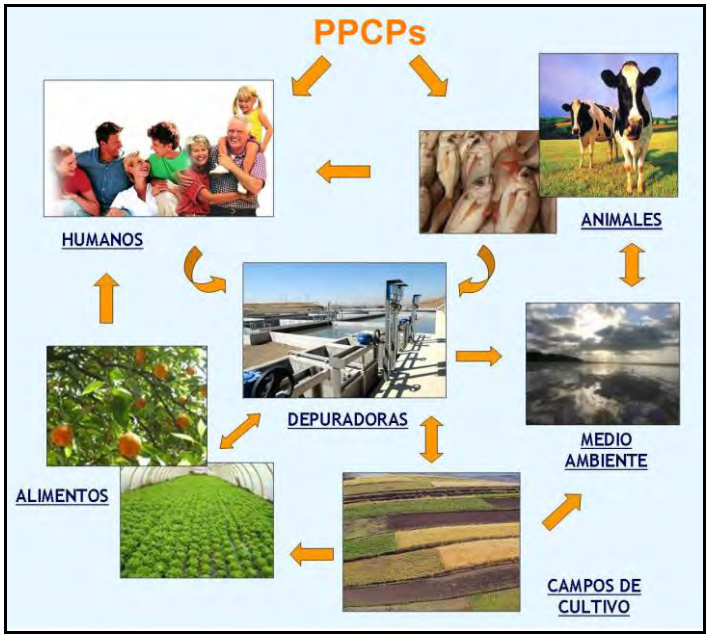

Figura 5. Ciclo de transporte en que pueden participar los PPCPs una vez son excretados por los animales o los seres humanos.

En esta Tesis Doctoral se han desarrollado procedimientos analíticos para dos grupos de compuestos representados dentro de los PPCPs: hemos escogido una familia de fármacos, concretamente antibióticos, las fluoroquinolonas, y un grupo de ingredientes utilizados en diversos productos de cuidado personal, los benzotriazoles estabilizadores de luz UV. De ambas familias de compuestos se hablará extensamente en los epígrafes I.3.1. y I.3.2.

I.3.1. Fármacos: Fluoroquinolonas

I.3.1.1. Características

Las fluoroquinolonas son antibióticos de origen sintético empleados para tratar distintos procesos infecciosos tanto en medicina humana como veterinaria. Poseen un amplio rango de actuación contra bacterias Gram-negativas y Gram-positivas, inhibiendo la ADN girasa y topoisomerasa IV, respectivamente [44]. Estas enzimas son esenciales en los procesos de replicación y transcripción del ADN.

Este grupo de compuestos pertenece a otra familia más amplia de antimicrobianos, las quinolonas. Todas las quinolonas parten de un núcleo común (o núcleo quinolónico), el acido nalidíxico, que fue descubierto fortuitamente en 1962 por Lesher y colaboradores durante la síntesis de la cloroquina, un compuesto para tratar el paludismo (Figura 6) [45].

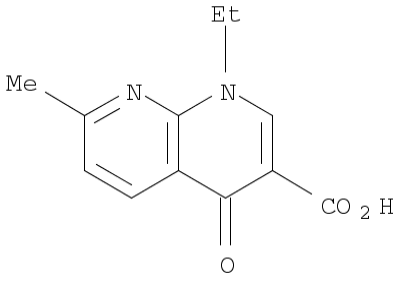

Figura 6. Estructura del ácido nalidíxico.

Su limitada aplicación a enfermedades urinarias, sus efectos secundarios y la rápida aparición de resistencia bacteriana, hicieron que pronto se desarrollaran otras sustancias derivadas del acido nalidíxico que resultaran más efectivas contra los microorganismos. Así, apareció el ácido pipemídico, introduciendo un radical

piperazinil en la posición 7, con mayor capacidad para atravesar la pared celular bacteriana (Figura 7) [46].

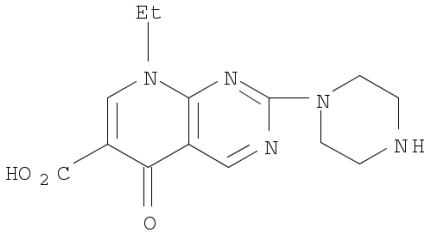

Figura 7. Estructura del ácido pipemídico.

El primer paso hacia las actuales fluoroquinolonas fue la síntesis en 1973 de la flumequina (Figura 8), la primera quinolona con un átomo de flúor que le confería una mayor efectividad contra bacterias Gram-positivas. Todos estos compuestos son conocidos como quinolonas de primera generación y fueron ampliamente usadas durante los años 70.

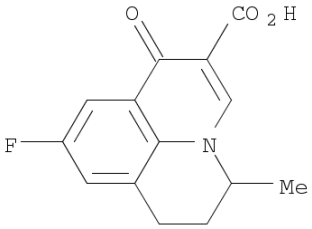

Figura 8. Flumequina.

A finales de los años 70, la síntesis de la norfloxacina, que combinaba el grupo piperacina en la posición 7 y un átomo de flúor en posición 6, supuso el nacimiento de las quinolonas de segunda generación, más efectivas contra bacterias Gram negativas. A éste le siguieron otros compuestos como la ciprofloxacina, la primera fluoroquinolona empleada para infecciones que no fueran del tracto urinario y el

agente microbiano mas empleado en el mundo a finales del siglo XX [47]. Estos nuevos sustituyentes les proporcionan un espectro de acción más amplio, mejores propiedades farmacocinéticas (mayor penetración intracelular, baja unión a proteínas plasmáticas, larga semivida de eliminación) y menor aparición de resistencias [48].

Posteriormente llegaron las quinolonas de tercera y cuarta generación, compuestos bi- o trifluorados cada vez más efectivos [49] y con más aplicaciones (infecciones urinarias, del sistema respiratorio, de transmisión sexual, cutáneas etc.).

Dado que la aparición de bacterias resistentes a los antibióticos supone una amenaza importante para el tratamiento efectivo de las enfermedades infecciosas, existe un interés creciente en conocer el transporte, la transformación, la acumulación y los efectos de los antibióticos en el medio ambiente. Existen estudios donde se correlaciona la presencia de contaminación por fluoroquinolonas con la de microorganismos resistentes, como por ejemplo *Escherichia Coli* en un río japonés [50]. Además, existen estudios que afirman que el alcantarillado hospitalario puede representar un ecosistema con condiciones óptimas para que se desarrolle resistencia a antibióticos por parte de las bacterias [51]. Además de éste, existen otros riesgos asociados a la presencia de fluoroquinolonas en el medio, como son problemas de salud generados por su movilidad a través de la cadena trófica (en consumidores de pescado u otros organismos acuáticos) [52].

Las fluoroquinolonas son normalmente administradas por vía oral y escasamente absorbidas en el tracto digestivo, por lo que entre un 60 y un 85% de la

dosis que toma el paciente es excretada sin metabolizar [53]. A continuación, su eliminación en las EDARs es solamente parcial [54,55].

Estos compuestos tienen propiedades anfotéricas ya que poseen un grupo amino que puede protonarse y un grupo carboxílico que puede deprotonarse. Así pueden estar presentes en disolución en forma aniónica, catiónica, neutra o zwitteriónica. Sin embargo, en los rangos pH que se dan normalmente en el medio ambiente, las fluoroquinolonas suelen estar en forma zwitteriónica, lo cual favorece su hidrofobicidad [56]. Así, cuando son vertidas en las masas de agua pueden moverse rápidamente hacia el suelo o sedimento, donde son fuertemente absorbidas por los minerales y la materia orgánica [57], siendo ésta su principal vía de eliminación en las aguas residuales. Presentan además una gran estabilidad térmica y química debida a su anillo heterocíclico, resistencia a la hidrólisis y son poco biodegradables. Todo esto hace que puedan tener un importante impacto medioambiental. Aunque sí presentan fotodegradación, la naturaleza, toxicidad y persistencia de los fotoproductos aún no están claras [58].

Los rangos de concentración en los que se encuentran las fluoroquinolonas van de $ng \cdot L^{-1}$ a $\mu g \cdot L^{-1}$ en ecosistemas acuáticos y de $\mu g \cdot kg^{-1}$ a $mg \cdot kg^{-1}$ en suelos. Sin embargo, aún no se han establecido límites de concentración de fármacos en el medio ambiente [58], aunque la Agencia Europea para la evaluación de productos médicos propuso ya en 1996 un umbral de 0.1 $\mu g \cdot L^{-1}$ en aguas subterráneas y de 10 $\mu g \cdot kg^{-1}$ en suelos y abonos para residuos de medicamentos empleados en veterinaria [59].

I.3.1.2. Técnicas de extracción

I.3.1.2.1 Muestras acuosas

La técnica de extracción más empleada para análisis de fluoroquinolonas en muestras acuosas es sin duda la SPE. En un trabajo de revisión bibliográfica publicado por Speltini y colaboradores en 2010, se recogen casi una treintena de métodos (publicados sólo en la última década) para su determinación en aguas medioambientales usando este procedimiento [60].

Destaca el uso de cartuchos HLB, empleado en más de la mitad de esos trabajos, debido a que las fluoroquinolonas tienen grupos funcionales tanto ácidos como básicos que pueden interaccionar con la parte hidrofílica o hidrofóbica del adsorbente. Usando este tipo de material se han obtenido buenas recuperaciones tanto en aguas residuales [61-64], como en aguas superficiales [62-66], aguas subterráneas [65,67] y agua de mar [67]. También se emplean frecuentemente los materiales de intercambio iónico y fase reversa llamados de modo mixto, como el MPC con base de sílica [68,69] ó el MCX de base polimérica [70-72]. A menudo se colocan dos materiales distintos en tándem para mejorar la eliminación de interferencias, sobre todo en muestras muy sucias como las aguas residuales [73-75]. Durante el uso de la SPE, se usan condiciones ácidas o básicas para la extracción y elución, en función del material ad- o absorbente escogido [60].

Siguiendo la tendencia que existe en la Química Analítica hacia la automatización, en los últimos años se han publicado trabajos para la extracción desde

distintas matrices (aguas superficiales, aguas depuradas y aguas residuales) empleando la SPE "en línea" acoplada a un sistema cromatográfico [76-79].

Nuevos materiales como los MIPs han sido desarrollados para la extracción de estos compuestos mediante la SPE, tanto en modo "en línea" como en modo convencional, siendo la robustez una de sus principales ventajas. Benito-Peña y colaboradores (2008) usaron como compuesto "molde" el enrofloxacino para la extracción de distintas fluoroquinolonas desde aguas de río, destacando los autores que el material adsorbente puede ser reutilizado hasta 80 veces [80]. Usando la combinación "en línea" la reutilización puede ser de hasta 200 veces [81].

También existen ejemplos de aplicaciones de técnicas de microextracción para la extracción de fluoroquinolonas en muestras medioambientales, como por ejemplo la SPME "en tubo" (*in tube SPME*) [82]. En esta variante de la SPME se usa como fase extractante una columna capilar de cromatografía de gases acoplada a un sistema cromatográfico, reduciéndose así la manipulación, el tiempo de análisis y aumentando la sensibilidad y reproducibilidad.

Otras técnicas miniaturizadas aplicadas con éxito son la microextracción en fase líquida con fibra hueca [83,84], la microextracción en fase líquida con fibra hueca empleando electromembranas [85] la microextracción liquido-liquido dispersiva asistida por ultrasonidos [86] y la microextracción con sorbentes empacados usando MIPs [87].

I.3.1.2.2 Muestras sólidas

Para la extracción de fluoroquinolonas en suelos, sedimentos y lodos, se han empleado diversas técnicas, desde las más tradicionales como agitación por ultrasonidos hasta las más sofisticadas como PLE.

La extracción asistida por ultrasonidos, por ser sencilla y de muy bajo coste, es la técnica con mayor número de aplicaciones en la determinación de estos antibióticos en muestras sólidas medioambientales [55,88-93]. Distintos extractantes han sido empleados, desde soluciones acuosas [89-91] hasta un 100% de acetonitrilo [92] con distintos aditivos. Frecuentemente se usan soluciones amortiguadoras de fosfato [55,88,93] para garantizar que la muestra se encuentre a pH neutro, donde predomina la forma zwitteriónica de las fluoroquinolonas, que es la menos hidrofílica y que tendrá mas afinidad por el disolvente orgánico [49].

Existen también numerosos ejemplos de la extracción de fluoroquinolonas usando la PLE desde suelos [54,94,95], sedimentos [94] y lodos procedentes de EDARs [54,96]. Vázquez-Roig y colaboradores (2010), en un enfoque hacia la llamada “química verde”, usaron agua a 90ºC como único extractante, proporcionando eficiencias de extracción comparables a las del metanol [94]. Aunque ésta es una técnica que permite aplicar temperaturas de hasta 200ºC, se ha observado que valores superiores a 100 ºC conlleva un importante problema de efecto matriz al aumentar la extracción de materia orgánica contenida en la muestra [58] .

En una revisión de procedimientos para la extracción de fluoroquinolonas desde matrices sólidas medioambientales, publicado por Speltini y colaboradores

(2011), se destaca la MAE como la técnica más ventajosa en términos de rapidez, eficiencia de extracción, coste y facilidad en la optimización [58]. Así, se han publicado muy buenos resultados para la extracción desde muestras agrícolas, sedimentos de lago y sedimentos marinos [97-100]. Con esta técnica también es posible emplear agua como extractante con excelentes recuperaciones (99%) [97]. En todas estas aplicaciones se obtuvieron mejores resultados empleando valores extremos de pH, debido a las características ácido-base de las fluoroquinolonas [58].

I.3.1.3 Técnicas de separación y sistemas de detección

Además de realizar una eficiente extracción y preparación de la muestra, es importante realizar una correcta separación de los analitos antes de su llegada al detector. Esto nos permitirá identificarlos de manera inequívoca, sobre todo cuando se analizan muchos compuestos a la vez.

La cromatografía líquida de alta resolución (LC, *High-performance liquid chromatography*) es la técnica más usada en el mundo de la Química Analítica para la separación de todo tipo de compuestos procedentes de muestras medioambientales. En el caso de las fluoroquinolonas, se emplea mayoritariamente la cromatografía líquida en fase reversa, usando columnas del tipo C_{18} [61-65,68,71,90,95,101,102] y C_8 [67,70,72,82,103].

Aunque existen algunos trabajos en los que se emplean columnas monolíticas [66,75], la mayoría de las aplicaciones que podemos encontrar en la bibliografía se han llevado a cabo con columnas convencionales.

Las columnas de fase reversa presentan problemas en la separación de compuestos básicos, ya que se producen fuertes interacciones con las formas aniónicas de los silanoles de la fase estacionaria, y esto causa un ensanchamiento de los picos [49]. Por ello, lo más apropiado es emplear una fase móvil a un pH bajo [60,66,68,69,72,75,80,82,104,105] ya que en medio ácido las fluoroquinolonas se encontrarán en forma catiónica [60].

No obstante, en ocasiones pueden aparecer problemas para separar algunas fluoroquinolonas, como por ejemplo la norfloxacina y la ciprofloxacina, debido a que la

estructura química de estos dos compuestos sólo difieren en que el primero tiene un grupo etilo mientras que el segundo tiene un grupo ciclopropilo [106].

Las fases móviles empleadas suelen consistir en una fase acuosa y un modificador orgánico (metanol o acetonitrilo) con una solución amortiguadora basada en ácido acético, acido cítrico o acido trifluoroacético [49].

La llegada de la cromatografía liquida de ultra resolución (UHPLC, *Ultra high-performance liquid chromatography*) ha permitido desarrollar métodos analíticos mucho más rápidos y con un importantísimo ahorro de disolventes, algo que está en concordancia con las nuevas tendencias en Química Analítica expuestas a lo largo esta Introducción.

Las partículas de la fase estacionaria de las columnas cromatográficas empleadas en UHPLC tienen un diámetro interno de menos de 2 µm, que en comparación con las de HPLC (entre 3 y 5 µm) permiten alcanzar mayores presiones que se traducen en picos más estrechos con mejor resolución, un aumento sensibilidad y por supuesto en tiempos de análisis menores [60].

Aunque se trata de una tecnología relativamente reciente y novedosa, y aún no existe una bibliografía muy numerosa al respecto, podemos encontrar ya algunos ejemplos de análisis de fluoroquinolonas empleando UHPLC [64,71,101,107]. Por ejemplo, Xiao y colaboradores (2008) realizan el análisis de 20 fluoroquinolonas en menos de 12 minutos empleando una columna con partículas de 1.7 µm de diámetro interno [101].

Los sistemas de detección que son generalmente acoplados a LC/UHPLC para la determinación de fluoroquinolonas están basados en técnicas ópticas (absorción de luz UV y emisión de fluorescencia) y en menor medida en espectrometría de masas.

El uso de detectores de UV ha resultado bastante útil, especialmente en muestras biológicas (sangre, orina, tejidos, etc.) [108-113]. Sin embargo, el número de interferencias que pueden aparecer debido a la absorción de luz UV por parte de otros compuestos de la muestra, hacen de ella una técnica con una selectividad muy limitada en matrices complejas. Además, pueden aparecer problemas durante la elución en gradiente, ya que ningún disolvente es totalmente transparente y pueden registrarse fluctuaciones en la absorción por parte de la fase móvil al modificarse su composición [49].

La detección por fluorescencia sí es ampliamente aceptada y empleada para la determinación de fluoroquinolonas en muestras procedentes del medio ambiente [63,66-69,75,80,104,105]. Estos compuestos presentan fluorescencia debido a su anillo aromático, y se ha demostrado que su intensidad depende del pH de la disolución, ofreciendo mayor señal en medio ácido [114-115]. Aunque se trata de una técnica mucho más sensible y selectiva que la detección por UV, en muestras muy complejas como las aguas residuales, parece ser más adecuada la detección por espectrometría de masas, ya que los espectros de fluorescencia pueden presentar mucho ruido de fondo e interferencias debido a la materia orgánica presente en la muestra.

Sin embargo, hay autores que sostienen que empleando un procedimiento adecuado de extracción y preconcentración, la detección por fluorescencia puede ser igual de válida ó incluso mejor que la espectrometría de masas [58,60]. Por ejemplo,

empleando espectrometría de masas con analizador por tiempo de vuelo (TOF, *Time of Flight*), aunque se consigue una óptima identificación de los compuestos, no se logran límites de detección tan competitivos [60,76].

Para lograr la sensibilidad necesaria en la determinación de analitos a nivel trazas, la mejor opción es usar espectrometría de masas con triple cuadrupolo (QqQ) [76], ya que ofrece mejores límites de detección. Además, y a pesar de que su selectividad no es tan buena como la del TOF, permite cumplir los criterios establecidos por la Unión Europea para la confirmación de residuos orgánicos [116].

En cuanto a las interfases empleadas entre el sistema de cromatografía liquida y el detector de masas, la más usada es la ionización por electrospray (ESI, *electrospray ionization*) [55,61,65,71,74,76,95]. En ella se produce la dispersión del analito dentro de un aerosol, y es muy útil para compuestos polares como las fluoroquinolonas ya que evita su tendencia a fragmentarse cuando son ionizados. La ionización suele hacerse en modo positivo ya que los grupos amina y cetona de las fluoroquinolonas son fácilmente protonados, y usando una fase móvil ácida para que se favorezca el proceso [49].

Para nuestro conocimiento, la ionización química a presión atmosférica (APCI, *Atmospheric Pressure Chemical Ionization*), no ha sido empleada en el análisis de fluoroquinolonas, probablemente porque los límites de detección que proporciona pueden ser hasta 10 veces más altos que los obtenidos con la ESI. A pesar de esto, la APCI puede ser más adecuada que la ESI para evitar problemas de supresión iónica [117].

I.3.2. Productos de cuidado personal: Benzotriazoles estabilizadores de luz UV

I.3.2.1 Benzotriazoles estabilizadores de luz UV (BUVSs) en Productos de Cuidado Personal: Metodologías recientes de extracción y determinación en muestras medioambientales y biológicas

En los últimos años, los cosméticos y otros productos de cuidado personal han ido incorporando en sus formulaciones compuestos estabilizadores de luz ultravioleta, debido a que ha aumentado la preocupación por los daños que la radiación solar puede provocar en la piel.

Una de las familias de compuestos más empleada como estabilizadores de luz ultravioleta es la de los benzotriazoles (BUVSs, *Benzotriazole UV stabilizers*) que tienen una estructura heterocíclica con un grupo fenólico capaz de absorber en un amplio espectro, tanto en el UV-A (320-400 nm) como en el UV-B (280-320 nm).

Se ha demostrado que estos productos no son eliminados en las EDARs, y por tanto, ésta es una de las principales vías por las cuales los BUVSs pueden alcanzar el medio ambiente. Dado que los BUVSs han sido descritos como contaminantes con características mutagénicas, estrogénicas, tóxicas, persistentes y con posibilidad de ser bioacumulados, existe un creciente interés en conocer todos los efectos negativos que pueden tener en el medio ambiente y sobre la salud de humanos y animales. Por ello, es vital desarrollar metodologías que permitan su extracción y determinación en distintas matrices complejas y a los niveles de concentración requeridos.

El siguiente trabajo ofrece una revisión de las metodologías que han sido empleadas para la extracción y determinación de estos compuestos en diferentes tipos

de muestras medioambientales y biológicas ofreciendo una discusión detallada de las principales ventajas y desventajas de cada una de las técnicas de extracción y detección empleadas.

Dicho trabajo ha sido enviado a la revista *Trends in Analytical Chemistry (TrAC)* y actualmente se encuentra en proceso de revisión.

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Editor of *Trends in Analytical Chemistry*

Dear Editor,

I´m enclosing you the manuscript entitled: **"Benzotriazole UV stabilizers (BUVSs) in Personal Care Products: Recent extraction and determination methodologies in environmental and biological samples"** for your consideration for publication in *Trends in Analytical Chemistry.*

In this paper we reviewed/overviewed the recent methodologies (new approaches) for extraction, preconcentration and determination of Benzotriazole UV stabilizers (BUVSs) in environmental and biological samples. This review is devoted mainly in BUVSs used in Personal Care Products, and it includes new extraction/preconcentation (SPE, microextraction -SPME, Stir-bar, MAE, USE, PLE, MSPD, etc.), procedures and determination techniques (GC and LC with different mass spectrometry detection, etc.), applied to environmental and biological samples.

Las Palmas de Gran Canaria, 28 February 2013.

Sincerely yours,

Prof. Dr. José Juan Santana Rodríguez

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Fecha: 1 Mar 2013 09:57:16 +0000
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Title: Benzotriazole UV stabilizers (BUVSs) in Personal Care Products: Recent extraction and determination methodologies in environmental and biological samples
Trends in Analytical Chemistry

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Thank you for submitting your work to Trends in Analytical Chemistry.

Kind regards,

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Benzotriazole UV stabilizers (BUVSs) in Personal Care Products: Recent extraction and determination methodologies in environmental and biological samples

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Abstract

Benzotriazole UV stabilizers (BUVSs) are a group of emerging contaminants that have been described as mutagenic, toxic, pseudo-persistent, and bioaccumulative; they also have significant estrogenic activity. In spite of this, these substances are extensively employed in diverse industrial activities. It has been reported that their use in personal care products (PCPs) is one of the primary ways that these substances enter the environment, although in both cases the emissions sources are often focused in "hot-spots" of discharge, such as wastewater treatment plants (WWTPs). Given the increasing interest of the scientific community regarding this topic, in this article we present an overview of the current methods employed in the trace analysis of BUVSs in different types of environmental and biological samples. We compare and discuss the potential advantages and disadvantages of each step involved in the analytical procedure, from different sample pre-treatments that have been employed in the conservation, extraction and purification of real samples to the detection and quantification techniques that have been used to analyse mixtures of these compounds over the last decade.

Keywords: Benzotriazole, UV stabilizers, liquid chromatography, gas chromatography, mass spectrometry, sample preparation.

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1. Introduction

Organic UV filters have been employed for decades in the formulation of personal care products (PCPs). Moderate exposure to solar radiation has both physical and psychological benefits [1], but the growing concern about the progressive and continued damage of the stratospheric ozone layer, resulting in increased incidences of erythema, burning, dehydration, photodermatoses, photoaging and skin cancer [2,3] has increased the use of these compounds in recent years. Although UV filters were initially designed to be used in sunscreen formulations, they are currently added to other daily cosmetic products to prevent the harmful effects of UV exposure that occur not only when sunbathing but also in daily life [1]. However, this class of organic filters has also received much interest in the scientific community in the last decade due to their potential to adversely affect the health of living organisms when they reach aquatic environments. Different studies have shown that organic UV filters can be absorbed through the skin after topical application, further metabolised in the body and bioaccumulated or eventually excreted [1]. Therefore, the entry of these compounds into the environment may occur via discharges from wastewater treatment plants (WWTPs) in addition to discharges from swimming pool water [4] or from recreational activities such as swimming and bathing in beaches, lakes or rivers (direct inputs) [5]. This continuous generation through anthropogenic activities allows UV filters to pseudo-persist in the environment [6].

One of the most commonly employed types of organic UV filters is benzotriazole UV stabilizers (BUVSs), which are derivative compounds of benzotriazole. Benzotriazole has a heterocyclic structure containing three N atoms which molecular form is $C_6H_5N_3$. BUVSs have a phenolic group attached to this benzotriazole structure (Figure 1 shows the structures of some BUVSs). These compounds absorb the full spectrum of UV light, both UV-A (320-400 nm) and UV-B (280-320 nm) [7], and are used in many PCPs such as sunscreen, soap, shampoo, toothpaste, scrubs, hair dye, nail polish, moisturising cream, lipstick, makeup formulations, and after-shave lotion, with typical concentrations between 0.1 and 10% [8]. For example, UV 360 or Bisoctrizole (commercially named Tinosorb M) is widely employed in sunscreen because of its triple action: UV absorption, light scattering and light reflection [2]. Moreover, UV 360 is used to stabilise other organic filters, such as avobenzone, due their extreme photostability [3]. BUVSs are also used for several other purposes, mainly as corrosion inhibitors in

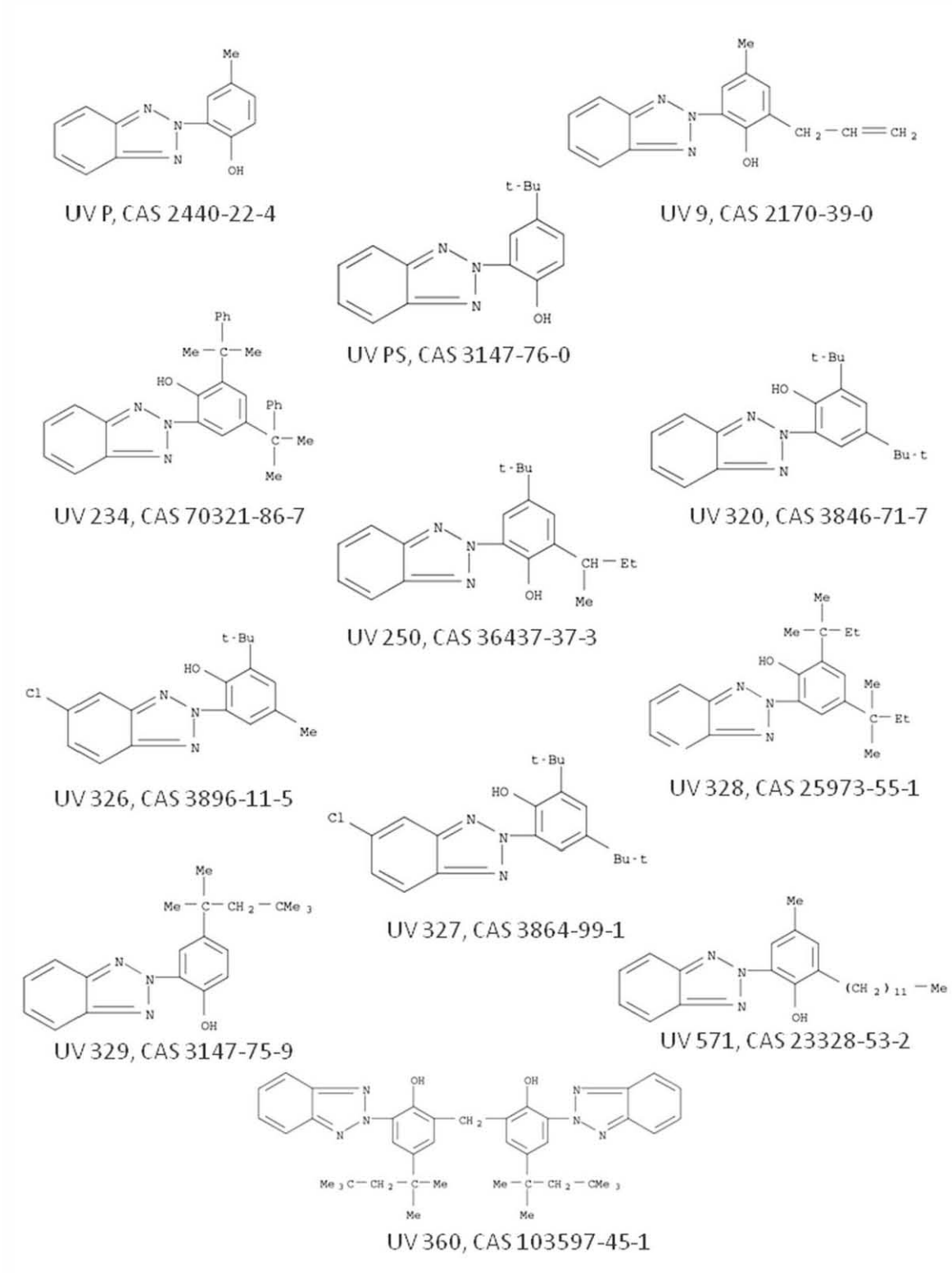

Fig. 1. Structures of some Benzotriazole UV Stabilizers (BUVSs).

dishwasher detergents, aircraft anti-icing fluids, automotive antifreeze formulations, industrial cooling systems, metal-cutting fluids, brake fluids, solid cooling lubricants, antifoggants in photography, and ultraviolet light stabilizers in plastics [9]; they are also used in specialised applications such as intraocular and contact lenses [10] or dental restorative materials [11]. Considering that percutaneous absorption processes may result in various adverse health effects, such as genotoxicity and estrogenicity, their maximum allowed concentrations have been regulated by legislation in various countries [1]. Some BUVSs were approved by the Cosmetics Regulation of the European Community [12] and have been considered by the International Nomenclature of Cosmetic Ingredients (INCI) [13] and the International Fragrance Organisation (IFRA) [14]. However, some of these compounds have also been identified by the US EPA (United States Environmental Protection Agency) as ingredients of unknown toxicity [15], and two benzotriazole derivatives were found to be mutagenic in bacterial systems [16].

Several studies have been conducted to clarify the estrogenic and toxic effects of BUVSs. Their potentially toxic effects on biota, particularly aquatic organisms, were investigated by Kim *et al.* [4], and the 24- and 48-h median lethal concentration (LC_{50}) values of UV 571 for *Daphnia pulex* were estimated to be 6.35 (5.08-8.39) and 2.59 (2.04-3.38) $mg \cdot L^{-1}$, respectively. No acute toxicity effects were observed at up to 10 $mg \cdot L^{-1}$ for other BUVSs such as UV 9, UV 320, UV 326, UV 327, UV 328, UV 329 and UV 360. Another study demonstrated that UV P, UV 326, UV 327 and UV 328 had no positive effect in *in vitro* assays [17], in agreement with Kawamura et al. [18], who found no estrogenic activity for UV P, UV 234, UV 326, UV 327, UV 328 and Uvitex OB. However, it has been demonstrated that benzotriazole derivatives are mutagenic in plants [19], and adverse effects on the fecundity and reproduction of fish have also been reported [20]. Moreover, they are soluble in water and resist biodegradation [21].

Although benzotriazole compounds have been included in several reviews regarding environmental contamination [19,22,23], limited information is available about the extent of the contamination of this class of UV stabilizers, especially concerning the most lipophilic compounds. The first reports describing the presence of BUVSs (named also Tinuvins) in the environment were presented by Jungclaus et al. in 1978 [24]. Afterwards, relevant concentrations of BUVSs have been

Table 1. Methods for the determination of BUVSs compounds in liquid samples. *Abbreviations*: See Appendix.

Compounds	**Matrix**	**Extraction technique, characteristics**	**Instrumental analysis**	**Recoveries (%)**	**LODs (ng·L^{-1})**	**Concentrations in environmental samples (ng·L^{-1})**	**Ref.**
UV P, UV 9, UV 320, UV 326, UV 327, UV 328	Raw and treated wastewater	SBSE-LD, n-pentane	GC-MS	24.9- 90.7 (Raw wastewaters) 24.6-83.7 (Treated wastewaters)	40-150*	22-85 (Raw wastewaters) 21-31 (Treated wastewaters)	[25]
UV 320, UV 326, UV 327, UV 328	Raw and treated wastewater	LLE, hexane	GC-MS	98-115	2.1-8.7	5.6-78 (Raw wastewaters) 2.1-4.5 (Treated wastewaters)	[26]
UV P, UV 9, UV 326, UV 327, UV 328	River, raw and treated wastewater	SPME-HD, PDMS-DVB coated	GC-MS/MS	86-108 (River) 28-144 (Raw wastewater) 89-109 (Treated wastewater)	0.5-2*	1-57 (Raw wastewater)	[28]
UV P, UV 234, UV 326, UV 327, UV 328, UV 329	Surface water	SPE, dichloromethane	GC-MS	79.9-129.6	0.1-0.7	1-701	[29]
UV 326, UV 329	Ground water and treated wastewater	SPE, methanol:dichloro-methane (50:50)	GC-MS/MS	90-110 (Ground water) 95-110 (Treated wastewater)	1.5-5.6 (Ground water) 3.3-4.8 (Treated wastewater)	-	[34]
UV 326, UV 329	Raw and treated wastewater	SPE, methanol:dicloro-methane (50:50)	GC-MS/MS	96-108 (Raw waters) 95-110 (Treated wastewater)	4.1-5.6 (Raw waters) 3.3-4.8 (Treated wastewater)	15-414 (Raw wastewater) 5-125 (Primary effluent) 55-98 (Secondary effluent)	[35]
UV P, UV 326, UV 327, UV 328, UV 329, UV 360, UV 571	Seawater and Treated wastewater	SPE on line	UHPLC-MS/MS	60-89 (Seawater) 56-82 (Treated wastewaters)	5.3-17 (Seawater) 5.4-18 (Treated wastewater	2.9-5.2 (Seawater) 0.4-1.3 (Treated wastewaters)	[36]
UV P, UV 326, UV 327, UV 328, UV 329, UV 360, UV 571	Seawater and Treated wastewater	SBSE-LD	UHPLC-MS/MS	18.4-92.2 (Seawater) 18.3-83.3 (Treated wastewaters)	18-51 (Seawater) 19-55 (Treated wastewater)	-	[37]

found in wastewater [25,26], surface waters [27,28], sediment samples [7,29] and sewage sludges [30]. In contrast to the polar benzotriazole species, its phenolic derivatives show hydrophobic character and the potential to accumulate in solid environmental matrices and to be accumulated and magnified through the trophic chain [29]. These findings explain reports of the presence of some BUVSs in different marine organisms such as fish [31]; lugworms, oysters, clams, crabs, ducks, and sharks [7]; and even birds and mammals [32,33].

The published literature is still too scarce to deeply understand the fate and distribution of these compounds in the environment, and most of the existing reports are very recent publications, so this field is expected to continue to grow in the coming years. In this work, we review the methods used for the analysis of BUVSs in liquid and solid samples, which are always based on liquid or gas chromatography coupled to mass spectrometry, and we discuss the different extraction and clean-up techniques currently employed.

2. Sample preparation methods

The analysis of emerging contaminants in environmental samples is characterised by the difficulty of the detection of low concentrations in complex matrices, which require an extraction/pre-concentration step prior to their determination. However, sometimes the most important steps in a sample preparation method are sampling and storage. To prevent the loss of analytes through physical or biological mechanisms, several procedures are usually employed to improve analyte stability in the environmental matrix.

For liquid samples, several papers report the addition of methanol in percentages between 5% [34,35] and 10% [36,37] to avoid the adsorption of the most lipophilic compounds to the glass flask, whereas other papers report the adjustment of the sample pH to 2 [34,25] or 3 [25] to inhibit microbial degradation. Once in the laboratory, the samples are normally passed through a glass fibre filter [26,26,27,28] to obtain the dissolved fraction. The samples are then refrigerated at 4ºC for immediate analysis in some cases [36,37] or for a maximum of 24 h [25,34,35] or 48 h [28] in others. Storage at -20°C until chemical analysis is also common [26]. Meanwhile, solid samples are often transported to the laboratory in aluminium containers [30] or packed in aluminium foil [38], and sodium azide can be added to suppress microbial activity [34,35]. The samples are freeze-dried in most cases

[7,26,27,30,32,34,35], although occasionally they may be air-dried in a hood for several days [39]. The samples are then stored in the dark at 4ºC and processed within 48 h [34] or stored at –20°C for later analysis [7,26,29,30,38,40]. Finally, tissues obtained from biological samples are always ground with anhydrous sodium sulphate [7,31-33,41,42] and stored at -20°C [7,33,42] or -25ºC [31,41] until chemical analysis.

2.1. Liquid environmental matrices

Although very sensitive and selective detection systems exist currently, extraction and purification are still required in dirty samples such as sewage water. In very dilute water samples, such as seawater, a pre-concentration procedure is also indispensable. There are some reports regarding the analysis of BUVSs in environmental liquid matrices, and half of them used solid phase extraction (SPE) as a preparation method. SPE was developed in the 1980s, and it has emerged as a powerful tool for chemical isolation and purification. It is an alternative to conventional liquid-liquid extraction (LLE) methods because it reduces the consumption of organic solvents and analysis time and because it can be automated. SPE is widely used in the environmental analytical field and has been applied to surface water [29], groundwater [34] and both raw [35] and treated wastewater [34,35] for the extraction of BUVSs with good pre-concentration factors and subsequent high sensitivity. Table 1 shows different cases of determination of these compounds in liquid samples. Good recoveries were obtained from surface water using two tandem cartridges, a monomerically bonded octadecyl phase (Discovery DSC-18LT, used for large hydrophobic molecules such as BUVSs) and a polymerically bonded aminopropyl phase (Discovery DSC-PH) [29]. As this method requires multiple analyses, the authors tried to identify the overall best cartridge for simultaneous analysis of all of the analytes. However, the recoveries for the other tested cartridges were not provided. In the same year, 2011, another paper including a detailed study of the efficiency of different sorbents for several BUVSs was published [34]. Four cartridges were compared (Oasis HLB 6 mL 500 mg, Supelco ENVI-18 6 mL 500 mg, Starta X-C-33 µm 6 mL 500 mg and Selby Biolab C18 6 mL 500 mg) using the same elution solvent (methanol/dichloromethane, 3×2 mL) and the best recoveries were obtained using an Oasis HLB cartridge, which is a universal polymeric reversed-phase sorbent that was developed for the extraction of a wide range of

acidic, basic, and neutral compounds. Other extraction parameters such as elution solvents, elution volumes and pH values were also studied, and the method was applied to groundwater and treated water with very satisfactory recoveries (90-110% and 95-110%, respectively). The same authors applied the same methodology for the analysis of BUVSs in both treated and raw wastewater [35], and the recovery for raw wastewater was between 96 and 108%.

Nevertheless, this technique presents some disadvantages, such as an increased chance of loss during sample handling and the requirement for large sample volumes. On-line SPE strategies coupled to an LC system appear to solve these problems. The sample volume required is lower, and the automation minimises sample loss or contamination during handling and improves the reproducibility of the analysis. Another advantage is a reduction in the analysis time because the on-line SPE system can utilise two extraction columns in parallel, and when a sample is being eluted from one of the extraction columns, the next sample can be loaded onto the other column. Moreover, an exhaustive wash at high pressure allows for improved cleaning of the extraction columns (compared with off-line SPE) and also reduces carryover. A Waters Corporation application note [43] demonstrated that the extraction columns yielded good results after 500 injections. A comparative study of the off-line and on-line SPE procedures, which were applied to seven BUVSs in seawater and treated wastewater [36], demonstrated that on-line SPE provides better sensitivity because the pre-concentration factor is 100 times higher. This difference occurs because the elution is carried out using the chromatographic mobile phase, and all injected mass comes to the detector [44].

Despite these new preparation methods, very recent publications continue to use conventional techniques such as liquid-liquid extraction (LLE). In 2010, a method based on LLE was used for the extraction of four BUVSs, employing 200 mL of hexane [26]. Although this procedure offered very good recoveries (107-115%), it is relatively time consuming, harmful (due to the use of large volumes of organic solvents that are frequently toxic), and very expensive. Therefore, there is an increasing tendency to replace LLE with SPE for liquid samples.

Over the last decades, several microextraction techniques have been developed that allow the simplification of the procedure and the minimisation of the volumes used [45]. One of these techniques is solid

phase microextraction (SPME), introduced by Arthur and Pawliszyn in 1990 [46]. This technique can integrate sampling, extraction, concentration and sample introduction into a single process [47] with a moderate or even null consumption of organic solvent [28]. SPME employing head space (HS) exposure as the absorption mode for 30 minutes has been applied to the analysis of five BUVSs in surface and wastewater [28]. Four fibres coated with different polymers, polyacrylate (PA), polydimethylsiloxane (PDMS), Carboxen–PDMS (CAR–PDMS) and PDMS–divinylbenzene (PDMS–DVB), were examined, with the last one proving to be the most appropriate for the target analytes. Thermal desorption with gas chromatography (GC) was employed at 270 Cº, which exhibited good repeatability (5-12%) and reproducibility (8.6-11.1%). The recoveries were high for river and treated water (86-108% and 89-109%, respectively), while low efficiencies (28-44%) were obtained in raw wastewater for the most apolar analytes studied (UV 326, UV 327 and UV 328). Although SPME has been widely applied since its introduction 23 years ago, the extremely small amount of sorbent employed is its most important disadvantage [48].

Another available technique is stir bar sorptive extraction (SBSE), developed by Baltussen et al. in 1999 [49], which overcame some of the limitations of SPME. SBSE involves the extraction of the analytes from the matrix by employing a magnetic stir bar with a coating, normally polydimethylsiloxane (PDMS), which is a non-polar polymeric phase that presents a large affinity for low polarity species such as BUVSs [25]. Although other coatings for polar compounds have been introduced recently [50], their use is not yet widespread. SBSE is based on the same principles as SPME, but the volume of the PDMS phase is higher, resulting in better sample capacity and extracting efficiency [51]. Moreover, SBSE provided efficient extraction without high solvent consumption [52], and the stir bar proved to be more robust and less expensive than SPME fibres [25] because it can be reused at least 100 times [53]. After extraction, the stir bar is removed from the aqueous sample and subjected to thermal desorption (TD) or liquid desorption (LD) [48]. As in the SPME procedure, for SBSE we must consider several parameters that will govern the partition coefficients of the analytes between the two phases, PDMS and water ($K_{PDMS,\ W}$). $K_{PDMS,\ W}$ is correlated with K_{OW} [54], which favours the extraction of BUVSs because they are non-polar.

Two methods using SBSE with liquid desorption have been proposed for BUVSs [25,37]. LD is generally more suitable than TD because it minimises contamination from the PDMS phase, is compatible with liquid chromatography and does not require expensive units for desorption or interfaces [55]. One of these studies examined raw and treated wastewater [25], and the other examined seawater and treated wastewater [37]. Both studies agreed that the extraction efficiency depends on the matrix characteristics and the polarity of the compounds. The worst results were obtained for dirty samples and less polar species. Although the PDMS fibre is appropriate for non-polar analytes, incomplete desorption can occur once compounds with very high octanol-water distribution coefficients, log K_{ow} (up to 10.3 and 14.5), are strongly absorbed into the PDMS phase [37]. To overcome this problem, stronger solvents such as n-pentane or diethyl ether have been used [25], but these solvents are very aggressive with the PDMS phase and they also are dangerous and contaminants products. Moreover, the use of highly non-polar solvents involves an extra step or evaporation and reconstitution prior to injection into the chromatographic system; this process is laborious, can introduce errors and increases the time required for analysis. Table 2 gives a description of each technique and list advantages and disadvantages of each of them for liquid samples.

2.2 Solid environmental matrices

The extraction of analytes from solid samples in environmental applications presents added complications because solute-matrix interactions are very difficult to predict and overcome [56]. In the case of BUVSs, compounds that present high lipophilicity (e.g.: the log K_{ow} of UV 360 is 14.5 at 25ºC according to SciFinder Scholar Database), tend to accumulate in this type of matrix. We found the following extraction methods for solid samples: soxhlet extraction, SE; ultrasonic assisted extraction, UAE; accelerated solvent extraction, ASE; microwave-assisted extraction, MAE; and supercritical fluid extraction, SFE. Table 3 summarises a description of each technique for solid samples and present their advantages and disadvantages and Table 4 shows different applications of these techniques for the determination of BUVs in solid samples.

2.2.1. Sludges and sediments

Conventional methods have been widely employed for the extraction of BUVSs

Table 2. Extraction techniques for liquid samples

	LLE	**SPE**	**SPME**	**SBSE**
Brief description	Analyte is partitioned between two inmiscible solvents	Analyte is retained on a solid sorbent	Partition of analytes between a polymeric stationary phase and matrix samples	Partition of the analyte between stir bar and matrix sample
Extraction time	Up to 24 h	Between 30-60 min	Up to 60 min	Between 60-240 min
Volume solvent used	≥ 150 mL	1-5 mL	Low amount	Low amount*
Cost	Low cost	Relative low cost	Relative low cost	Relative moderated cost
Ease of operation	Easy	Relative easy	Relative easy	Relative easy
Disadvantages	Large consumption of volume and time	Insufficient retention of polar compounds; large volume of sample	Poor retention and stability of fiber	Poor desorption of analytes highly non-polar

* In liquid desorption

Table 3. Extraction techniques for solid samples

	Soxhlet extraction	**UAE**	**ASE**	**MAE**	**SFE**
Brief description	Analyte is placed in a Soxhlet apparatus with hot organic solvent	Sample is placed in a ultrasonic bath with organic solvent	Sample is extracted in high pressure and temperature conditions	Sample is placed in a vessel in a microwave oven	Sample is placed in a high pressure cartridge with a solvent in supercritical state
Extraction time	Up 48 h	Between 30-60 min	15 min	Between 10-30 min	Between 30-60 min
Volume solvent used	High amount	High amount	Low amount	Low amount	Lower than Soxhet and UAE
Cost	Low cost	Low cost	High cost	Moderate cost	Moderate cost
Ease of operation	Easy	Easy	Relative easy	Relative easy	Relative easy
Disadvantages	Evaporation step is necessary; large volume is used	Evaporation step is necessary; large volume is used	High dependence of sample matrices	Clean up step is necessary	Clean up step is necessary; high dependence of sample matrices

from sludges and sediments and offer good results in terms of repeatability and recovery, but their main disadvantage is the requirement of a large volume of strong solvents; such as ultrasonication (20 mL of dichloromethane and 20 mL of acetone) [27], shaking (50 mL of ethylacetate/dichloromethane, 1:1, v/v) [30], 50 mL of acetonitrile/hexane, 60:40, v/v [36]) or Soxhlet (dichloromethane/hexane, 8:1, v/v) [7,26,32] (see Table 4a). Moreover, an additional evaporation step is needed for each of these methods.

Ruan et al. [38] used accelerated solvent extractor (ASE) and obtained good recoveries (83-100%). After ASE, the extract was concentrated by evaporation, fractionated on a gel permeation chromatographic column, concentrated again, passed through a Florisil column and concentrated once again prior to injection. In other studies employing ASE, a clean-up step based on silica, copper and anhydrous sodium sulphate was incorporated to remove interfering components such as sulphur and lipids in the extracts [34,35]. In most cases, extraction procedures for solid samples require the use of a clean-up step for purification, which often causes the method to become even more tedious and time-consuming. On-line systems such as SPE have been coupled to chromatographic systems as clean-up procedures after extraction methods to minimise sample loss or contamination during handling and to improve repeatability [44].

Among the relatively modern sample preparation techniques that have been satisfactorily applied to BUVSs in solid samples, we found that MAE [44] reduces the extraction time, allows the preparation of multiple samples in a single step and requires lower volumes of organic solvents compared with conventional procedures [57]. Moreover, a weaker organic solvent can be sufficient to extract the compounds [44].

Another modern sample preparation technique is matrix solid-phase dispersion (MSPD), which has a low cost and combines a limited consumption of organic solvents, the use of mild extraction conditions and the potential to integrate extraction and purification in the same step. This technique has been satisfactorily employed for the extraction of six BUVSs in coastal and river sediments, with recoveries between 78 and 110% [39]. In MSPD, samples are dispersed with a suitable sorbent and then packed in a polypropylene syringe that contains a clean-up sorbent to retain co-extracted interfering species. Then, analytes are recovered with a small volume of an adequate organic solvent.

Table 4. Methods for the determination of BUVSs compounds in a) solid environmental samples, b) biological samples. Abbreviations: See Appendix.
a)

Compounds	Matrix	Extraction technique, characteristics	Instrumental analysis	Recoveries (%)	LODs ($ng \cdot g^{-1}$)	Concentrations in environmental samples ($ng \cdot g^{-1}$)	Ref.
UV 320, UV 326, UV 327, UV 328	Sludges from WWTP	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	98-115	0.0021-0.0087	120-1800	[26]
UV 326, UV 329	Sludges from WWTP	ASE, n-hexane:dichloromethane (50:50)	GC-MS/MS	81-152	0.3-8.2	49.9-122.9	[34]
UV 326, UV 329	Sludges from WWTP	ASE, n-hexane:dichloromethane (50:50)	GC-MS/MS	81-152	0.3-8.2	27-114	[35]
UV P, UV PS, UV 234, UV 320, UV 326, UV 327, UV 328, UV 329, UV 350	Sludges from WWTP	ASE, hexane:dichloromethane (7:3)	LC-MS/MS	83-100	0.15-0.77 *	0.57-24700	[38]
UV P, UV 234, UV 326, UV 327, UV 328, UV 329	River and lake sediments and sludges from WWTP	Ultrasonication, dichloromethane Secuencial SPE, hexane: acetone (100:0, 95:5, 0:100)	GC-MS	69.8-125.6	0.05-10	0.7-117 (sediments) 0.5-1266 (sludges)	[27]
UV 326, UV 327, UV 328, TBHPBT	River sediments and sludges from WWTP	Shaking, ethyl acetate:dichloromethane (1:1)	GC-MS	82-106	0.1-0.5	0.22-224 (sediments) 0.73-5920 (sludges)	[30]
UV P, UV 326, UV 327, UV 328, UV 329, UV 360, UV 571	Marine sediments and sludges from WWTP	MAE, acetonitrile + On-line SPE	UHPLC-MS/MS	50.1-87.1 (sediments) 46.1-83.9 (sludges)	0.053-0.106 (sediments) 69.9-108 (sludges)	0.18-24 (sediments) 0.94-12.2 (sludges)	[44]
UV P, UV 9, UV 320, UV 326, UV 327, UV 328	Coastal and river sediments	MSPD, dichloromethane	GC-MS/MS	78-110	3-15*	5.6-32	[39]
UV 320, UV 326, UV 327, UV 328	Coastal and river sediments	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	110-122	0.05-0.15	0.3-720	[7]
UV P, UV 326, UV 327, UV 328	Coastal and river sediments	Shaking, acetonitrile, hexane (60:40)	GC-MS	90-110	20	29-5200 (river sediments)	[29]

b)

Compounds	Matrix	Extraction technique, characteristics	Instrumental analysis	Recoveries (%)	LODs ($ng \cdot g^{-1}$)	Concentrations in environmental samples ($ng \cdot g^{-1}$)	Ref.
UV 320, UV 326, UV 327, UV 328	Marine organisms (mollusc, crustaceans, fish and birds)	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	110-122	0.05-0.15	0.06-55	[7]
UV 320, UV 327, UV 328	Blubber of marine mammals	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	110-114	0.05-0.15	4.27-66.1	[33]
UV 320, UV 326, UV 327, UV 328,	Marine organisms (mollusc, crustaceans, fish, birds and mammals)	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	110-122	-	-	[32]
UV 320, UV 326, UV 327, UV 328	Mussels	Soxhlet, dichloromethane:hexane (8:1)	GC-MS	110-122	0.05-0.15	7.6-450	[42]
UV P, UV 9, UV 234, UV 320, UV 326, UV 327, UV 328, UV 329	Fishes	HSSE, hexane:acetone (1:1)	UHPLC-MS/MS	70.9-112	0.0002-0.009	0.78-105	[31]
UV P, UV 9, UV 234, UV 320, UV 326, UV 327, UV 328, UV 329	Fishes	HSSE, hexane:acetone (1:1)	UHPLC-MS/MS	70.9-112	0.0002-0.009	0.02-211	[41]

* LOQs

2.2.2. Biological samples

The development of extraction methods to monitor BUVSs in biological samples is very important because, due to their lipophilicity, these analytes have a tendency to bioaccumulate in aquatic ecosystems and can reach trophic levels as high as marine mammals through the food chain. The contaminant levels in the tissues of bivalves, which are sessile organisms that filter and accumulate particles from water, are a good indicator of the level of local pollution [42]. To the best of our knowledge, only two research groups, Nakata et al. and Kim et al., have studied BUVS residues in organisms; thus, only their methodologies can be compared (Table 4b). Nakata et al. have published four papers between 2009 and 2012 analysing the occurrence of several BUVSs in mussels [42], the blubber of marine mammals [33] and tissues from a selection of different marine organisms (mollusc, crustaceans, fish, birds and marine mammals) [7,32]. An identical extraction procedure was carried out for all of the samples using a mixture of dichloromethane and hexane (8:1) for 5 h for a conventional Soxhlet extraction, which was followed by gel permeation chromatography (GPC) and passage through a deactivated silica gel column to remove impurities. Satisfactory recoveries (110-122%) were calculated from salad oil spiked with the target analytes, but Soxhlet extraction is a very time- and solvent-consuming method when a great number of samples are analysed. A more modern technique, high speed solvent extraction (HSSE), has been employed by Kim et al. to identify eight BUVSs in fish muscle tissues from three different species [31]. The extraction was performed using a mixture of hexane and acetone (1:1), and then the extract was cleaned up with deactivated silica gel. An equivalent procedure was applied by these authors for the determination of these BUVSs in twenty-two fish species from the same region [41]. HSSE is a faster method than Soxhlet extraction, but the achieved recoveries (70.9-112%) were lower than those in the Nakata et al. studies.

3. Analysis

Currently, the vast majority of the current literature regarding the chemical analysis of BUVSs in environmental and biological samples is focused almost exclusively on gas chromatography (GC) and liquid chromatography (LC). Moreover, mass spectrometry (MS) has been, in its different modalities, the unique detection system employed to date.

Due to the reported difficulties, frequently some BUVSs (e.g. UV 327 and UV 328) can only be distinguished by MS, and other detection techniques, such as fluorescence detection (FD) or ultraviolet-diode array detection (UV-DAD), are not recommended for the determination of these compounds.

3.1. Liquid Chromatography

Chromatographic separation of BUVSs presents some singularities that make it complicated to choose an appropriate analytical column and mode of separation in LC and ultra-high performance liquid chromatography (UHPLC). As in the extraction/pre-concentration protocols, the highly lipophilic behaviour of these compounds (K_{ow} between 3.0 and 10 [32]) requires the use of high percentages of organic solvents in the mobile phases when working with reversed phase (RP), which is the most widespread mode of separation [31, 36-38, 41, 44]. Due to their pKa (>7), BUVSs also show markedly basic behaviour, which can also exert an influence on chromatographic separation parameters (retention times, peak shape, tailing, etc.), especially for the less lipophilic benzotriazoles. Among all of the RP chromatographic columns (e.g. C_{18} and C_8 silica based, C_{18} alumina based or polyethylene-coated alumina), the C_{18} silica-based package is the only one that has been employed for BUVSs in environmental and biological samples.

J.W. Kim and co-workers [31,41] tested two commercially available octadecilsilica-based reversed-phase LC columns, Zorbax Extend-C_{18} (1.8 μm, 100 x 2.1mm) and Asentis express C_{18} (2.7 μm, 100 x 2.1 mm), for a multi-residual analysis of antimicrobials, preservatives, BUVSs, flame retardants and plasticisers in fish by UHPLC-MS/MS. They reported that the Asentis column seemed to be more appropriate for the separation of the selected compounds because of its high efficiency and low backpressure. However, they achieved elution of BUVSs from the column only when 100% methanol was employed as the mobile phase; otherwise, co-elution of some of the selected BUVSs was observed. T. Ruan et al. [38] reported the development and application of another analytical methodology based on LC-MS/MS for the determination of twelve BUVSs in sewage sludge samples. They also employed a C_{18} package for chromatographic separation (Symmetry Shield 5 μm, 150 x 4.6 mm). The gradient employed was methanol/water (80:20, v/v) with a flow rate of 1 mL/min and a linear increase to 100% methanol over 20 min. The authors did not report the retention times of the

analytes or any related chromatograms. Nevertheless, considering the analytical conditions employed, co-elution of some of the analytes is highly probable. Montesdeoca-Esponda et al. also developed and applied analytical methodologies based on octadecilsilica-based reversed-phase UHPLC columns (Acquity BEH C_{18} UPLC column, 1.7 µm, 100 x 2.1 mm) coupled to an MS/MS detector [36,37,44]. In this particular case, an isocratic elution based on 100% methanol for 1 minute was sufficient to determine seven BUVSs; however, the co-elution of three of them (UV 326, UV 327 and UV 328) was unavoidable, as they were detected spectrometrically [36,37, 44].

This observed co-elution of BUVSs in most of the reported LC methodologies severely compromises the quantification of these analytes because the response factors of each BUVS can vary significantly. In addition, this phenomenon also leads to competitive ionisation during the electrospray processes [58], resulting in signal suppression and impairing the proper quantification of the analytes when MS detection systems are employed [31]. Therefore, the appropriate separation and quantification of BUVSs continues to be an exceptional chromatographic challenge, given the matrix effects associated with complex materials such as biological, solid or WWTP-related samples.

Based on these facts, we suggest that further investigation of different types of column packages, sizes, or even combined separation mechanisms, such as mixed-modes columns (e.g., based on both size exclusion and polarity retention simultaneously [59]), is required to overcome the main drawbacks observed in current publications, including the use of high volumes of organic solvents and the co-elution of various compounds during chromatographic separation.

3.1.1. Detection systems

The application of advanced LC-MS technologies has become an important tool for the identification and quantification of BUVSs over the last decade. Particle beam (PB) and thermospray (TSP) were the first interfaces employed in this combined technique in the early 1990s [60]. However, the recent interest of the scientific community in these pollutants has led, at least to our knowledge, to the exclusive use of atmospheric pressure interfaces (API) in the determination of BUVSs. These types of interfaces allow successful elimination of the mobile phase from the column and realisation of proper ionisation of the analytes at the high vacuum

conditions required for their determination by MS.

Today, electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) interfaces are the most widely employed interfaces for LC-MS and LC-MS/MS analyses of BUVSs. Theoretically, both ESI and APCI interfaces offer a soft ionisation mode compared to the abovementioned PB, TSP or even MALDI (Matrix-Assisted Laser Desorption Ionisation); thus, they are more appropriate for quantitative analysis in both single ion monitoring (SIM) and multiple reaction monitoring (MRM) detection modes. It has been reported on countless occasions that ESI provides better sensitivity for compounds of a wide range of molecular weights and medium to high polarity, whereas APCI provides an optimum interface for the ionisation of chemicals of a wide range of molecular weights, but primarily those showing medium to low polarity, which is the case of most BUVSs (Figure 2).

Regardless of the ionisation method employed, BUVSs are determined in positive ion (PI) mode as $[M-H]^+$, although there are no reports in the literature describing the formation of adducts for their analysis. Moreover, these substances are amenable to fragmentation in the collision cells of triple quadrupole mass spectrometers, forming stable and reproducible product ions [36,37]; thus, their determination by MS/MS working in multiple reaction monitoring (MRM) mode is highly recommended. This acquisition mode allows more selective and sensitive detection, resulting in LODs that are far lower than those reported by single quadrupole systems working in single ion monitoring (SIM), regardless of whether LC, UHPLC or even GC is employed as the separation technique (Tables 2 and 5).

J.W. Kim and co-workers [31,41] tested the feasibility of UHPLC-MS/MS for the determination of BUVSs in fish, employing an ESI interface. BUVSs were determined in MRM in PI, employing nitrogen as the nebuliser and drying gas and argon as the collision gas. These authors reported LODs between 0.0002 and 0.009 $ng \cdot g^{-1}$. Montesdeoca-Esponda et al. [36,37,44] also employed UHPLC-ESI-MS/MS to detect and quantify BUVSs in different types of samples, including marine sediments, sludges from WWTP, seawater and treated wastewater, reporting LODs below 87.1 $ng \cdot g^{-1}$ and 0.018 $ng \cdot L^{-1}$ for solid and liquid matrices, respectively. T. Ruan et al. [38] employed an LC-MS/MS system for the determination of twelve BUVSs in sewage sludge samples, using an APCI interface for the ionisation of the analytes. They also determined BUVS under PI,

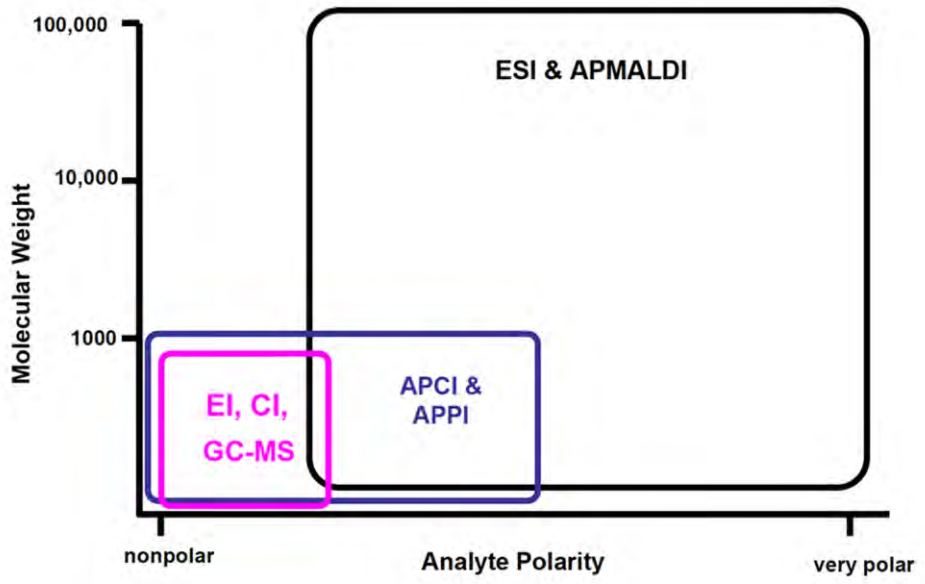

Fig. 2. Relative applicability of API interfaces in liquid chromatography-mass spectrometry related techniques. *Abbreviations*: See Appendix

employing nitrogen as the nebuliser and drying gas and argon as the collision gas. Their LODs (between 0.15 and 0.77 $ng \cdot g^{-1}$) were higher than those reported by Kim et al. [31,41], although *a priori* APCI should work better than ESI for these types of analytes.

Other examples for the determination of BUVSs in liquid, solid and biological samples can be found in Tables 2, 4a and 4b, respectively.

Taking into consideration the physico-chemical properties and fragmentation behaviour of BUVSs, the use of other MS techniques such as ion trap (IT), time of flight (TOF), and even novel hybrid-MS systems such as quadrupole-time of flight (Q-TOF) or quadrupole-ion trap (Q-IT) is also plausible (coupled to both LC and GC separation technologies). These detectors may offer additional and more versatile recognition of degradation products and metabolites due to their highly accurate mass measurements, low LODs, speed and sophisticated MS-scanning techniques [61,62].

3.2. Gas chromatography

Gas chromatography (GC) has been the major instrumental technique employed in the

environmental analysis of BUVSs thus far. GC coupled to different detectors, such as electronic capture detectors (ECD), nitrogen-phosphor detectors (NPD), flame ionisation detectors (FID), or mass spectrometry detectors (MS and MS/MS), has been the preferred technique for the determination of volatile and semi-volatile compounds (boiling points lower than 450 °C). However, their application field can be extended to "non-volatile" compounds if a proper derivatisation step is included in the analytical protocol. This procedure enhances the volatility and thermal stability of the analysed species, which is still the main drawback of GC analysis [63].

However, from the perspective of analytical chemistry, most BUVSs are amenable to gas chromatography-mass spectrometry (GC-MS or even GC-MS/MS) determination without any derivatisation of the analytes [25,28,34,35]. In addition, to our knowledge, these are the only ones employed for the determination of BUVSs in environmental and biological samples [7,35-29,30,32,34,35,38,39,42,44].

Currently, there are countless types of GC columns commercially available. However, only a few of them, mainly based on fused silica- (5%-phenyl)-methylpolysiloxane, have been used for BUVSs [21,27,32,40]. With respect to the injection mode, the split-less mode is preferred by most researchers for the determination of these substances in environmental and biological samples (e.g., [25, 28,34,35]).

3.2.1. Detection Systems

The analysis of BUVSs in complex environmental and biological samples by GC-MS and LC-MS/MS often reveals matrix effects [64-66]. However, gas chromatography-tandem mass spectrometry (GC–MS/MS) has increasingly been applied in the determination of trace organic contaminants due to the extremely high selectivity and sensitivity of its multiple reaction monitoring techniques (MRM) in tandem mass spectrometry, and it has several advantages, such as reduced matrix effects and interferences [67-69].

Among all of the ionisation sources employed in these hybrid techniques (e.g., electron ionisation (EI), cold electron ionisation (cold-EI), or chemical ionisation (CI)), electronic ionisation (EI) has been the most frequently used technique [25,28,34,35]. With respect to MS detectors, single quadrupole detectors (QD) [7,25,32,33,40], triple quadrupole detectors (TQD) [20,35] and ion traps (ITs) [28] have been the only ones used to date. IT detection offers some advantages with respect to single and triple quadrupole detection.

For example, it allows the possibility of working in MS^n mode without any additional cost. To do this, the selected precursor ion is isolated in the trap, and once there, it can be fragmented several times (n) by colliding it with helium molecules. Subsequently, the product ions obtained are registered during each fragmentation stage (n), and therefore, more precise and complete information regarding the chemical structures of the analysed compounds can be obtained. However, ITs allow an instrumental technique that generally obtains less linear response and worse limits of detection and quantification compared with those obtained when using TQD in MRM mode [70].

Nakata and co-workers have developed and applied a GC-MS technique for the determination of BUVSs in different biological samples, including molluscs, crustaceans, fish, birds, and even the blubber of marine mammals [7,32,33,42]. These authors employed an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, U.S.), using helium as the carrier gas. The oven temperature was programmed from 80 to 160 °C at a rate of 10 °C/min and was held for 10 min; then, the temperature was increased to 300 °C at a rate of 3 °C/min, with a final hold time of 15 min. The temperatures of the injector and detector of the GC-MS were set at 270 and 300 °C, respectively. The authors employed both SCAN and SIM modes for BUVS detection and they reported LODs between 0.05 and 0.15 $ng \cdot g^{-1}$, which is low enough to monitor these pollutants in the environment.

Carpinteiro et al. [25,28] developed different GC-MS methods for BUVS determination. In one study [25], they combined stir-bar sorptive extraction and liquid desorption with large volume injection-gas chromatography and mass spectrometry detection (single quadrupole) under SIM acquisition mode (SBSE-LD/LVI-GC-MS) for the determination of six BUVSs in treated and raw wastewater. They reported limits of quantification between 0.004 and 0.015 $ng \cdot mL^{-1}$, two orders of magnitude lower than those reported for other systems such as LC-MS and in the same range of values as those attained by tandem systems (GC-MS/MS) [25]. These authors have also been the only ones to employ IT detection [28]. They used a GC-MS/MS system consisting of a Varian (Walnut Creek, CA, USA) 450 GC instrument connected to an ion-trap Varian 240 mass spectrometer (MS) that was furnished with an electron impact (EI) ionisation source to assess these compounds in environmental samples (indoor dust). In this

report, they achieved limits of quantification (LOQs) below 10 $ng \cdot g^{-1}$, very close to another GC-MS/MS (employing triple quadrupole methods in MRM) method developed for the determination of BUVSs in solid samples (e.g., LOD 0.3-8.2 $ng \cdot g^{-1}$ by Liu et al. [34,35]).

More details and examples regarding another methodologies employing GC-MS and GC-MS/MS methodologies for BUVSs analysis have been highlighted in Tables 1 and 4.

4. Conclusions and future trends

Only a few studies have determined real concentrations of BUVSs in environmental samples. Global levels of BUVS contamination still cannot be defined, but local points of their occurrence have been described. Therefore, wide spatial monitoring must be used to draw an adequate map of the occurrence of BUVSs. Moreover, the presence of analytes in coastal birds and mammals [7,33] suggests bioaccumulation in higher trophic species in the aquatic food chain that must be closely observed. As the main sources of environmental contamination, the input and output concentrations of BUVSs in WWTPs must also be studied in detail. Several studies analysing both influents and effluents of treatment plants in different locations have suggested that BUVS compounds are conveniently removed from the water phase, reaching, in some cases, a 90% removal rate [26]. However, high concentrations of BUVSs detected in sewage sludge samples indicate their adsorption with organic carbon in these types of matrices. Although UV 326 and UV 328 are usually the dominant compounds measured in influents, it is necessary to extend these types of studies to other benzotriazole derivatives and compare the results to those of other WWTPs.

One of the most critical steps in the determination of BUVS compounds is sample preparation, especially in liquid samples, due to their hydrophobic character. Moreover, taking into account that the main source of BUVS contamination are effluents from WWTPs and their dilution in environmental samples, extraction and pre-concentration procedures are necessary. Although the presence of BUVSs in the environment was reported many years ago, it has only been in the last decade that multiple papers have been published on this topic. Thus far, few modern methods have been chosen to characterise the contamination of BUVSs in environmental samples. Therefore, future trends in this field will be oriented toward the development of new extraction protocols for the pre-concentration and clean-up of these samples.

Regarding the current instrumental techniques employed for the determination of BUVSs, there have only been reports in the literature on LC, UHPLC and GC separation techniques coupled with different mass spectrometry detectors (single quadrupole, triple quadrupole, and ion trap) for the chemical analysis of these pollutants in environmental and biological samples. Thus, further investigation into liquid chromatography is required to avoid the co-elution of other substances when the reversed-phase separation mode is employed, as co-elution clearly impairs the proper quantification of BUVSs in complex matrices when MS detection systems are used. Moreover, the use of other MS techniques such as ion trap (IT), time of flight (TOF), or even novel hybrid-MS systems could offer additional and more versatile recognition of degradation products and metabolites. Given these facts, GC-MS and GC-MS/MS are still the most suitable techniques for the determination of BUVSs in complex matrices at the moment because no derivatisation is required. Moreover, gas chromatography-tandem mass spectrometry (GC–MS/MS) has increasingly been applied in the determination of trace organic contaminants, including BUVSs, due to its extremely high selectivity and sensitivity in multiple reaction monitoring mode (MRM); GC–MS/MS also has several advantages, such as reduced matrix effects and interferences, when compared to GC-MS and LC-MS/MS.

Acknowledgements

This work was supported by funds provided by the Spanish Ministry of Science Education, Research Project CTQ2010-20554. Sarah Montesdeoca Esponda would like to thank the Canary Agency of Investigation, Innovation and Information Society (ACIISI) for her PhD Student Grant.

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I.4. Tratamiento de datos

I.4.1. Análisis estadístico

Todas las metodologías analíticas presentadas en esta Tesis han sido evaluadas en términos de precisión, linealidad, límites de detección, límites de cuantificación y recuperación.

· Precisión

La precisión fue evaluada mediante el cálculo de las desviaciones estándares relativas (*RSD, relative standard deviation*). Los resultados, expresados como porcentajes, fueron estimados a partir de un tamaño de muestra n = 6.

La RSD se calcula según la siguiente expresión:

$$RSD = \frac{\sigma}{\overline{x}} \; x \; 100$$

donde,

$$\overline{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$$ (*Media aritmética*)

$$\sigma = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}$$ *(Desviación estándar)*

· Linealidad

La linealidad de cada uno de los métodos desarrollados fue estimada en base a los coeficientes de correlación de Pearson (r), calculados a partir de las curvas de calibrado para cada analito. Los coeficientes de determinación estimados (r^2) fueron superiores a 0.990 en todos los casos.

El coeficiente de correlación de Pearson se define como:

$$r_{x,y} = \frac{\sigma_{XY}}{\sigma_X \sigma_y} = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n\sum x_i^2 - (\sum [x_i)^2]}\sqrt{n\sum y_i^2 - (\sum [y_i)^2]}}$$

· Límites de detección

Los límites de detección (*LODs, limits of detection*) en las diferentes metodologías, y para cada uno de los analitos, han sido calculados en base a la siguiente definición: *"El límite de detección viene dado por la concentración de analito que genera una relación señal/ruido (signal/noise) igual a tres"*, y que se calcula según la expresión:

$$\text{LOD} = \frac{\overline{X}_{signal}}{\overline{X}_{noise}} = 3$$

Los cálculos de dicho valor se realizaron por triplicado (n=3) a partir del nivel de concentración más bajo estimado en las curvas de calibrado.

· Límites de cuantificación

Por su parte, los límites de cuantificación (*LOQs, limits of quantification*) se determinaron en base a la siguiente definición: *"El límite de cuantificación viene dado por la concentración de analito que genera una relación señal/ruido (signal/noise) igual a diez",* y que se calcula según la expresión:

$$LOQ = \frac{\overline{X}_{signal}}{\overline{X}_{noise}} = 10$$

Los cálculos de dicho valor también se realizaron por triplicado (n=3) a partir del nivel de concentración más bajo estimado en las curvas de calibrado.

· Recuperación

Las cantidades de analito que somos capaces de extraer empleando cada una de las técnicas desarrolladas durante esta Tesis Doctoral han sido expresadas en porcentaje. Los valores obtenidos han sido calculados por triplicado, expresándose además las desviaciones estándares relativas obtenidas para cada compuesto.

I.4.2. Análisis de los resultados en muestras reales

Las concentraciones de los analitos en las muestras reales han sido determinadas haciendo uso de curvas de calibrado preparadas en agua Milli-Q (apartados III.1.2., III.1.3. y III.1.5.) ó aplicando el método de las adiciones estándar (apartados III.2.1., III.2.2. y III.2.3.), después de llevar a cabo el método analítico completo.

En ambos casos, la regresión lineal se realizó empleando seis niveles de concentración diferentes, estimándose cada uno de ellos por triplicado. Los rangos lineales variaron en función de la metodología analítica empleada y del analito a determinar, estando éstos indicados de forma específica en cada uno de los apartados del capítulo III.

El método de las adiciones estándar ha sido definido por algunos autores como la mejor estrategia para corregir el efecto matriz [77]. Éste efecto matriz debe tenerse en cuenta especialmente cuando se analizan muestras muy complejas, como las aguas residuales. Cuando se usa espectrometría de masas con ionización por ESI, pueden darse problemas de supresión iónica, ya que este tipo de interfase es muy susceptible a otros compuestos presentes en la muestra [117].

En muchas ocasiones, no se emplea el método de adiciones estándar para realizar la calibración, ya que se trata de un procedimiento muy laborioso y que emplea mucho tiempo. Sin embargo se trata de una herramienta muy efectiva y exacta para la cuantificación en Química Analítica [117].

I.4.3. Diseño factorial

La eficiencia de extracción de algunas técnicas puede estar influenciada por un gran número de variables que, además, están relacionadas entre sí.

Por ello, para la optimización de prácticamente todas las metodologías desarrolladas en esta Tesis Doctoral se han empleado diseños factoriales construidos con el programa Statgraphics Plus versión 5.1 (Manugistic, Rockville, MD, USA). Para evitar la influencia de factores no controlados que pudieran tener efecto sobre los resultados finales, los experimentos fueron generados de forma aleatoria.

Según Miller y Miller [118], esta herramienta matemática permite reducir significativamente el número de ensayos requeridos durante el proceso de optimización, y permite obtener la influencia de cada variable sobre el proceso de extracción y las correlaciones con el resto de variables.

Dependiendo del tipo y número de variables involucradas, durante el desarrollo de cada metodología de extracción se han empleado los diseños apropiados, detallándose cada uno de ellos en los apartados III.1.2., III.1.3., III.1.4., III.1.5., III.2.2. y III.2.3.

A su vez, los cálculos de las correlaciones bivariadas y parciales entre las diferentes variables se realizaron empleando el programa SPSS versión 11.0 (SPSS Inc., Illinois, USA). Las correlaciones parciales miden la influencia de cada variable sobre otra, mientras que las correlaciones bivariadas dan idea de cómo influye cada parámetro en la recuperación final de cada analito.

Los valores de cada una de estas correlaciones pueden ir desde -1 (máxima correlación de manera negativa) hasta +1 (máxima correlación en forma positiva). Un valor de 0 indica que una variable no tiene ningún efecto sobre otra variable o sobre la recuperación.

I.5. Referencias

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II. Objetivos

En las últimas décadas se ha realizado un importante esfuerzo para desarrollar metodologías de extracción desde matrices complejas que mejoren la selectividad y la sensibilidad en la determinación de contaminantes. Las nuevas tendencias han ido dirigidas hacia minimizar los volúmenes de muestra necesarios y reducir o eliminar el uso de disolventes orgánicos caros y tóxicos. Además, se apuesta cada vez más por métodos que puedan ser fácilmente automatizables. Existe una demanda de métodos más rápidos, efectivos y respetuosos con el medio ambiente que mejoren los convencionales, algo que ha estimulado la investigación en este sentido durante las últimas décadas.

Por otro lado, la presencia de compuestos emergentes en el medio ambiente, así como el desconocimiento de sus efectos negativos sobre el mismo y sobre la salud de las poblaciones animales y humanas, representan un problema de interés creciente en la comunidad científica y en la sociedad. No obstante, hoy en día no existe una

normativa específica que regule los niveles de emisión de estos contaminantes, por lo que las plantas de tratamiento de aguas residuales están exentas de toda responsabilidad.

Para lograr la regulación de estos compuestos, además de establecer sus niveles de toxicidad, es indispensable desarrollar metodologías que permitan detectarlos en el medio y establecer los niveles en los que se encuentran.

Suponiendo una futura normativa acerca de las concentraciones de estas familias de compuestos, es de interés tanto a nivel internacional como local, conocer y referenciar por primera vez su presencia en los influentes y efluentes de las EDARs de la isla de Gran Canaria (España), así como medir sus concentraciones una vez alcanzan el medio ambiente.

Con todo ello, en esta Tesis Doctoral pretende optimizar y aplicar técnicas de extracción miniaturizadas, automatizadas y donde se reemplazan los disolventes orgánicos por medios micelares, para la determinación de fluoroquinolonas, pertenecientes a la familia de los fármacos, y benzotriazoles estabilizadores de luz UV como productos de cuidado personal, en distintas muestras medioambientales líquidas y sólidas tomadas en la isla de Gran Canaria.

De una manera más concreta, los objetivos que se presentan en la presente Tesis Doctoral son los siguientes:

a) Optimizar y desarrollar una metodología basada en la técnica de microextracción en fase sólida (SPME) utilizando medios micelares como agente desorbente, que

permita la extracción de fluoroquinolonas desde muestras de agua de mar, aguas residuales y aguas subterráneas.

b) Optimizar y desarrollar una metodología basada en la técnica de extracción en fase sólida (SPE) empleando por primera vez los medios micelares como eluyentes, para la extracción de fluoroquinolonas desde las aguas resultantes de los distintos tratamientos de una estación depuradora y en aguas procedentes de un complejo hospitalario.

c) Comparar el uso de medios micelares con el de disolventes orgánicos en las técnicas de SPME y SPE para la extracción de fluoroquinolonas en términos de sensibilidad y repetibilidad.

d) Optimizar y desarrollar una metodología basada en la técnica de extracción asistida por microondas (MAE) usando medios micelares como extractantes, para la extracción de fluoroquinolonas desde sedimentos costeros y lodos de depuradora.

e) Optimizar y desarrollar una metodología basada en la técnica de extracción en fase sólida “en línea” (On-line SPE) para benzotriazoles estabilizadores de luz UV en agua de mar y aguas residuales.

f) Comparar el uso de la SPE “en línea” con la SPE convencional (no “en línea”) para benzotriazoles estabilizadores de luz UV en agua de mar y aguas residuales, en términos de sensibilidad, repetibilidad, rapidez y volumen de muestra y eluyente requeridos.

g) Optimizar y desarrollar una metodología basada en la MAE seguida de un paso de purificación por SPE "en línea" para la extracción de benzotriazoles estabilizadores de luz UV en sedimentos marinos y lodos de depuradora.

h) Optimizar y desarrollar una metodología basada en la técnica de extracción por absorción con barras agitadoras (SBSE) para benzotriazoles estabilizadores de luz UV en agua de mar y aguas residuales.

i) Establecer las condiciones de separación, detección y determinación de fluoroquinolonas empleando la cromatografía líquida de alta resolución (LC) acoplada a detectores de fluorescencia (FD) y de espectrometría de masas de triple cuadrupolo (MS/MS).

j) Establecer las condiciones de separación, detección y determinación de benzotriazoles estabilizadores de luz UV empleando la cromatografía líquida de ultra resolución (UHPLC) acoplada a un detector de espectrometría de masas de triple cuadrupolo (MS/MS).

k) Aplicar las metodologías de extracción y determinación optimizadas para flouroquinolonas a muestras medioambientales (aguas residuales, lodos de depuradora y sedimentos marinos) de la isla de Gran Canaria.

l) Aplicar las metodologías de extracción y determinación optimizadas para benzotriazoles estabilizadores de luz UV a muestras medioambientales (aguas residuales, aguas costeras, lodos de depuradora y sedimentos marinos y costeros) de la isla de Gran Canaria.

III. Parte experimental y Resultados

III.1. Fármacos: Fluoroquinolonas

III.1.1. Extracción y determinación de fluoroquinolonas mediante la combinación de microextracción en fase sólida con desorción micelar y LC con detección por fluorescencia.

En esta comunicación corta se exponen, de forma breve, los primeros ensayos del proceso de optimización de un método basado en la microextracción en fase sólida (SPME) para la determinación de fluoroquinolonas en muestras de agua.

Concretamente se estudió la afinidad de los analitos por diferentes fibras empleadas en SPME. Se probaron diferentes recubrimientos y volúmenes de fase (cantidad de recubrimiento), siendo la fibra compuesta de Carbowax (polietilenglicol), la que proporcionó las mejores eficiencias de extracción para las cinco fluoroquinolonas estudiadas. En posteriores ensayos se optimizaron el resto de parámetros que afectan a la extracción y a la desorción, incorporándose la desorción micelar (usando surfactantes en lugar de disolventes orgánicos) como una alternativa menos tóxica y menos costosa.

Tras la extracción se empleó la cromatografía líquida en fase reversa seguida por detección por fluorescencia, lográndose la adecuada separación de todos los analitos.

Esta primera parte de la optimización fue publicada en *Luminescence 23 (2008) 250-251,* y sirvió como base para llevar a cabo el trabajo presentado en el siguiente apartado de este capítulo (III.1.2.).

Among the 39 biopsies examined, the percentages of samples that resulted positive for high risk HPV DNA were: 22.2% in non-neoplastic lesions, 75.0% in low grade CIN (CIN1) and 94.7% high grade CIN (CIN2/3).

In the study of the correlation among a) $p16^{INK4A}$ over-expression determined by values over the cut-off value of 0.89 b) HPV positivity determined by CL-ISH and c) histological grade of lesions, it was shown that non neoplastic lesions proved mainly negative both for $p16^{INK4A}$ and HPV (75.9%); low grade CIN proved mostly negative for $p16^{INK4A}$ and positive for HPV (58.3%), while 16.5% proved positive for both $p16^{INK4A}$ and HPV; high grade CIN proved mostly positive for both $p16^{INK4A}$ and HPV (78.9%).

Conclusions

Our results confirmed that an increased expression of $p16^{INK4A}$ in the epithelium of cervical biopsy sections is a valuable indicator for high-grade preneoplastic lesions (CIN2–3). On the other hand, the presence of high oncogenic risk HPV DNA in low-grade preneoplastic lesions (CIN1) is an important indicator of the risk of progression to higher grade lesions. The quantitative evaluation of $p16^{INK4A}$ expression combined with the localization of HR-HPV DNA showed a statistically significant discrimination among different lesions (non neoplastic, low grade and high grade CIN), thus offering an accurate and objective diagnostic test providing important information for counselling, selection of therapy, follow up and vaccine monitoring.

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Extraction and determination of fluoroquinolones by coupling solid phase microextraction with micellar desorption and HPLC—fluorescence detection

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Introduction

Fluoroquinolones (FQs) are a class of relatively new and synthetic antibiotics/antibacterials (1). These compounds are widely used, which are applied as both human and veterinary medicine. Fluoroquinolones are rather resistance to microbial degradation and may be persisting within environmental waters (2) Environmental concentrations of fluoroquinolones are very low, for that the analytes need to be extracted and preconcentrated prior to analysis. In the present work, a simple method combining extraction by solid phase microextraction with micellar desorption (SPME-MD) and chromatographic determination of five fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin and sarafloxacin) in water samples is proposed (3). The method involves pre-concentration by SPME and desorption using a micellar medium as desorbing agent, followed by HPLC determination with fluorescence detection. Parameters for the extraction and desorption procedures are optimized as well as the chromatographic conditions. Finally, the method is applied to the extraction and determination of this kind of compounds in environmental water samples.

Experimental

Chromatographic separation

Fluoroquinolones were analysed by reversed-phase HPLC in the isocratic mode, with a Symmetry C_{18} column. Mobile phase consisted of methanol/water (adjusted at pH = 2.5 with acetic acid), 15:85, v/v at flow rate of 1 mL/min. Fluorescence detector operated at an excitation wavelength of 280 nm and an emission wavelength of 450 nm. Chromatogram of five fluoroquinolones in these conditions is shown in Fig. 1.

Optimisation of SPME

Variables affecting to SPME process were studied: fiber type, extraction time and temperature, ionic strength and pH.

In the other hand, the desorption step was carried out using a surfactant like desorbing agent. We used three different non-ionic surfactants: Polyoxyethylene 10 lauryl ether (POLE), Polyoxyethylene 9 lauryl ether (Polidocanol) and Polyoxyethylene 6 lauryl ether ($C_{12}E_6$) to different concentrations.

Initial experimental conditions were: absorption time, 60 min, temperature, 20°C at constant stirring speed of 1200 rpm and 10 min of desorption time in a desorption volume of 60 μl of methanol.

Results

Optimization of SPME parameters

To maximize the extraction efficiency of target analytes, seven fibers were tested for comparison of peak area: Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) 60 and 65 μm, Polydimethylsiloxane

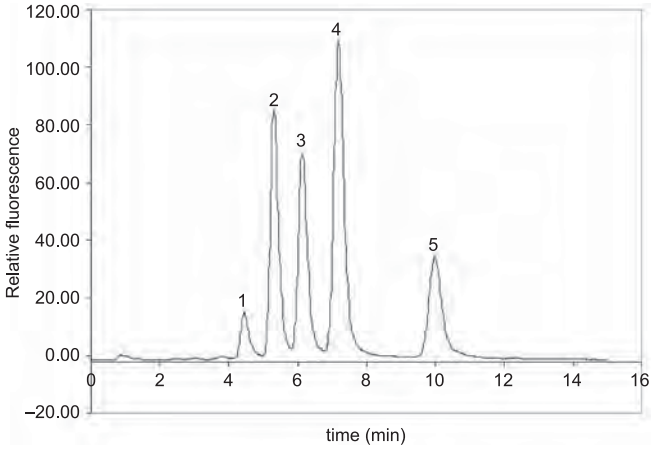

Figure 1. Chromatogram of five fluoroquinolones (500 ng·mL^{-1} for each analyte in Milli-Q water) under optimun conditions. (1) Levofloxacin, (2) Norfloxacin, (3) Ciprofloxacin, (4) Enrofloxacin, (5) Sarafloxacin.

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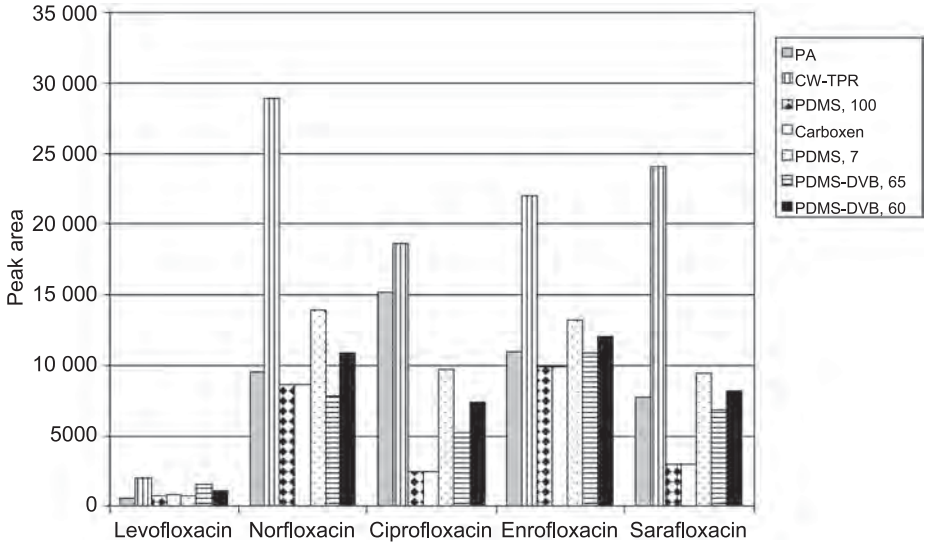

Figure 2. Relative extraction efficiencies of fluoroquinolones with different fibers (1 μg·mL^{-1} for each analyte in Milli-Q water).

(PDMS) 100 and 7 μm, Polyacrylate (PA), Carboxen-PDMS 75 μm and Carbowax-TPR/100 (CW-TPR).

As can be seen in Fig. 2, the best extracting efficiencies were obtained with the most polar fiber, CW-TPR for all compounds, including the case of Levofloxacin, which shows very low areas in comparison with others fluoroquinolones.

When the extraction efficiency was optimized, desorption process was performed using a surfactant as desorbing agent. The nature and concentration of surfactant also must be optimised.

The use of surfactants, instead of the organic conventional solvents, presents some advantages like less toxicity and reduction in price; moreover, the surfactants are commercially available and they are compatible with the mobile phase used in HPLC analysis (4). For that, SPME-MD-HPLC can be a good alternative to determination of Fluoroquinolones in natural waters.

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Determination of norfloxacin by flow injection analysis using photoinduced chemiluminescence detection

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Norfloxacin (NOR) is an oral broad-spectrum fluoroquinolone antibacterial agent widely used in the treatment of urinary tract infections and to treat eye and gastrointestinal infections. In this work we propose a new method for the determination of NOR by flow injection analysis (FIA) based on the application of photoinduced chemiluminescence (PIC) detection. We have selected the peroxyoxalate (PO) reaction, in which a PO is oxidized in presence of H_2O_2 and an intermediate transfers its energy to an energy-accepting fluorophore, which becomes electronically excited and subsequently emits light. The usefulness of this reaction is based on the possibility of detecting native fluorophores, fluorescent derivatized compounds or fluorescent products derived from a photochemically induced decomposition. This last option has been selected because the native fluorescence of NOR is drastically increased by UV irradiation in ethanolic:water media (1), observing high PIC signal when the derivative participates in the PO reaction. We have used a FIA device with two-injection valves for the introduction of both the PO and the derivatized analyte solutions in the flow system, avoiding the problems arising from the use of e.g. acetonitrile as solvent, as neither special tubes nor special pumps are required (2). Furthermore, the use of micellar media (sodium dodecyl sulphate, SDS) as carrier in the PO solutions increases both the solubility and stability of POs, avoiding their rapid degradation in water. The method has been satisfactorily applied to the monitoring of NOR in pharmaceutical compounds.

Experimental

Chemicals

All the reagents or solvents were of analytical reagent or HPLC grade. Ultrapure water (Mili-Q plus system, Milipore Corporation) was used. A stock solution of NOR (100 mg l^{-1}) was prepared in ethanol avoiding exposure to direct light and maintaining the solution stored at 4°C. A 10 mM sodium dodecyl sulphate (SDS, Panrec) solution was prepared in 0.1 M sodium dihydrogenphosphate buffer (Panreac) pH 5.0 and used as carrier and solvent. A 1 M stock solution of imidazole (Sigma-Aldrich) was prepared in deionized water every two weeks and proper working solutions were prepared daily by dilution with

III.1.2. Microextracción en fase sólida con desorción micellar y LC con detección por fluorescencia para el análisis de residuos de fluoroquinolonas en muestras de agua.

En esta publicación se presenta la optimización, desarrollo y aplicación de una metodología analítica para la determinación de cinco fluoroquinolonas (levofloxacina, norfloxacina, ciprofloxacina, enrofloxacina y sarafloxacina) en distintas muestras líquidas medioambientales (agua residual, agua subterránea y agua de mar). El método de extracción empleado está basado en la microextracción en fase sólida (SPME) y la identificación y cuantificación de los compuestos se realizó mediante el uso de cromatografía líquida de alta resolución con detector de fluorescencia.

La SPME es una técnica de extracción miniaturizada que requiere sólo unos pocos mililitros de muestra y que puede ser empleada para muestreos *in situ*. Basándonos en los ensayos presentados en el apartado anterior, se optimizaron todos los parámetros que afectan al proceso de extracción (tiempo, temperatura, pH y fuerza iónica) y al de desorción (tiempo y naturaleza del extractante). Una vez optimizadas las condiciones de extracción, se reemplazó el disolvente orgánico empleado en la desorción de los analitos desde la fibra (metanol), por un surfactante, comparándose todos los parámetros analíticos calculados en ambos casos.

Además de las ventajas inherentes al uso de medios micelares (menores costes, toxicidad y generación de residuos peligrosos), el surfactante escogido, POLE al 5% (v/v) en agua, proporcionó mayor sensibilidad que el disolvente orgánico. Esto puede deberse a que la presencia de los medios micelares incrementa la señal de

fluorescencia de los analitos. Así, los límites de detección obtenidos para los distintos compuestos estuvieron en el rango 0.02-0.23 y 0.01-0.19 $ng \cdot mL^{-1}$, usando metanol y POLE, respectivamente. Además, la repetibilidad no superó el 9% en ninguno de los casos.

Una vez optimizado todo el procedimiento, éste se aplicó a muestras liquidas de distinta naturaleza, recogidas en la isla de Gran Canaria, obteniéndose buenas recuperaciones para todas ellas: 81-92% en agua residual, 85-112% en agua de mar y 81-116% en agua subterránea.

La optimización completa y la aplicación del procedimiento ha sido publicada en la revista *Analytical and Bionalytical Chemistry 394 (2009) 927-935.*

Anal Bioanal Chem (2009) 394:927–935
DOI 10.1007/s00216-009-2629-8

ORIGINAL PAPER

Solid-phase microextraction with micellar desorption and HPLC-fluorescence detection for the analysis of fluoroquinolones residues in water samples

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Received: 30 October 2008 / Revised: 18 December 2008 / Accepted: 15 January 2009 / Published online: 11 February 2009

Abstract A sensitive and useful method based on solid-phase microextraction with micellar desorption (SPME-MD) coupled to HPLC with fluorescence detection was developed for the determination of five fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, and sarafloxacin) in environmental water matrices. The SPME extraction efficiency was optimized with regard to time, temperature, pH, and ionic strength using a CW-TPR fiber. A detailed study about the optimum conditions for micellar desorption (surfactant type, concentration, and desorption time) were made. Among different surfactants studied, Polyoxyethylene 10 lauryl ether showed the best responses to extract fluoroquinolones using SPME-MD. Relative standard deviations of the developed method were below 9%. Limits of detection and quantification were between 0.01–0.2 and 0.03–0.6 ng mL^{-1}, respectively. The recoveries achieved for all five compounds were in the 81–116% range. The proposed method was compared using conventional desorbing agent as methanol. Finally, the SPME-MD methodology was applied to the determination of these target analytes in several environmental liquid samples, including seawater, groundwater, and wastewater samples with excellent results.

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Keywords Solid-phase microextraction · Surfactants · Fluoroquinolones · High-performance liquid chromatography · Fluorescence · Water analysis

Introduction

Pharmaceutical compounds are an important group of emerging contaminants, which have recently attracted the attention of the international scientific community because they can be present in different aquatic environments. Between them, antibiotic residues have been found in a wide range of environmental samples, including surface waters, groundwaters, and wastewaters. Fluoroquinolones (FQs) are a synthetic generation of quinolones antibacterials family, used in human and veterinary medicine for therapeutic purpose as against both gram-positive and gram-negative bacteria [1]. However, their extensive administration may give rise to an increase in the antibiotic resistance of pathogenic bacteria, which may result in health problems [2].

These compounds have been detected at the micrograms per liter levels in different aquatic environment of the world, including municipal wastewaters effluents and surface waters [3–5]. Fluoroquinolones are rather resistant to microbial degradation and they may persist within environmental waters [6].

Thus, the development of sensitive multiresidue methods is important in order to provide their determination with respect to the required levels. Since FQs are highly fluorescent, reversed-phase liquid chromatography with fluorimetric detection is the determination technique mainly used for their analysis, although other detection systems

have been used such as UV [7–9], liquid chromatography/mass spectrometry (LC/MS) [10–12], or LC tandem mass spectrometry (LC/MS/MS) [13–15].

Commonly used methods for determining antibiotics typically include extraction for both clean-up and enrichment of aqueous environmental samples due to low concentration levels present.

Although different techniques have been employed to their extraction, the most frequently used is solid-phase extraction (SPE) using polymeric cartridge [16–18]. Organic solvents are employed for elution in the most cases.

Recently, several authors have started to use solid-phase microextraction (SPME) in environmental samples due to its advantages with respect to SPE (e.g., little manipulation of samples or lower volume samples) [6, 19–21]. After equilibration, extracted analytes are desorbed into an organic solvent, followed by their determination.

Ultimately, an alternative to the use of organic solvents has been developed. The methodology known as solid-phase microextraction with micellar desorption (SPME-MD) uses aqueous surfactant solutions as desorbing agent [22, 23], and its suitability has been demonstrated for the extraction of pharmaceutical compounds [24].

Taking account that some surfactant solution enhances the fluorescence of many compounds of environmental interest [25], in this work, we have developed a method to extract and determine five FQs in environmental water samples using a micellar medium like desorbing agent in SPME followed by HPLC with fluorescence detection. We have optimized several variables that affect to the extraction process (extraction time, temperature, pH, and ionic strength), and we have studied the use of surfactants solutions like desorbing agents optimizing variables that influence in the desorption process: time and type and concentration of surfactant.

Optimized SPME-MD method coupled to HPLC system was applied to the analysis of five fluoroquinolones in different spiked water samples: seawater, sewage, and groundwater.

Experimental

Reagents

Fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, and sarafloxacin) were obtained from Sigma-Aldrich (Madrid, Spain). Their stock solutions (1,000 μg mL^{-1}) were prepared by dissolving appropriate amounts of the commercial products in methanol and stored in glass-stoppered bottles at 4 °C prior to use.

Ultra-high-quality water obtained by a Milli Q (Millipore, Bedford, MA, USA) water purification system was used to prepare the mobile phase and the working aqueous standard solutions. Appropriate volumes of the stock solutions were diluted daily to prepare solutions containing fluoroquinolones at 50 ng mL^{-1}. They are listed in Table 1 (numbers identify the compounds in the chromatograms).

Methanol used to dissolve standard and to prepare the mobile phase was HPLC grade, and it was obtained from Panreac Química (Barcelona, Spain). It was filtered through a 0.22 μm acetate membrane filter. Glacial acetic acid used to adjust the pH of mobile phase was obtained from Scharlau Chemie S.A. (Barcelona, Spain).

Finally, the surfactants used in this study, oligoethylene glycol monoalkyl ether (Genapol X-080), polyoxyethylene 10 lauryl ether (POLE), and polyoxyethylene 9 lauryl ether (Polidocanol) were obtained from Sigma-Aldrich and they were prepared in deionized water.

Instrumentation and chromatographic conditions

The chromatograph system consists of a Varian pump fitted with a Varian Autosampler 410 with a volume selector, a Column Valve Module with an internal oven, and detection was performed on a Varian Fluorescence detector. The system and the data management were controlled by Star software from Varian (Varian, Madrid, Spain).

For the separation, the stationary-phase column was a 150 mm×3.9 mm, 4 μm particle Waters Symmetry C_{18} column. Separation and quantification of FQs were achieved by an isocratic mobile phase that consisted of methanol/water (adjusted at pH=2.5 with acetic acid; 15:85, *v*/*v*) at flow rate of 1 mL min^{-1}.

Fluorescence detector was operated at an excitation wavelength of 280 nm and an emission wavelength of 450 nm. The retention time for each compound is listed in Table 1.

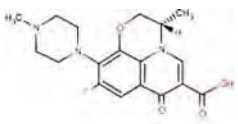
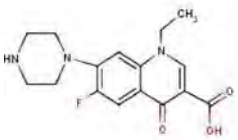
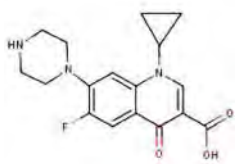
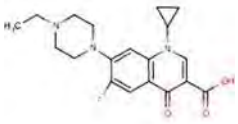
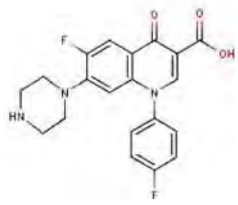
Quantification of FQs was performed in the range of 0.01–50 ng mL^{-1} by injecting 50 μL of the surfactant solution into the liquid chromatograph.

Solid-phase microextraction

SPME was carried out by introducing 4 mL of aqueous samples containing 50 ng mL^{-1} of each fluoroquinolone into glass vials. Samples were saturated with NaCl (30% *w*/*v*) without pH adjustment. The fiber was then immersed in the sample for 60 min. During the extraction, the samples were heated at 40 °C, and they were stirred with a magnetic stir bar at a constant speed of 1,100 rpm.

After the extraction step, the fiber loaded with the analytes was introduced into a conical glass insert of 100 μL contained in a 4-mL glass vial with 60 μL of selected desorbing solvent. External micellar desorption was made using three different surfactants to select the optimum one. The external setup has been described in a previous work [22].

Table 1 List of fluoroquinolones, chemical structure, pK_a values, and retention times (t_r)

N°	Compound	Abbreviation	Structure	pK_a*	t_r (min)
1	Levofloxacin	LEVO		6.05	3.5
2	Norfloxacin	NOR		5.94	4.2
3	Ciprofloxacin	CIPRO		5.86	4.9
4	Enrofloxacin	ENRO		5.88	5.8
5	Sarafloxacin	SARA		5.62	7.2

*Obtained from [27] and [28]

After each desorption, fibers were rinsed with Milli Q water and then in methanol to avoid damage due the use of NaCl. Finally, they were dried before next use. Blanks were run to confirm the carryover absence.

Spiked water samples

Prior to the analysis, different environmental liquid samples (seawater, wastewater, and groundwater) were filtered

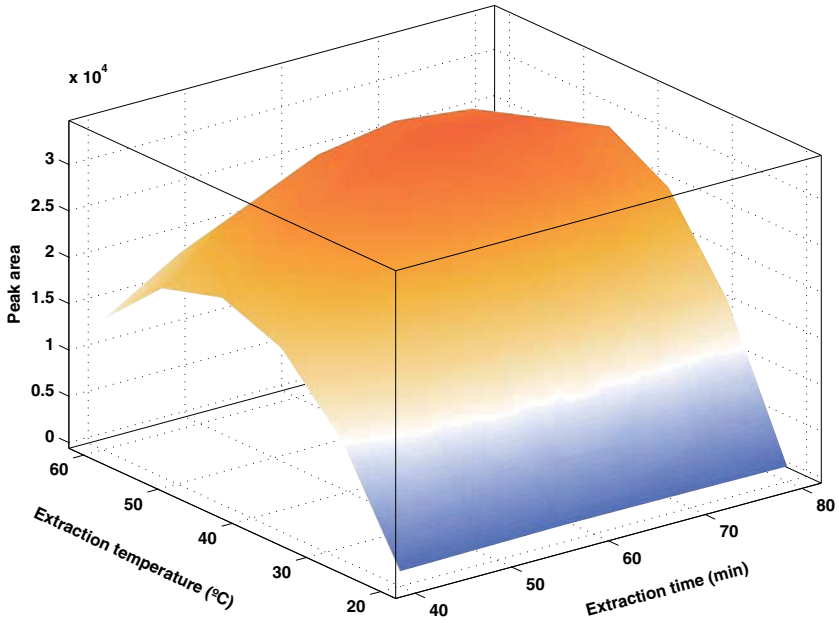


Fig. 1 Response surface for the effect of extraction time and temperature on the extraction on norfloxacin (50 ng mL^{-1}) using SPME procedure

through a 0.22 μm cellulose acetate filter, and they were stored in the dark and refrigerated until analysis. Water (4 mL) samples were spiked with fluoroquinolone mixture and analyzed according to the procedure described using CW-TPR fiber and micellar desorption. Three replicate analyses of each water sample were carried out to confirm the results.

Statistical analysis

Experimental designs were performed using Statgraphics Plus software, version 5.1 Manugistic, Rockville, MD, USA). Statistical test was done using SPSS 11.0 (Chicago, IL, USA).

Results and discussion

Optimization of extraction process in SPME

Our aim is to find the conditions suitable for extraction of five FQs for environmental water samples.

In a SPME procedure, we must evaluate several experimental variables in order to achieve the *maximum* extraction efficiency of target analytes. Several parameters were optimized: fiber type, extraction time and temperature, matrix pH, and ionic strength. In a previous work, we have verified that the CW-TPR *fiber* was suitable for the selected analytes [26].

Initial experimental conditions were as follows: extraction time 60 min, temperature 20 °C at constant stirring speed of 1,100 rpm and 10 min of desorption time in a desorption volume of 60 μL of methanol to a solution containing 50 ng mL^{-1} of each fluoroquinolone in 4 mL of Milli Q water.

Extraction time and temperature

One of the main steps is to determine the optimum extraction time and temperature. The optimization consisted of a factorial design 2^2 + star with three central points involving 11 runs (Table 2). Extraction time was studied in the range of 40–80 min and temperature in the range of 20–60 °C. Effect of these variables for norfloxacin is shown in Fig. 1. The studies carried out for the rest of analytes showed similar results.

It can be seen that the maximum extraction efficiency is reached about 60 min. Slightly longer extraction times were observed. These extraction times are consistent with other observed for extraction of polar compounds by CW-TPR [20].

With respect to the temperature, it is observed that the maximum peak area is obtained at 40 °C.

Table 2 Experimental design for the optimization of temperature and time of extraction

Run	Temperature (°C)	Time (min)
1	20	20
2	20	40
3	20	60
4	40	20
5	40	40
6	40	40
7	40	40
8	40	60
9	60	20
10	60	40
11	60	60

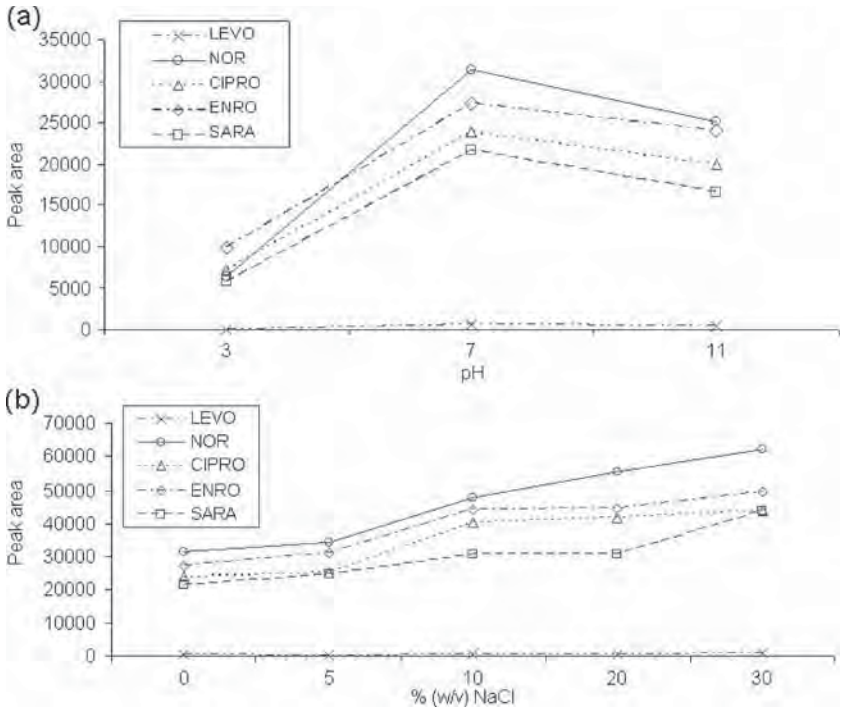


Fig. 2 Effect of **a** pH and **b** percent NaCl (*w*/*v*) in the extraction of selected FQs. Milli Q water containing 50 ng mL^{-1} for each analyte

As a compromise, an extraction time of 60 min and an extraction temperature of 40 °C were chosen for subsequent experiments.

pH and ionic strength

The extraction of many analytes in the fiber depends to a certain extent on the pH solutions. If the pH is such that the analyte is present in an ionic form, the partitioning from the solution to the fiber will be incomplete. In our case, a change of pH induces large changes on the adsorption features of FQs, which they are dramatically reflected in the fluorescence emission [27]. FQs can be present in aqueous solution as cationic, anionic, or intermediate forms due to the presence of carboxylic groups and changed amino group of the piperazine moiety. Compounds under study present their undissociated form at pH<7 just as it is observed in the pK_a values included in Table 1 [28, 29]. For that, effect of pH was investigated at three pH values: 3.0, 7.0, and 11.0. Obtained results showed that to pH value 7.0 presented the best extraction efficiencies. Therefore, this behavior agreed with expected results (Fig. 2a).

On the other hand, addition of salt to aqueous samples generally improves the extraction efficiency of the studied analytes when they are in the neutral form. The ionic strength effect in the recovery of the compounds under study was performed by the addition of sodium chloride to the aqueous medium ranging from 0% to 30% (*w*/*v*). Increments of ionic strength produced an increase in the response areas of compounds (Fig. 2b). Because of this, we chose 30% (*w*/*v*) of salt like optimum value.

Desorption time

After, to optimize the extraction process, we made the desorption time optimization using methanol like desorbing

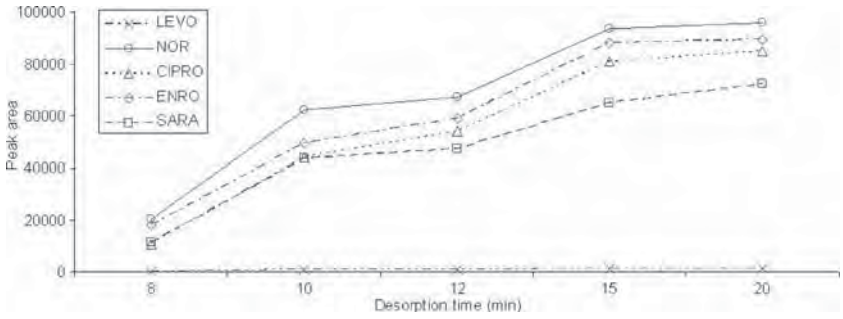


Fig. 3 Effect of desorption time in the SPME procedure. Milli Q water containing 50 ng mL^{-1} for each analyte

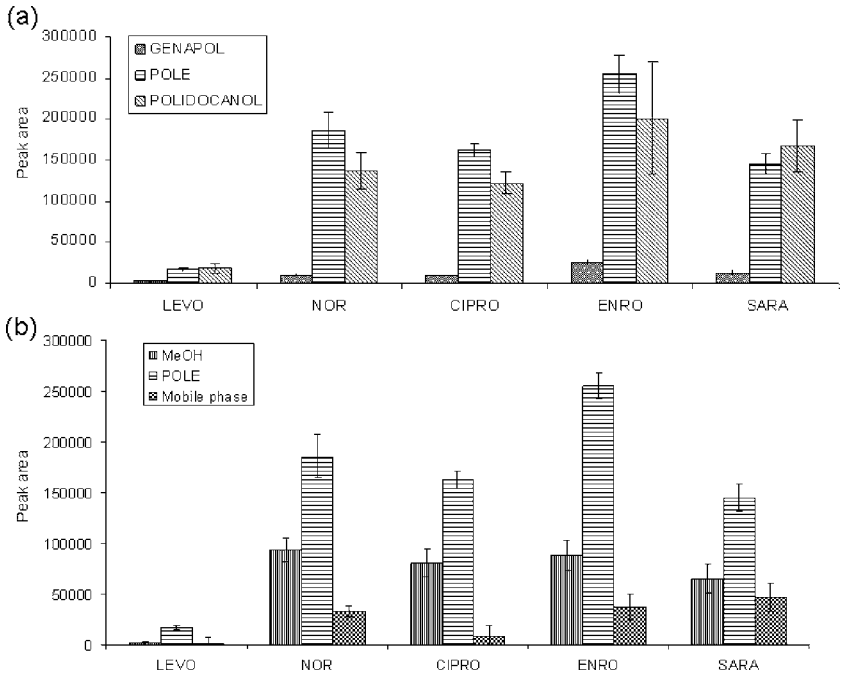


Fig. 4 **a** Response of analytes to the presence of different surfactants in the desorption process. **b** Response of different mode desorptions in SPME: methanol, mobile phase, and micellar desorption. Milli Q water containing 50 ng mL^{-1} for each analyte

agent. The volume used for the process was 60 μL. It was the lowest amount sufficient to cover the fiber coating. Desorption time was evaluated in a range between 8 and 20 min.

The results (Fig. 3) indicated that the FQ concentration increased over the first 15 min; times longer than this did not increase the signal significantly. We selected 15 min optimum desorption time with organic solvent.

In summary, the SPME procedure adopted for the analysis of FQs in liquid samples was as follows: direct exposition of a CW-TPR fiber in a sample volume of 4 mL, without pH adjustment, and saturated with NaCl, 30% (*w*/*v*). The extraction was performed during 60 min under magnetic stirring rate of 1,100 rpm at 40 °C. Finally, the fiber was then statically desorbed in 60 μL of methanol during 15 min.

Optimization of external micellar desorption process in SPME

In order to improve the desorption efficiency in the SPME process, we have replaced the organic solvents by a micellar medium in the desorption step. Nature, concentration of surfactant, and desorption time are important parameters to optimize in this process.

Surfactant type

After the extraction process, three non-ionic surfactants were tested: oligoethylene glycol monoalkyl ether (Genapol X-080), POLE, and polyoxyethylene 9 lauryl ether (Polidocanol). All surfactants solutions were prepared at 5% (*v*/*v*), and desorption time of 15 min was selected in order to compare the response areas. Desorption volume of surfactant was 60 μL.

As it can be seen in Fig. 4a, all FQs can be desorbed by the selected surfactants, although Genapol X-080 presented worse responses. In general, analyte responses increased with the surfactant polarity. POLE allows the best desorption efficiencies for the selected analytes, and therefore, it was selected like the most suitable surfactant in this process of micellar desorption.

Finally, we have made a comparison of the obtained results with different desorption solvents: POLE, methanol, and mobile phase. If we compared the area responses obtained, we

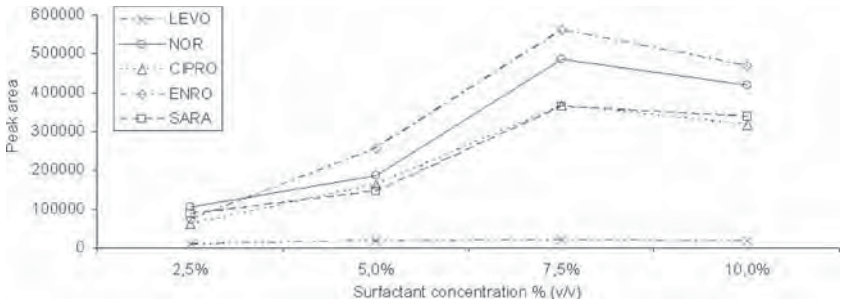


Fig. 5 Response of analytes to the different surfactants concentrations in the desorption process. Milli Q water containing 50 ng mL^{-1} for each analyte

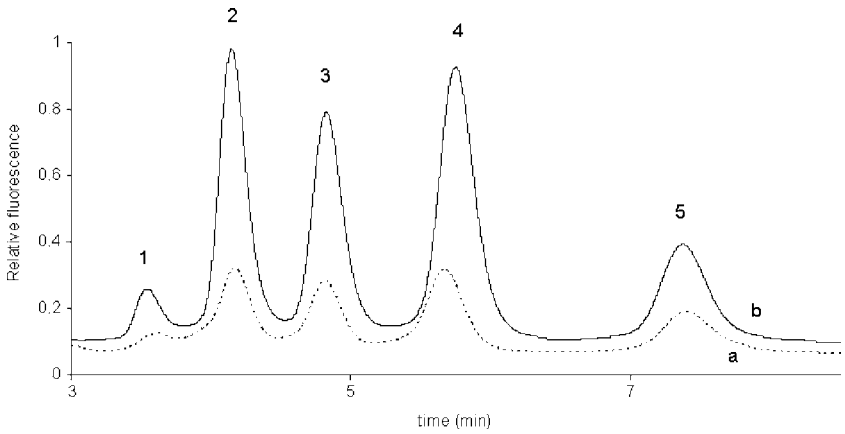


Fig. 6 Comparison of chromatograms using **a** conventional SPME and **b** SPME-MD procedure applied to a FQs mixture (50 ng mL^{-1} for each analyte) in Milli Q water. The *numbering* refers to Table 1. Extraction and chromatographic conditions specified in the text

can observe that micellar desorption improves significantly the response of all fluoroquinolones with respect to the desorption with mobile phase or methanol (Fig. 4b).

Surfactant concentration and desorption time

Once POLE surfactant was selected, other parameters which affect the desorption process were optimized. POLE concentration in a range of 2.5–10% (*v*/*v*) was studied. As it can be seen in Fig. 5, the desorption efficiency increased with the surfactant concentration until it reach the 7.5% (*v*/*v*) value. Values higher than 7.5% (*v*/*v*) give place to a decrease in analyte responses. This may occur because solutions with high concentration are very viscous, and this may cause a lower diffusion of analytes to the surfactant solution. For that, a 7.5% (*v*/*v*) concentration was chosen for the micellar desorption.

To check if the desorption time changes with the surfactant concentration, we have studied this parameter by obtaining the same results than for methanol.

Therefore, in the micellar desorption for FQs, we have selected a desorption volume of 60 μL of POLE, 7.5% (*v*/*v*) during a desorption time of 15 min.

In Fig. 6, we have compared the chromatograms obtained for both optimized methods, SPME with conventional desorption using methanol (a) and SPME-MD with POLE (b) for 50 ng mL^{-1} FQs mixture. We can observe that FQ signals improve significantly when micellar solution is used like desorbing agent. We think that the improvement of the chromatographic signal in the micellar desorption process is due to a combination of two processes: (1) We get the desorption of a great amount of analytes retained in the fiber due to their high affinity by the surfactant, and (2) the presence of the micellar media increases the fluorescence signal of the compounds because it increases the rigidity of the molecule [30]. It has been experimentally verified for injecting directly the standards prepared in methanol and in a solution of POLE 7.5% (*v*/*v*).

Analytical parameters

The performance characteristics of the conventional SPME and SPME-MD coupled with HPLC methodologies were calculated in order to compare both methods. Linearity, precision, limits of detection (LOD) and quantification (LOQ), and recovery were studied (Table 3).

Table 3 Analytical parameters for SPME procedure with desorption in methanol and POLE

	MeOH				POLE			
	R^a	LOD[b] (ng mL^{-1})	LOQ[c] (ng mL^{-1})	RSD[d] (%)	R^a	LOD[b] (ng mL^{-1})	LOQ[c] (ng mL^{-1})	RSD[d] (%)
LEVO	0.9910	0.23	0.77	2.0	0.9983	0.19	0.62	3.6
NOR	0.9922	0.02	0.08	6.8	0.9954	0.01	0.03	4.2
CIPRO	0.9998	0.05	0.12	8.5	0.9903	0.03	0.09	7.7
ENRO	0.9914	0.18	0.57	4.4	0.9981	0.01	0.04	6.1
SARA	0.9929	0.05	0.18	6.6	0.9910	0.02	0.05	5.8

[a] Correlation coefficient
[b] Detection limits are calculated as signal to noise ratio of three times
[c] Quantification limits are calculated as signal to noise ratio of ten times
[d] Relative standard deviation (n=6)

Table 4 Recovery percentages and RSD (percent) obtained for different real liquid samples spiked with 1 and 50 ng mL^{-1} of each fluoroquinolone

Compound	1 ng mL^{-1}			50 ng mL^{-1}		
	Sewage	Sea water	Groundwater	Sewage	Sea water	Groundwater
LEVO	87±11	100±15	114±7	91±8	104±7	85±9
NOR	89±4	88±7	82±5	92±3	88±7	90±9
CIPRO	90±7	89±9	81±16	81±3	85±6	83±4
ENRO	91±7	112±22	116±17	89±4	93±5	88±4
SARA	90±12	93±11	88±11	86±6	94±3	87±9

Mean of three determinations

The linearity was evaluated for each process in the range of 0.01–50 ng mL^{-1}. Calibration curves were established for each compound from the spiked deionized water by plotting the peak area versus the FQ concentration after the application of the whole procedure described in the experimental section. Each point of the calibration curves corresponds to the mean value obtained from three area of measurements. Obtained correlation coefficients were equal or higher than 0.9903 in all cases.

To evaluate the precision, six replicates samples with 10 ng mL^{-1} were extracted and analyzed following both methodologies. The reproducibility was expressed as relative standard deviation (percent RSD). Satisfactory results were achieved for all FQs with RSDs lower than 9% for both methodologies.

LODs and LOQs were calculated from the signal to noise ratio of the individual peaks, assuming a minimum detectable signal-to-noise level of 3 and 10, respectively. LODs obtained were in the range of 0.02–0.23 ng mL^{-1} using methanol and between 0.01–0.19 ng mL^{-1} using POLE like desorbing agent; LOQs were between 0.08 and 0.77 ng mL^{-1} for methanol and 0.03−0.62 ng mL^{-1} for POLE.

As we can observe, the LODs and LOQs using SPME-MD were better than those calculated in the conventional SPME using methanol as desorbing agent. They were enhanced by a factor between 1.5 and 3.5 times.

Application to water samples

SPME-MD methodology was applied to water samples of different origin (seawater, sewage, and groundwater) in order to study the influence of matrix effects. Blanks of real samples were run to verify the absence of interferences. These samples were spiked with two levels of concentration: 1 and 50 ng mL^{-1} of each fluoroquinolone and analyzed by SPME-MD coupled to HPLC procedure.

Recoveries of the analytes were calculated using calibration curves obtained according to the procedure described in the experimental section. They were expressed by the average of three determinations (Table 4). In general, it can be observed that the extraction efficiency was satisfactory for all compounds in all samples in both levels, being the recoveries in the range 81–116%.

Figure 7 shows the chromatogram obtained for a non-spiked (a) and a spiked (b) seawater sample analyzed by SPME-MD-HPLC using CW-TPR fiber.

Obtained results with SPME-MD for the spiked levels showed that, in general, recoveries were satisfactory for

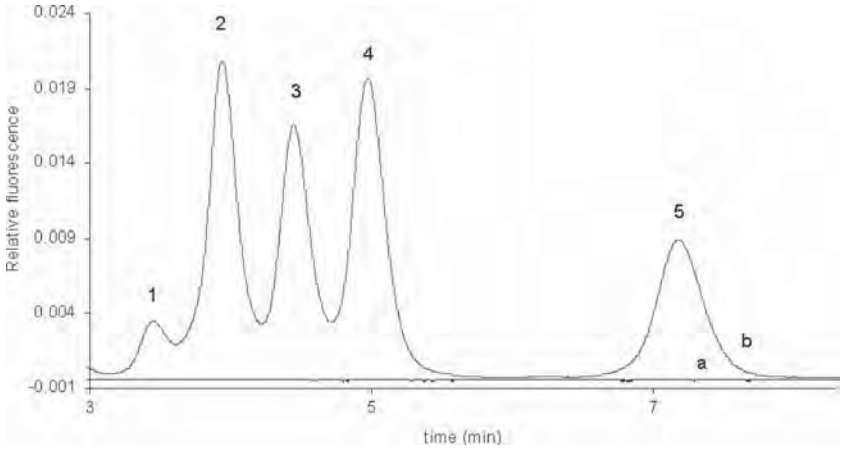


Fig. 7 Chromatogram of blank (**a**) and spiked (**b**) wastewater sample (1 ng mL^{-1} for each analyte) using SPME-MD procedure. The *numbering* refers to Table 1. Extraction and chromatographic conditions specified in the text

different kind of samples. Therefore, we can say that the matrix has no effect in the SPME-MD analysis of FQs in environmental water samples.

Conclusions

In the present work, a simple and sensitive SPME-MD method, based on the micellar desorption using POLE, 7.5% (*v*/*v*) followed by HPLC with fluorescence detection, has been developed for the extraction and analysis of five fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, and sarafloxacin) in different water samples. Under optimized experimental conditions, it has been shown that SPME-MD could represent a viable and easy analytical approach for routine analysis obtaining better analytical parameters than conventional SPME using methanol as desorbing agent. About that, this method showed a good linearity and reproducibility. Recoveries were in the range from 81% to 116% for all studied FQs. Fluorescence detection provided good sensitivity and selectivity with detection limits ranging from 0.01 to 0.2 ng mL^{-1} in environmental water samples. The proposed SPME-MD method proved to be a valuable tool in the analytical characterization of water samples like effluents from hospitals or sewage waters of the treatment plants. It may be used for routine analysis because HPLC-fluorescence detection is an inexpensive analytical technique compared with others one like LC-MS.

Acknowledgment This work was supported by funds provided by the Spanish Ministry of Education of Science. Research Project CTQ2006-06507.

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III.1.3 Comparación de extracción en fase sólida usando desorción micelar combinada con LC-FD y LC-MS/MS en la determinación de residuos de fluoroquinolonas.

En este trabajo se introduce una importante variación en la técnica de extracción en fase sólida convencional, llevándose a cabo el paso de elución de los analitos con un medio micelar. A diferencia de la SPME, donde el uso de surfactantes sí había sido empleada con éxito, para nuestro conocimiento, ésta fue la primera vez en que fueron utilizados en la SPE.

En este caso, las cinco fluoroquinolonas analizadas también en el trabajo anterior, fueron determinadas en muestras líquidas por LC con espectrometría de masas, además de con detección por fluorescencia. Así se demostró que los surfactantes también son compatibles con la detección por espectrometría de masas.

Para el desarrollo de la SPE, se optimizó el tipo de material adsorbente, el pH, la fuerza iónica, el volumen de muestra y la naturaleza, concentración y volumen de surfactante.

Los parámetros analíticos fueron comparados para los cuatro procedimientos desarrollados: elución con disolvente orgánico o surfactante (POLE al 7.5%, v/v), y detección por fluorescencia y espectrometría de masas. Empleando fluorescencia, los límites de detección estuvieron entre 0.072 y 0.200 $ng \cdot mL^{-1}$ utilizando metanol y entre 0.010 y 0.034 $ng \cdot mL^{-1}$ usando POLE. Con detección por espectrometría de masas, los límites de detección obtenidos estuvieron en el rango 0.007-0.013 $ng \cdot mL^{-1}$ con

metanol y 0.005 - 0.011 $ng \cdot mL^{-1}$ con POLE. Al igual que en el artículo anterior (III.1.2.), se observó un aumento de la sensibilidad cuando se emplean medios micelares y detección por fluorescencia, si bien en la detección por espectrometría de masas fueron similares.

Después de determinarse los porcentajes de recuperación en varias matrices acuosas (obteniéndose valores entre el 73 y el 97%), se analizaron diferentes aguas residuales. Por un lado, se tomaron muestras de las aguas residuales de un hospital antes de su llegada al alcantarillado público, en las que se detectaron tres de las cinco fluoroquinolonas estudiadas, en concentraciones entre 46 y casi 700 $ng \cdot mL^{-1}$.

Además, se analizaron muestras procedentes de las distintas etapas de tratamiento de una EDAR. En este caso se detectaron los mismos analitos (levofloxacina, norfloxacina y ciprofloxacina) y se observó una disminución en la concentración de todos ellos desde la entrada hasta la salida de la planta de tratamiento.

Este trabajo ha sido publicado en la revista *Journal of Liquid Chromatography and Related Technologies 35 (2012) 2081-2096.*

Journal of Liquid Chromatography & Related Technologies, 35:2081–2096, 2012

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2011.627622

COMPARISON OF SOLID PHASE EXTRACTION USING MICELLAR DESORPTION COMBINED WITH LC-FD AND LC-MS/MS IN THE DETERMINATION OF ANTIBIOTICS FLUOROQUINOLONE RESIDUES IN SEWAGE SAMPLES

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□ *A new "green" methodology, free of hazardous solvents, was developed for the extraction and determination of five fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, and sarafloxacin) in environmental water matrices. The method is based on solid-phase extraction (SPE) coupled to liquid chromatography (LC) with fluorescence and mass spectrometry detection. In this work, we replaced an organic solvent by a micellar media in the desorption step of the SPE process. This combination has never before been optimized and applied to environmental samples analysis. After SPE extraction optimization, a detailed study on the optimum conditions for micellar desorption (i.e., surfactant type, concentration, and volume) was performed. The proposed method was compared to the conventional SPE procedure using methanol as the desorbing agent and tested on several aqueous environmental samples, including seawater, groundwater, and wastewater. Extraction efficiencies of 73%–97% were obtained for all five compounds with relative standard deviations below 11% in all matrices. Finally, real sewage samples from a hospital and wastewater treatment plant were analyzed with both conventional and micellar desorption using fluorescence and mass spectrometry detection.*

Keywords antibiotics, fluoroquinolones, high-performance liquid chromatography, micellar desorption, solid-phase extraction, water analysis

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) have recently attracted the attention of the international scientific community because they are continuously being found in different aquatic environments. Many of these PPCPs are highly bioactive, can enter municipal sewage systems,

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and even survive passage through the sewage treatment plants[1] to seawater, surface water, or groundwater.[2] They can therefore be considered "pseudo" persistent pollutants due to their continuous introduction into the environment.[3] However, these compounds are normally found at trace concentration levels;[4] as a result, an extraction/preconcentration step is necessary prior their determination.

Among the PPCPs, fluoroquinolones (FQs) are a class of relatively new and entirely manmade non-steroidal antibiotics/antibacterials used in human and veterinary medicine for therapeutic purposes.[5] The FQs exhibit high activity against a broad spectrum of gram-negative and gram-positive bacteria through inhibition of their DNA-gyrase or topoisomerase II.[6] They are used to treat infection in many parts of the body by killing the harmful bacteria or preventing their growth.[7] However, their extensive administration may give rise to an increase in the antibiotic resistance of pathogenic bacteria, which may result in health problems.[8,9] Unfortunately, sewage treatment plants are not able to completely remove these compounds from their effluent, resulting in significant quantities of the active ingredients transported to environmental aquatic systems. It has also been reported that the irrigation of crops with treated wastewater can introduce antibiotics into surface waters through agricultural runoff.[10] These compounds have been detected at the $\mu g \cdot L^{-1}$ level in different aquatic environments around the world, including municipal wastewater and surface waters,[11–13] with a risk of accumulating up the food chain. In fact, the European Union has established maximum residue limits (MRL) for FQs in animal foodstuffs.[14]

Sensitive multiresidue methods are needed to determine FQ concentrations and compare to the required levels. The methods commonly used for the determination of antibiotics include extraction for both clean-up and enrichment of aqueous environmental samples. The extraction technique most frequently used is solid phase extraction (SPE) using polymeric cartridge and organic desorption solvents.[15–18] However, these solvents are also pollutants themselves. Green chemical approaches seek to develop methods free of such solvents for extraction/preconcentration and analysis of these pharmaceutical compounds.

Micellar media are an alternative to organic solvents. The surfactants or surface-active agents are amphipathic substances that can associate to form molecular aggregates called micelles in aqueous solutions. One of the most important properties of micellar systems is their high capacity in solubilizing different types of solutes. We can exploit this extractant capacity in conventional sample pretreatment methods, as they provide several advantages such as lower toxicity, easy handling, and low cost. Moreover, these surfactants are commercially available and compatible with the mobile phase used in LC analysis.[19]

In a previous work, we used surfactants as the desorbing agents in solid phase microextraction (SPME), a method similar to SPME with micellar desorption (SPME-MD), for the extraction of fluoroquinolones.[20] The determination of these compounds was improved compared to conventional SPME using methanol as the desorbing agent. However, SPME is not an exhaustive extraction process and because of the low concentrations of the target compounds in environmental water samples, it is necessary to obtain a high preconcentration of the analytes.

Alternatively, SPE is widely used in the environmental analytical field because it extracts and preconcentrates in a single step. The primary goal of this work was to establish a new extraction process that applied a micellar agent in the desorption step of SPE, considering its innate advantages over organic solvents. To our knowledge, this combination has never been developed and applied before to environmental samples. We optimized several variables that affect the extraction process: cartridge type, pH, ionic strength, and sample volume. We also studied the variables that influence the desorption process: nature, volume, and concentration of surfactant. To verify matrix effects in the method, the optimized SPE with micellar desorption (SPE-MD) procedure was tested on different spiked water samples including seawater, groundwater, and wastewater.

Once the satisfactory extraction efficiencies were achieved, the extraction method was coupled to an LC system with fluorescence and mass spectrometry detectors. It was then used to analyze the five selected FQs (by their wide administration) in hospital and municipal wastewaters, comparing both conventional and micellar desorption.

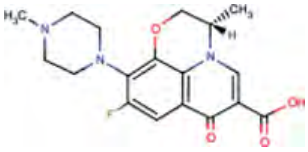
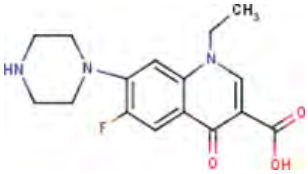
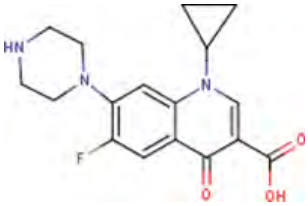
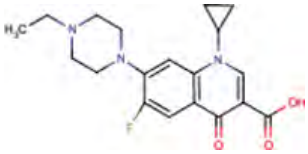
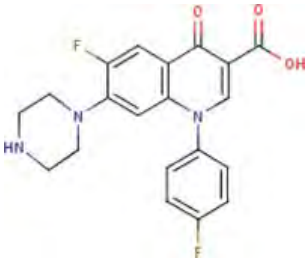
EXPERIMENTAL

Reagents

Fluoroquinolones (Table 1, numbers identify the compounds in the chromatograms) were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions ($1000\,\mu g \cdot mL^{-1}$) were prepared in methanol and stored in glass-stoppered bottles at 4°C prior to use. The mobile phase was prepared with water and methanol. Ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA). The LC-grade methanol and LC-MS grade methanol and water were obtained from Panreac Química (Barcelona, Spain). Glacial acetic acid that was used to adjust the pH of the LC-FD mobile phase was obtained from Scharlau Chemie S.A. (Barcelona, Spain), and the LC-MS mobile phase contained formic acid and ammonium formate supplied by Panreac Química (Barcelona, Spain).

The SPE cartridges tested included the following: 500-mg EnvirElut Pesticide and 500-mg Bond Elut SCX from Varian (Madrid, Spain),

TABLE 1 List of Fluoroquinolones, Identification Number, Abbreviation, and Chemical Structure

Compound	Identification number	Abbreviation	Structure
Levofloxacin	1	LEVO	
Norfloxacin	2	NOR	
Ciprofloxacin	3	CIPRO	
Enrofloxacin	4	ENRO	
Sarafloxacin	5	SARA	

500-mg Sep-Pak C18 and 200-mg Oasis HLB from Waters (Madrid, Spain), and 100-mg LiChrolut ENV from Merck (Darmstadt, Germany).

Surfactants used in this study, polyoxyethylene 10 lauryl ether (POLE), hexaethylene glycol monododecyl ether ($C_{12}E_6$), hexadecyltrimethylammonium bromide (HTAB), and sodium dodecyl sulfate (SDS), were obtained from Sigma-Aldrich (Madrid, Spain) and prepared in Milli-Q water.

Instrumentation

Two chromatographic systems were provided by Varian (Varian Inc., Madrid, Spain). The Varian Prostar consisted of a pump fitted with an

autosampler and a column valve module with an internal oven and fluorescence detector. A 320-MS LC/MS/MS system (triple quadrupole) was equipped with an electrospray ionization (ESI) interface, two pumps, and a column valve module with an internal oven and an autosampler.

Chromatographic Conditions

A Waters Symmetry C_{18} column (150 mm × 3.9 mm, 4-μm particle size, Waters Cromatografía, Barcelona, Spain) under a methanol/water (15:85 v/v) isocratic mobile phase adjusted to pH = 2.5 at a flow rate of 1 mL·min^{-1} was used for the separation of FQs using LC-FD detection. The sample volume injected was 30 μL, and the fluorescence detector operated at an excitation wavelength of 280 nm and an emission wavelength of 450 nm.

In LC-MS/MS analysis, a Varian Pursuit UPS 2.4 C_{18} (50 × 2.0 mm, 2.4-μm particle size) stationary phase and a methanol/water (15:85 v/v) mobile phase with 0.2% (v/v) formic acid and 5 mM ammonium formate were used. The flow-rate was 0.2 mL/min, and the sample volume injected was 10 μL under pick-up conditions. The electrospray ionization parameters were fixed as follows: the housing temperature at 60°C, the nebulizing gas pressure at 50 psi, the drying gas pressure at 30 psi, a shield voltage of 0.6 kV, and a needle voltage of 5 kV. Nitrogen was used as the nebulizing and drying gas, and argon was employed as the collision gas. The detailed MS parameters for each FQ are shown in Table 2 and were optimized by direct injection of a standard solution of each analyte at 1 mg/L into the detector at a flow rate of 10 μL/min. The analysis was carried out in multiple reaction monitoring (MRM) mode, and the detector was fixed at maximum extended dynamic range (EDR) with a peak mass width of 2 and 1.5 amu for the first and third quadrupole, respectively. The dwell time for all transitions was 0.1s.

Spiked Water Samples

Prior to analysis, the different aqueous environmental samples (i.e., seawater, groundwater, and wastewater) were filtered through a 0.22-μm cellulose

TABLE 2 Mass Spectrometer Parameters for Fluoroquinolones Detection

	Precursor ion *(m/z)*	Capillary voltage (V)	Quantification ion (Collision Potential, V)	Confirmation ion (Collision Potential, V)
LEVO	362.0	56	318.0 (17)	261.0 (24)
NOR	320.1	56	301.9 (19)	230.8 (37)
CIPRO	332.1	52	313.9 (19)	230.8 (36)
ENRO	360.2	60	342.0 (19.5)	316.0 (16.5)
SARA	386.1	52	299.0 (21.5)	342.0 (15.5)

acetate filter, stored in the dark and refrigerated. Water samples (200 mL) were spiked with the fluoroquinolone mixture ($5\,ng \cdot mL^{-1}$) and processed according to the optimum extraction conditions using micellar desorption. Three replicates of each water sample were analyzed with LC and fluorescence detection, and the corresponding extraction efficiencies were calculated.

Solid-Phase Extraction

Cartridges were conditioned with 5 mL of methanol and 5 mL of Milli-Q water at a flow-rate of $5\,mL \cdot min^{-1}$ before each run. Then, the sample was passed through the cartridge at a flow-rate of $10\,mL \cdot min^{-1}$. A wash step was carried out using 2×5 mL of Milli-Q water to remove impurities retained on the cartridge.

Subsequently, the cartridge was dried under vacuum for 10 min, and the retained analytes were eluted at a low flow rate ($\sim 1\,mL \cdot min^{-1}$). Blanks were run to confirm carryover absence.

Experimental designs were performed using Statgraphics Plus software, version 5.1 (Manugistic, Rockville, MD, USA). Statistical tests were performed with SPSS 11.0 (Chicago, IL, USA).

RESULTS AND DISCUSSION

In a SPE procedure, we must evaluate several experimental variables to achieve maximum extraction efficiency of target analytes. Once the extraction parameters are determined, conditions for the desorption process must be optimized. We monitored this process using LC with fluorescence detection.

Optimization of Extraction Procedures

Before implementation of micellar desorption, we optimized all of the parameters that affect the extraction process in SPE: cartridge type, pH, ionic strength, and sample volume. For this, we used an initial sample volume of 100 mL containing $5\,ng \cdot mL^{-1}$ of each fluoroquinolone, and desorption was by 2 mL of methanol.

SPE Cartridge Type and pH

The most important step in SPE method development is to determine the best cartridge that achieves maximum extraction of all analytes. We tested five SPE cartridges with different characteristics: EnvirElut Pesticide, Bond Elut CSX, Sep-Pak C18, Oasis HBL, and LiChrolut.

The extraction efficiency of many analytes is determined by pH. Additional considerations are required when fluorescence detection is used. Large changes in the adsorption features of FQs and, consequently, in their fluorescence emissions are observed with a change in pH.[21] These compounds can be present in aqueous solution as cationic, anionic, or intermediate forms due to the presence of amino groups in the piperazine moiety, as well as carboxylic groups. The FQs present their undissociated form at pH < 7 (see Table 1). We decided to check each cartridge at several pH values to study both variables in complementary forms. Thus, four pH values were studied: 3.0, 6.0, 9.0, and 12.0.

The behavior of each tested cartridge at the different pH values for the enrofloxacin extraction is shown in Figure 1. We can observe that the Oasis HLB cartridge at a pH value of 3.0 yielded the best extraction efficiencies. The studies carried out for the rest of analytes had similar results.

Ionic Strength

The effect of ionic strength on compound recovery was evaluated by the addition of sodium chloride to the aqueous medium at concentrations ranging from 0 to 30% (w/v). Incremental increases in ionic strength did not produce increases in analyte response areas. Therefore, we chose a solution without added salt for our experiments.

Sample Volume

The volume of sample that passes through the cartridge is another important variable to be optimized. We chose to evaluate volumes between 25 and 250 mL.

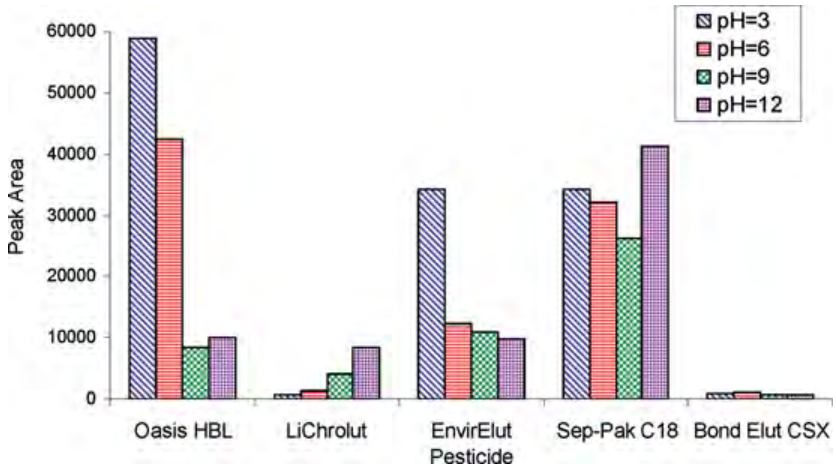


FIGURE 1 Relative extraction efficiencies with different SPE cartridges at several pH values for enrofloxacin (Milli-Q water spiked with 5 ng · mL^{-1} using methanol for the desorption and LC-FD). (Color figure available online.)

Logically, a larger sample volume at the same concentration should yield a larger response signal if the same desorbing volume is used. However, if we normalize the data to take into account the preconcentration step, we obtain similar responses over the 25 to 200 mL sample volume range. After 200 mL, a decrease in signal response occurs because the breakthrough volume had been reached, where the cartridge was not able to retain any more of the analyte. We therefore chose 200 mL as the sample volume, obtaining a greater chromatographic signal and preconcentration factor of 100.

In summary, the best conditions for FQ extraction were as follows: an Oasis HBL cartridge, the solution at a pH of 3, no addition of salt, and a 200 mL sample volume.

Optimization of the Micellar Desorption Process

To replace the organic solvent with a micellar medium in the desorption step, we must optimize the type, concentration, and volume of surfactant.

Four different surfactants were tested for analyte desorption: two non-ionic surfactants [Polyoxyethylene 10 lauryl ether (POLE) and Hexaethylene glycol monododecyl ether ($C_{12}E_6$)], one cationic [Hexadecyltrimethylammonium bromide (HTAB)], and one anionic [Sodium dodecyl sulfate (SDS)].

Several values of concentration and volume for each surfactant were used to observe the combined effect of these variables. We used a 2^2 factorial design to observe the influence of each variable on extraction efficiencies and the variables' correlation with each other. All experiments were performed randomly to minimize the effects of uncontrolled factors. Upper and lower values were chosen for each variable: 2.5 and 7.5% (v/v) for the surfactant concentration and 2 and 6 mL for the volume of surfactant. The peak area of each fluoroquinolone was selected as the elemental response value of the design.

The FQs were eluted more efficiently with the non-ionic surfactants compared to the ionic ones. Between the two non-ionic surfactants, the greatest response areas were obtained with POLE. Considering these results, we chose POLE as the desorbing agent in subsequent experiments. Figure 2 shows the results obtained for norfloxacin as a representative example.

To find the optimum values of surfactant concentration and volume, we used another 3^2 factorial design with duplication of the central point, where the recovery was the dependent variable. This experimental matrix combined values of 2, 4, and 6 mL for surfactant volume with surfactant concentrations of 2.5, 5, and 7.5% (v/v). Figure 3 shows the surface response obtained for enrofloxacin, where we observe that a surfactant concentration of 7.5% (v/v) and volume of 5 mL were the best conditions. Under these conditions, we obtained a preconcentration factor of 40.

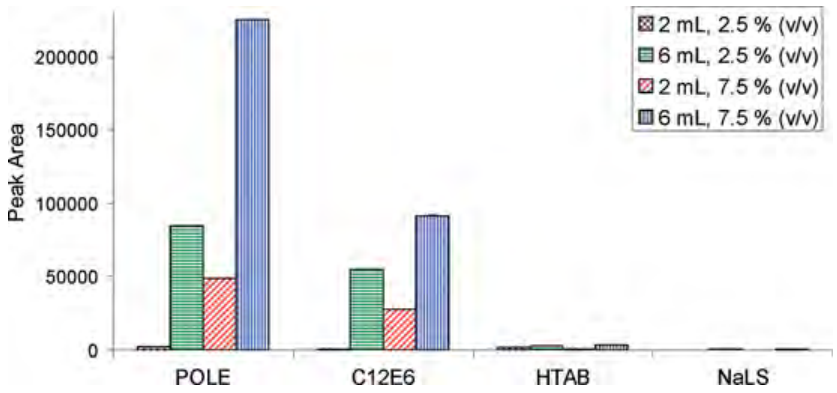


FIGURE 2 Relative desorption efficiencies with different volume and concentration for four different surfactants solutions for norfloxacin (Milli-Q water spiked with 5 ng · mL^{-1} using LC-FD). (Color figure available online.)

Analytical Parameters

The performance characteristics of the conventional SPE process with methanol and SPE using micellar media for the desorption coupled to LC were calculated for comparison. Figure 4 shows the LC-FD chromatograms for the desorption of FQs in methanol (a) and 7.5% (v/v) POLE (b). The resolution and signal obtained with POLE were improved, although the effect on preconcentration was minor. The increase in signal may have been due to an increase in rigidity of the analyte molecules in the presence of surfactant.[22]

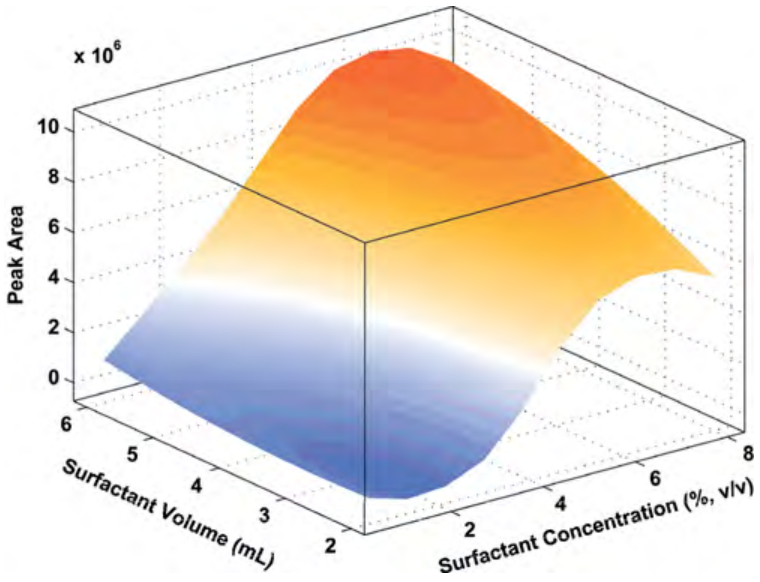


FIGURE 3 Response surface for the effect of POLE concentration and volume on the desorption of enrofloxacin (Milli-Q water spiked with 5 ng · mL^{-1} using LC-FD). (Color figure available online.)

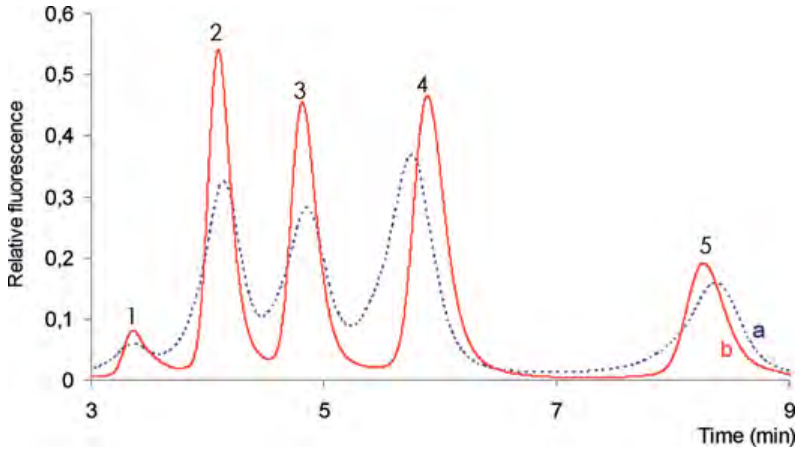


FIGURE 4 Comparison of chromatograms using (a) conventional SPE and (b) SPE-MD procedure applied to a FQs mixture (5 ng · mL^{-1} for each analyte) in Milli-Q water using LC-FD. Numbers refers to Table 1. (Color figure available online.)

Calibration curves for the different compounds were built from 0.05–500 ng · mL^{-1}, and each point corresponds to the mean value obtained from three area measurements. Satisfactory linear ranges were obtained with correlation coefficients greater than 0.992 for all analytes in both cases.

The detection (LODs) and quantification limits (LOQs) for the two desorption procedures and the two detection methods were calculated from the signal-to-noise ratios of the individual peaks, assuming minimum detectable signal-to-noise levels of 3 and 10, respectively. In LC with fluorescence detection, the LODs obtained were between 0.072–0.200 ng · mL^{-1} using methanol and 0.010–0.034 ng · mL^{-1} using POLE. The LOQs were between 0.240–0.668 ng · mL^{-1} for methanol, but were quite lower for POLE, between 0.032 and 0.112 ng · mL^{-1}. This demonstrates that the response signal is improved by the surfactant presence.

With MS detection, the LODs were between 0.007 and 0.013 ng · mL^{-1} when using methanol and between 0.005 and 0.011 ng · mL^{-1} when using POLE as the desorbing agent. The LOQs ranged from 0.023 to 0.050 ng · mL^{-1} for methanol, and between 0.017 and 0.037 ng · mL^{-1}, for POLE. In this case, no enhanced effect exists for the use of POLE as the desorbing agent.

To evaluate precision, six replicate samples containing 5 ng · mL^{-1} of the target compounds were extracted following both conventional and micellar procedures, then analyzed by LC-FD and LC-MS/MS. Reproducibility was expressed as relative standard deviation (% RSD). Satisfactory results were achieved for all FQs with RSDs lower than 11% for both methods. Table 3 summarizes these results.

Before analyzing real samples, SPE using micellar desorption was applied to different spiked samples to calculate method recoveries and

TABLE 3 Analytical Parameters for SPE Procedure with Elution in Methanol and POLE Using HPLC with Fluorescence Detection (FD) and Mass Spectrometry Detection (MS)

	MeOH elution			POLE elution		
FD	LOD[a] ($ng \cdot mL^{-1}$)	LOQ[b] ($ng \cdot mL^{-1}$)	RSD[c] (%)	LOD[a] ($ng \cdot mL^{-1}$)	LOQ[b] ($ng \cdot mL^{-1}$)	RSD[c] (%)
LEVO	0.200	0.668	9.36	0.034	0.112	8.47
NOR	0.112	0.372	6.18	0.013	0.044	8.52
CIPRO	0.072	0.240	7.44	0.013	0.044	6.78
ENRO	0.088	0.292	9.29	0.010	0.032	7.00
SARA	0.140	0.468	10.82	0.014	0.048	9.79
	MeOH elution			POLE elution		
MS	LOD[a] ($ng \cdot mL^{-1}$)	LOQ[b] ($ng \cdot mL^{-1}$)	RSD[c] (%)	LOD[a] ($ng \cdot mL^{-1}$)	LOQ[b] ($ng \cdot mL^{-1}$)	RSD[c] (%)
LEVO	0.007	0.023	8.06	0.009	0.030	6.82
NOR	0.011	0.037	7.79	0.008	0.027	8.08
CIPRO	0.013	0.043	7.51	0.008	0.027	5.31
ENRO	0.007	0.023	7.04	0.005	0.017	5.99
SARA	0.010	0.050	8.11	0.011	0.037	8.61

[a]Detection limits are calculated as signal-to-noise ratio of three times.
[b]Quantification limits are calculated as signal-to-noise ratio of ten times.
[c]Relative Standard Deviation ($n = 6$).

evaluate sample matrix effects. None of the FQs were detected in seawater, groundwater, or wastewater blanks. These samples were spiked with $5\,ng \cdot mL^{-1}$ of each fluoroquinolone and analyzed by SPE-MD coupled to LC-FD.

Recoveries of the analytes were calculated using the calibration curves and expressed as the average of three determinations (Table 4). With extraction efficiencies ranging from 73–97%, the extraction efficiency was deemed satisfactory for all compounds in different kinds of samples.

TABLE 4 Recovery Percentages and RSD (%) Obtained for Different Real Liquid Samples Spiked with $5\,ng \cdot mL^{-1}$ of Each Fluoroquinolone Using SPE-MD-HPLC-FD[a]

	Seawater	Groundwater	Wastewater
LEVO	88 ± 12	73 ± 2	82 ± 14
NOR	97 ± 10	83 ± 3	85 ± 5
CIPRO	88 ± 8	86 ± 1	86 ± 2
ENRO	82 ± 6	92 ± 2	94 ± 6
SARA	89 ± 9	84 ± 10	86 ± 11

[a]Mean of three determinations.

TABLE 5 FQs Concentrations[a] Measured in Sewage Samples from Hospital and Wastewater Treatment Plant Using Conventional and Micellar SPE Procedure and HPLC with Fluorescence Detection (FD) and Mass Spectrometry Detection (MS)

	MeOH elution				POLE elution			
		Treatment plant waters ($ng \cdot mL^{-1}$)				Treatment plant waters ($ng \cdot mL^{-1}$)		
FD	Hospital waters ($ng \cdot mL^{-1}$)	Primary treatment	Secondary treatment	Final effluent	Hospital waters ($ng \cdot mL^{-1}$)	Primary treatment	Secondary treatment	Final effluent
LEVO	94.11 ± 9.32	8.550 ± 2.88	3.001 ± 0.77	2.323 ± 0.41	100.1 ± 12.2	11.23 ± 2.33	3.323 ± 0.62	2.931 ± 0.33
NOR	568.8 ± 11.8	5.391 ± 2.81	<LOQ	<LOQ	601.2 ± 10.3	7.563 ± 1.82	0.314 ± 0.08	0.062 ± 0.02
CIPRO	46.01 ± 5.91	39.21 ± 4.05	10.17 ± 2.74	8.131 ± 3.17	46.94 ± 4.62	42.02 ± 3.97	10.95 ± 2.01	10.07 ± 3.34
ENRO	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
SARA	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

	MeOH elution				POLE elution			
		Treatment plant waters ($ng \cdot mL^{-1}$)				Treatment plant waters ($ng \cdot mL^{-1}$)		
MS	Hospital waters ($ng \cdot mL^{-1}$)	Primary treatment	Secondary treatment	Final effluent	Hospital waters ($ng \cdot mL^{-1}$)	Primary treatment	Secondary treatment	Final effluent
LEVO	111.8 ± 11.5	11.31 ± 2.44	2.972 ± 0.75	2.983 ± 0.08	128.1 ± 9.33	13.84 ± 3.81	3.911 ± 1.44	3.271 ± 1.09
NOR	651.2 ± 18.5	8.670 ± 3.88	0.831 ± 0.20	0.104 ± 0.04	698.8 ± 13.4	9.332 ± 2.13	0.930 ± 0.12	0.110 ± 0.03
CIPRO	49.33 ± 5.66	44.99 ± 4.79	11.33 ± 4.06	10.97 ± 3.31	49.07 ± 3.72	46.71 ± 6.91	14.08 ± 3.98	13.62 ± 2.95
ENRO	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
SARA	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

[a]Mean of three determinations.
Nd: not detected.

Therefore, the matrix had little effect on the analysis of FQs in environmental water samples using this method.

Analysis of Fluoroquinolones in Sewage Samples

Once the effectiveness of the proposed method was verified, it was applied in the determination of the target analytes in different sewage water samples from hospitals and wastewater treatment plants (Table 5). The samples were filtered through a 0.22-μm cellulose acetate filter and analyzed immediately after sampling.

We took three samples from a wastewater treatment plant at the exit of each treatment stage: primary treatment (i.e., bulk removal), secondary treatment (i.e., activated sludge), and the final effluent (i.e., after flocculation and chlorination). We detected three of the five FQs in all of these samples, and, as expected, their concentrations were decreasing from input to output of the treatment plant. Most elimination occurs between primary and secondary treatment: 62–72% for levofloxacin, 89–96% for norfloxacin, and 70–74% for ciprofloxacin. Between secondary treatment and the final effluent, however, the difference in concentration was very low. The three compounds were detected and determined with the four optimized

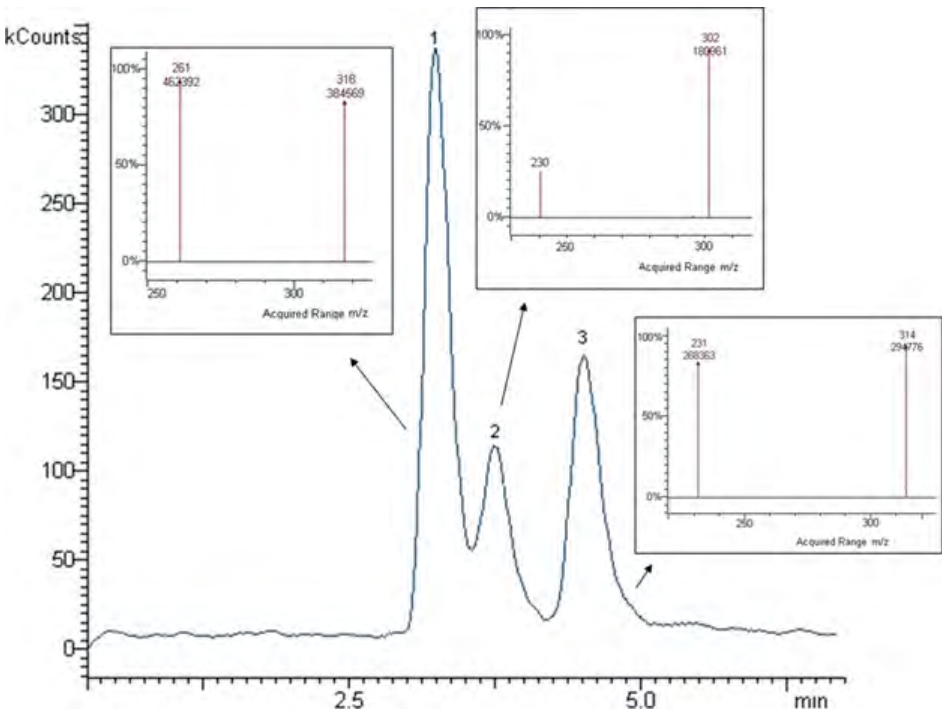


FIGURE 5 Chromatogram and mass spectra of fluoroquinolones in sewage hospital water using SPE-MD-LC-MS/MS procedure. Numbers refers to Table 1 and detection parameters specified in Table 2. (Color figure available online.)

procedures. However, norfloxacin could not be quantified using SPE with conventional desorption and LC-FD in the secondary treatment and final effluent because its concentration was below the quantification limit. Nevertheless, its determination was possible when we used LC-MS/MS, and slightly better results were obtained when POLE was the desorption agent.

When we analyzed sewage water samples from hospitals, we detected three FQs. As seen in Table 5, these samples contained higher amounts of these FQs than the treatment plant samples did. The three FQs could all be determined by the different optimized procedures, but concentrations were slightly higher with SPE-MD-LC-MS/MS. Figure 5 shows the chromatogram of the hospital sewage sample analyzed by SPE-MD-LC-MS/MS and the mass spectra of the FQs detected. These data suggest that desorption with POLE and determination by LC-MS/MS are choice analysis methods for this class of compounds.

CONCLUSIONS

A novel and rapid method based on solid phase extraction using micellar desorption followed by liquid chromatography with fluorescence or mass spectrometry detection has been developed for the extraction and determination of antibiotic fluoroquinolone residues in water samples.

Its application to spiked environmental water samples yielded high extraction efficiencies, between 73% and 97% with RSD values lower than 11%, for all FQs studied. Matrix effects were not observed.

Although the solid phase microextraction (SPME) method developed in a previous work[20] presents some benefits in that it allows *in situ* analysis and less sample manipulation, the limits of detection obtained by solid phase extraction using micellar desorption (SPE-MD) indicate that this procedure is more sensitive for the extraction and determination of FQs. The LODs obtained using fluorescence detection were lower (0.010–0.034 $ng \cdot mL^{-1}$) than those of SPME-MD-LC-FD (10–200 $ng \cdot L^{-1}$).

Aside from the advantages that the micellar media offered the fluorescence signal in terms of sensitivity, mass spectrometry allowed us to reach even lower LODs and LOQs, ranging between 9–11 and 17–37 $ng \cdot L^{-1}$, respectively. Using either mass spectrometry or fluorescence detection with micellar media as desorbing agent, we determined very low concentrations of FQs in sewage water samples, while some compounds could not be quantified using SPE with conventional desorption and fluorescence detection.

The SPE using micellar desorption is a suitable method for the extraction prior the analysis of aqueous environmental samples in which analytes

are at trace concentrations, and it may even be considered a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure.[23]

ACKNOWLEDGMENT

This work was supported by funds provided by the Spanish Ministry of Science Education, Research Project CTQ2010-20554. Sarah Montesdeoca Esponda would like to thank the Canary Agency of Investigation, Innovation and Information Society for her PhD Student Grant.

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III.1.4. Extracción asistida por microondas con medios micelares para la determinación de fluoroquinolonas en sedimentos marinos costeros seguida por LC con detección por fluorescencia.

En este apartado se presenta una publicación corta en la que se expone una parte de la optimización de un método de extracción para cinco fluoroquinolonas. En este caso la matriz es sólida y se emplea la extracción asistida por microondas, usando nuevamente los medios micelares como agente extractante. La determinación se realizó con LC-FD.

Se aplicó un diseño factorial de 2^4 (cuatro variables con dos niveles, con un total de 16 experimentos) para observar el efecto de la potencia, tiempo, volumen y concentración de surfactante, calculándose las correlaciones parciales y bivariadas de cada uno de estos parámetros. Se estudiaron tres medios micelares y se observó que las variables que más afectan a la extracción son la potencia y la concentración del extractante, mientras que las más relacionadas entre sí son potencia/tiempo y potencia/concentración.

Por tanto, esas parejas de variables fueron estudiadas más detenidamente en el siguiente trabajo (presentado en el apartado III.1.5.), en el cual se presenta el desarrollo del método al completo. Además, con los resultados obtenidos en esta publicación corta, pudimos fijar el hexaethylene glycol monododecyl ether (HTAB) como el surfactante más adecuado para los siguientes estudios.

Esta parte de la optimización fue publicada en *Luminescence 25 (2010) 239-240.*

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Microwave assisted extraction with micellar media for determination of fluoroquinolones in coastal marine sediments followed by HPLC with fluorescence detection

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Introduction

Contamination by pharmaceutical and personal care products (PPCPs) in sediments samples has recently been studied for the scientific community. Fluoroquinolones (FQs) are a class of antibiotics employed in human and animals medicine [1] that are accumulated in the wastewater treatment plants because there aren't mechanisms for remove it completely. Moreover, FQs are resistant to microbial degradation and may be persistent within environment [2].

Table 1. Experimental design for the optimization of extraction time, power, surfactant volume and concentration volume

RUN	POWER (V)	EXTRACTION TIME (min)	VOLUME (mL)	CONCENTRATION (%)
1	100	10	5	0,5
2	500	2	15	5
3	100	2	5	5
4	500	10	15	0,5
5	500	10	5	5
6	100	2	15	0,5
7	500	2	5	0,5
8	100	10	15	5
9	100	2	15	5
10	100	2	5	0,5
11	500	10	15	5
12	500	2	5	5
13	100	10	15	0,5
14	500	10	5	0,5
15	100	10	5	5
16	500	2	15	0,5

The aim of this work is to optimize a microwave assisted extraction method using micellar media as extractants (MAME) for the determination of FQs in different coastal marine sediment samples using high performance liquid chromatography with fluorescence detection.

Experimental

Chromatographic separation

A chromatograph system (C_{18} column) with fluorescence detector (excitation and emission wavelength 280 and 450 nm) from Varian (Madrid, Spain) was used. Analytes separation was carried out employed an isocratic mobile phase (15:85 v/v, methanol/water) at a flow rate of 1 mL.min^{-1}.

Microwave Assisted Micellar Extraction (MAME)

Microwave oven used was a Multiwave of Anton Paar (Graz, Austria).

Sediment samples were sieved to a particle size of lesser than 0.3 mm and we taken 2 g, which were spiked with a solution of FQs to obtain a concentration of each analyte of 2 µg/g. Then the sample was transferred to the vessel, the extractant agent was added and was subjected to the MAME process. The surfactants used as extractans were: Polyoxyethylene 10 lauryl ether (POLE), Hexaethylene glycol monododecyl ether ($C_{12}E_{6)}$, Hexadecyltrimethylammonium bromide (HTAB) and Sodium dodecyl sulphate, (NaLS). The extract solution was filtered through a 0.45 µm syringe filter.

The experimental design used for the optimization was obtained using Statgraphics Plus software 5.1 and the statistics study was done with SPSS 17.0.

Results

We optimize the extraction time, power, volume and concentration of surfactant with a 2^4 factorial design (Table 1) for researching the influence of each variable on the recovery and the variables correlation each other.

In the first experiments we observed that NaLS showed a very low amount of analyte extracted, so we not included it in following studies. In table 2 are shown the bivariate and partial correlations between each parameter for HTAB, which presents the major responses. The variable that more affects the process is the

Table 2. Relevant variables and correlation between variables using HTAB as extractant. The maxima correlations are +1 and −1

CORRELATION	Levofloxacin	Norfloxacin	Ciprofloxacin	Enrofloxacin	Sarafloxacin
Power	0.463	0.577	0.553	0.472	0.532
Time	0.157	0.339	0.346	0.024	0.045
Volume	–0.392	–0.251	–0.088	–0.308	–0.364
Concentration	0.449	0.968	0.331	0.521	0.465
Power/Concentration	–0.263	–0.265	–0.233	–0.327	–0.330
Time/Concentration	–0.080	–0.136	–0.129	–0.015	–0.024
Power/Time	–0.083	–0.255	–0.245	–0.013	–0.028
Volume/Concentration	0.214	0.097	0.031	0.198	0.205
Volume/Time	0.068	0.094	0.033	0.008	0.018
Volume/Power	0.0223	0.183	0.059	0.173	0.245

power and the concentration. The same trend was observed for POLE and $C_{12}E_6$.

Discussion

The use of surfactants as extractants instead of the organic conventional solvents presents some advantages like less toxicity and reduction in price. We will design an experiment 3^2 for power and time and other for power and concentration using HTAB to achieve the optimum conditions for the extraction of FQs.

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Determination of Bisphenol A by laser induced fluorescence spectroscopy

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Bisphenol A (BPA) is classified among Endocrine Disrupter Compounds (EDCs), susceptible to have hormono-mimetic properties disrupting hormonal balance and leading to adverse health effects. It is widely used for the production of polycarbonate water bottles or baby's bottles used to warm up water; it is also used in epoxy resin industry for internal coverage of beverage and food cans; consequently, BPA may be a potential contaminant for food [1, 2].

In this study, absorbance and fluorescence properties of BPA have been examined; this compound presents a maximum excitation-emission couple at 226 / 305 nm, a fluorescence quantum yield of 0.010 and a short fluorescence lifetime of 4 ns. By irradiation at 230 nm (200 µJ) a photo-induced dimer or polymer compound is rapidly produced that shows fluorescence emission at 410 nm (Figure 1). Its formation increases proportionally to the BPA concentration (Figure 2), and with the irradiation time. Mass spectrometric determinations are in progress to characterize this compound.

In a second part, an analytical application has been undertaken to evaluate direct determination of BPA traces in water, based on the determination of the photo-induced compound by laser induced fluorescence spectroscopy [3, 4]. To perform direct analysis, we used a pulsed YAG laser with an Optical Parametric Oscillator as a high energy excitation source and an ICCD camera for highly sensitive fluorescence detection [5]. As the fluorescence quantum yield of the photo-induced compound is greater than BPA compound, it can be used to determine indirectly BPA concentration with a higher sensitivity. Moreover, as its formation is specific of BPA irradiation at 230 nm, its appearance permits to identify BPA in the sample.

The analysis of BPA photo-induced compound by laser induced fluorescence spectrometry has permitted to propose a new and innovating method for BPA trace analysis in water by improving both sensitivity and specificity.

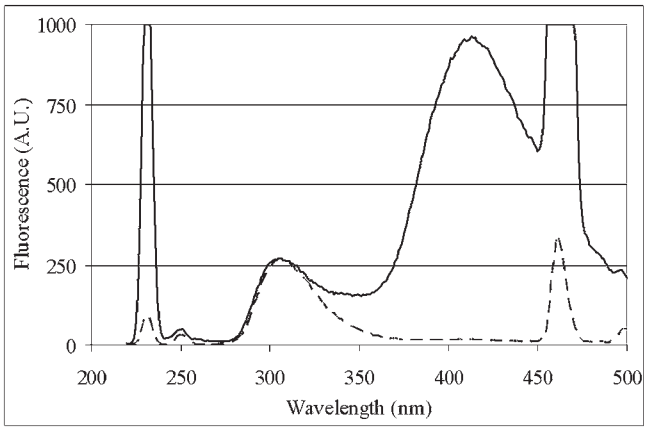

Figure 1. Fluorescence spectrum of BPA (dotted line 6 mg/L) and of its photo-induced compound (continuous line) after 1 minute irradiation at 230 nm (200 µJ).

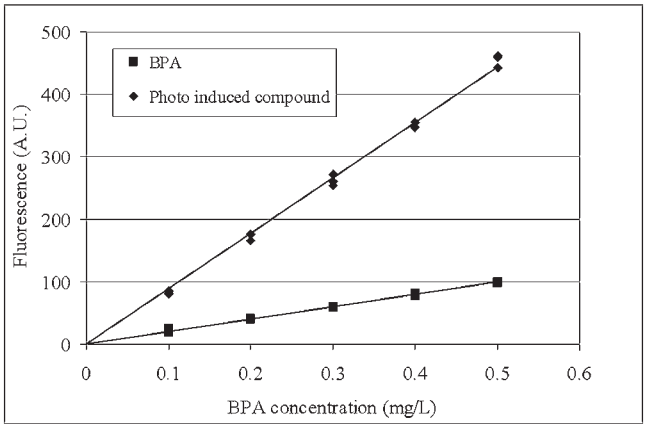

Figure 2. Fluorescence intensity of BPA and of its photo-induced compound after 5 minute irradiation at 230 nm (200 µJ) versus the concentration of BPA.

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Generic test of fluoroquinolones by superquenching-based fluorometric method

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Fluoroquinolones (FQs) are broad-spectrum antibiotics with particularly active against Gram-negative pathogens and novel among antimicrobial agents in clinical use because they directly inhibit DNA synthesis. They are also used to treat and prevent the veterinary diseases in animals produced for human consumption. The extensively use of these antibiotics has raised questions about the impact of veterinary medicines on organisms in the environment and on human health. Methods that are characterized by multi-analyte screening, sensitive, rapid, high-throughput, economical are needed for routinely monitoring of residue levels of FQs in foods of animal origin. Herein we demonstrate that superquenching-based signal amplification of fluorescence conjugate polyelectrolytes (FCPs) gives a new probability to sensitively generic test of FQs.

III.1.5 Combinación de la extracción micelar asistida por microondas con cromatografía liquida y espectrometría de masas en tándem para la determinación de fluoroquinolonas en sedimentos marinos costeros y lodos de depuradora.

Continuando la optimización presentada en el apartado anterior (III.1.4.), este artículo recoge el desarrollo completo y la aplicación de un método de extracción asistida por microondas combinada con LC-MS/MS para la determinación de fluoroquinolonas en muestras medioambientales sólidas.

Después de escoger el surfactante más apropiado para extraer los analitos (HTAB) y determinar las variables más influyentes y más correlacionadas entre sí, se aplicaron dos diseños factoriales de 3^2 para estudiar potencia/tiempo y potencia/concentración, con un total de nueve experimentos cada uno. De este diseño obtenemos una superficie de respuesta que nos permite escoger la mejor combinación de valores.

Se calculó la eficiencia y repetibilidad del método, obteniéndose recuperaciones entre el 73.2 y el 95.6%, con valores de RSD iguales o menores al 8% para todos los analitos. Usando LC-MS/MS, se obtuvieron limites de detección entre 0.15 y 0.55 $ng \cdot g^{-1}$.

La aplicación de este procedimiento en muestras sólidas tomadas en la isla de Gran Canaria permitió determinar importantes concentraciones de algunas de las fluoroquinolonas estudiadas. En lodos procedentes de una EDAR se detectaron hasta 206 $ng \cdot g^{-1}$ de ciprofloxacina.

También se tomaron muestras de sedimentos marinos en cuatro puntos cercanos a un emisario submarino de aguas residuales en la isla de Gran Canaria, detectándose las mayores concentraciones en el punto más cercano al mismo y siendo gradualmente más bajas hacia la costa (en el punto más alejado del emisario). Se detectaron cuatro de los cinco analitos estudiados, en el rango de 0.69-34.3 $ng \cdot g^{-1}$. Todas estas concentraciones fueron confirmadas usando LC con detección por fluorescencia y por espectrometría de masas.

El trabajo completo ha sido publicado en la revista *Biomedical Chromatography 26 (2012) 33-40.*

Research article

Biomedical Chromatography

Received 19 November 2010, Revised 19 January 2011, Accepted 19 January 2011 Published online in Wiley Online Library: 4 March 2011

(wileyonlinelibrary.com) DOI 10.1002/bmc.1621

Combination of microwave-assisted micellar extraction with liquid chromatography tandem mass spectrometry for the determination of fluoroquinolone antibiotics in coastal marine sediments and sewage sludges samples

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ABSTRACT: In this work, a method for the analysis of fluoroquinolone antibiotics using microwave-assisted micellar extraction combined with liquid chromatography–mass spectrometry detection has been developed. Different surfactants were tested for use as extractants in the isolation of the analytes from solid samples, and several experimental designs were evaluated for the determination and optimization of the variables that affect recovery from the matrix. Under optimal conditions, we obtained recoveries greater than 73% with relative standard deviations below 8%. Compounds were detected by liquid chromatography–electrospray ionization tandem mass spectrometry with detection limits between 0.15 and 0.55 ng g^{-1} and quantification limits between 0.49 and 1.85 ng g^{-1}. Finally, the optimized method was applied in the determination of antibiotics in real solid samples. Four fluoroquinolones (levofloxacin, norfloxacin, ciprofloxacin and enrofloxacin) were found in coastal marine sediments taken close to a marine outfall and in sewage sludge samples from a wastewater treatment plant. Concentrations ranged between 0.81 and 34.3 ng g^{-1} in the sediments and 3.43 and 206.1 ng g^{-1} in the sludge. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: microwave-assisted extraction; fluoroquinolones; liquid chromatography; mass spectrometry; micellar media

Introduction

Environmental residues of pharmaceuticals and personal care products (PPCPs) have attracted the interest of the scientific community for many years. Thus, many studies report the analysis of different PPCPs in a wide range of liquid samples, such as seawater, groundwater, surface water and sewage wastewater. However, only recently has attention been given to the potential contamination of solid samples (Hernando *et al.*, 2006).

Among PPCPs, fluoroquinolones (FQs) are a class of antibiotics used in human and animal medicine (Kaur *et al.*, 2008) that accumulate in wastewater treatment plants (WWTPs) after their excretion because there are no mechanisms to eliminate them completely. Because of their hydrophobic properties, they can accumulate in the solid products that are generated during wastewater treatment. In fact, any reduction in FQs achieved during treatment is mainly due to sorption onto sewage sludge (Li and Zhang, 2010), which is the major reservoir of FQ residues. Studies indicate that approximately 70% of the total FQs that enter the WWTPs can be found in the sludge (Lindberg *et al.*, 2007). Thus, a management strategy to determine whether most of the human-excreted FQs enter the environment is vital (Golet *et al.*, 2003). One of the ways that these compounds are introduced into the environment is through the use of this sludge as fertilizer in forest soils (Andreu *et al.*, 2007; Turiel *et al.*, 2006), a use which can affect soil-dwelling organisms (Nakata *et al.*, 2005). Additionally, the pollutants contained in the treated water can be deposited in coastal sediments around wastewater marine outfalls. FQs are resistant to microbial degradation and may be persistent in the environment (Mitani and Kataoka, 2006). Thus, to prevent the growth of resistant bacteria, monitoring FQ concentrations in different kinds of environmental samples is necessary (Ferding *et al.*, 2005).

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Abbreviations used: FD, fluorescence detection; FQs, fluoroquinolones; HTAB, hexadecyltrimethylammonium bromide; MAE, microwave-assisted extraction; MAME, microwave-assisted micellar extraction; NaLS, sodium dodecyl sulfate; POLE, polyoxyethylene 10 lauryl ether; PPCPs, pharmaceuticals and personal care products; WWTPs, wastewater treatment plants.

The extraction of analytes from solid samples is much more complicated than extraction from liquids, especially in environmental applications, because the solute–matrix interactions are very difficult to predict and overcome (Camel *et al.*, 2001). There are several methods of extraction, but the conventional procedure is Soxhlet extraction. Other frequently used techniques include sonication and automated Soxhlet extraction, but supercritical fluid extraction (Garrigós *et al.*, 2004), accelerated solvent extraction (Mielke *et al.*, 2004), which is also known as pressurized solvent extraction, pressurized fluid extraction, pressurized fluid extraction microwave-assisted extraction (PFE-MAE) (Barriada Pereira *et al.*, 2004) or focused microwave-assisted extraction (Morales Muñoz *et al.*, 2005) have been used more recently.

Among the methods available for solid samples, we selected MAE. This method represents a viable alternative to other methodologies because it requires much lower volumes of organic solvents, reduces extraction time and allows the preparation of multiple samples in one step (Esteve-Turillas *et al.*, 2004). The main advantage of MAE over other procedures is the replacement of organic solvents by micellar media as the extraction agent (Cueva-Mestanza *et al.*, 2008). Microwave-assisted micellar extraction (MAME) can be used prior to sample introduction into the chromatographic system because the micellar media or surfactants are compatible with the mobile phase used in liquid chromatography (LC). Besides being non-toxic, biodegradable and less expensive than organic solvents, the surfactants can enhance the signal of many compounds in fluorescence detection, which has been demonstrated for FQs in a previous paper (Montesdeoca-Esponda *et al.*, 2009).

In this work, we optimized the parameters affecting the microwave extraction process (i.e. extraction time, power, volume and concentration of surfactant) to achieve the maximum extraction efficiency for five FQs: levofloxacin, norfloxacin, ciprofloxacin, enrofloxacin and sarafloxacin. This optimization process was carried out using LC with fluorescence detection (FD) because it is a less expensive and simpler technique than LC with mass spectrometry (MS) detection. However, once the extraction process was optimized, analysis was performed using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) because of its higher sensitivity and selectivity. We evaluated the FQ concentrations in sediments from several coastal points along the Gran Canaria island (Spain) that had not been studied previously and in sludge samples from a wastewater treatment plant.

Experimental

Reagents

FQs (Table 1, numbers identify compounds in chromatograms) were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions (1000 µg mL^{-1}) were prepared in methanol and stored in glass-stoppered bottles at 4°C prior to use. The mobile phase was prepared with water and methanol. Ultrahigh-quality water was provided by a Milli-Q system (Millipore, Bedford, MA, USA). LC-grade methanol and LC-MS-grade methanol and water were obtained from Panreac Química (Barcelona, Spain) and filtered through a Millipore 0.22 µm acetate membrane filter (Bedford, MA, USA) prior to use. The glacial acetic acid used to adjust the pH of the LC-FD mobile phase was obtained from Scharlau Chemie S.A. (Barcelona, Spain), and the LC-MS mobile phase contained formic acid and ammonium formate supplied by Panreac Química (Barcelona, Spain). The surfactants tested, including polyoxyethylene 10 lauryl ether (POLE), hexaethylene glycol monododecyl ether ($C_{12}E_6$), hexadecyltrimethylammonium bromide (HTAB) and sodium dodecyl sulfate (NaLS), were obtained from Sigma-Aldrich (Madrid, Spain). Solutions were prepared in 5–15 mL of Milli-Q water at surfactant concentrations of 0.5–5% (v/v). The 0.45 µm, syringe-driven filter used for purification of the extract solutions was provided by Scharlau Chemie S.A. (Barcelona, Spain).

Instrumentation

The microwave oven used for the extraction was a multiwave with a 6 EVAP rotor and 6 MF100 vessels (Anton Paar, Graz, Austria). The chromatographic systems were provided by Varian (Varian Inc., Madrid, Spain). A Varian Prostar was used in the optimization of the extraction conditions and consisted of a pump, an autosampler, a column valve module with an internal oven and a fluorescence detector. A 320-MS LC/MS/MS system (triple quadrupole) equipped with an electrospray ionization (ESI) interface was used for sample analysis.

Chromatographic separation and detection

Separation of FQs by the LC-FD system was achieved on a 150 × 3.9 mm (4 µm particle size) Waters Symmetry C_{18} column (Waters Cromatografía, Barcelona, Spain) with an isocratic mobile phase consisting of methanol–water (15:85 v/v) adjusted to a pH of 2.5 at a flow rate of 1 mL min^{-1}. The injected sample volume was 30 µL, and the fluorescence detector was operated at an excitation wavelength of 280 nm and an emission wavelength of 450 nm. The retention times for each compound are listed in Table 2. A Varian Pursuit UPS 2.4 C_{18} 50 × 2.0 mm (2.4 µm particle size) stationary phase and a mobile phase consisting of methanol:water with 0.2% (v/v) formic acid and 5 mM ammonium formate (15:85 v/v) were used for LC-MS/MS analysis. The FQs were eluted at a flow rate of 0.2 mL min^{-1}, and the sample volume injected was 10 µL under pick-up conditions. The electrospray ionization parameters were fixed as follows: 60°C housing temperature, 345×10^3 Pa nebulizing gas pressure, 207×10^3 Pa drying gas pressure, 0.6 kV shield voltage and 5 kV needle voltage. Nitrogen was used as the nebulizing and drying gases, with argon as the collision gas. The detailed mass spectrometer parameters for each FQ are shown in Table 2 and were optimized by directly infusing a standard solution of each analyte (1 mg/L) into the detector at a flow rate of 10 µL/min. Analyses were carried out in multiple reaction monitoring mode, and the detector was fixed at maximum Extended Dynamic Range (EDR) with peak mass widths of 2 and 1.5 amu for the first and third quadrupoles, respectively. The dwell time for all transitions was 0.1 seconds.

Preparation of spiked samples

The sediment samples were sifted to a particle size of less than 300 µm and spiked with a solution of FQs prepared in methanol to obtain a final concentration of 2 µg g^{-1} for each analyte. The samples were stirred and air-dried for 24 h in the dark at room temperature to obtain dry and homogeneous samples. Before sample preparation, analysis of blank marine sediments showed that they did not contain any signals in the chromatogram for the FQs under investigation.

Coastal marine sediments and sludge samples

For the analysis of real samples, we selected a marine outfall located in the southern region of Gran Canaria island that discharges the depurated waters from a sewage treatment plant. Sediments around these locations were taken at different distances from the coast, between 250 m (sample 4) and 1000 ms (sample 1). Sample locations are shown in Fig. 1. Additionally, we analysed two sewage sludge samples procured directly from a wastewater treatment plant. All samples were dried in the same manner as the spiked samples and analysed in triplicate.

Table 1. List of fluoroquinolones, identification number, abbreviation and chemical structure

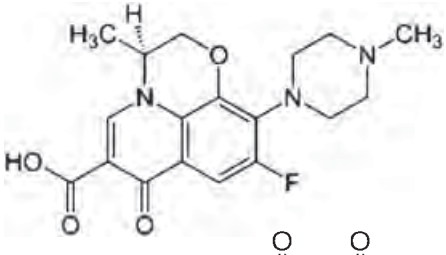
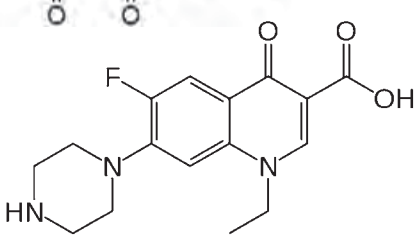
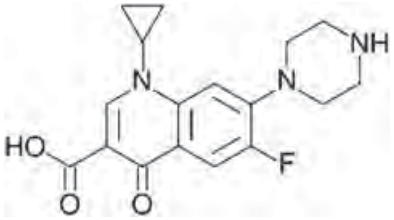
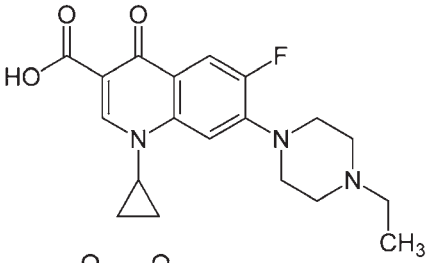
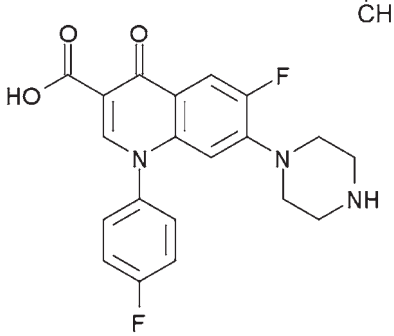
Compound	Identification number	Abbreviation	Structure
Levofloxacin	1	LEVO	
Norfloxacin	2	NOR	
Ciprofloxacin	3	CIPRO	
Enrofloxacin	4	ENRO	
Sarafloxacin	5	SARA	

Table 2. Mass spectrometer parameters for fluoroquinolones detection

	Retention time (min)	Precursor ion (*m/z*)	Capillary voltage (V)	Quantification ion (collision potential, V)	Confirmation ion (collision potential, V)
LEVO	5.3	362.0	56.0	318.0 (17.0)	261.0 (24.0)
NOR	5.5	320.1	56.0	301.9 (19.0)	230.8 (37.0)
CIPRO	6.1	332.1	52.0	313.9 (19.0)	230.8 (36.0)
ENRO	9.6	360.2	60.0	342.0 (19.5)	316.0 (16.5)
SARA	13.5	386.1	52.0	299.0 (21.5)	342.0 (15.5)

MAME procedure

Two grams of the spiked sediment samples were transferred to the MAME vessels. The extractant agent was added to the mixture, and the closed vessels were subjected to the MAME process. Once the extraction time had elapsed, the vessels were allowed to cool for 10 min in the presence of the microwave fan and for an additional 10 min at room temperature outside of the microwave oven before they were opened. The extracted solution was filtered through a 0.45 µm syringe filter. The experimental design for optimization was obtained using Statgraphics Plus software 5.1, and statistics were calculated with SPSS 17.0.

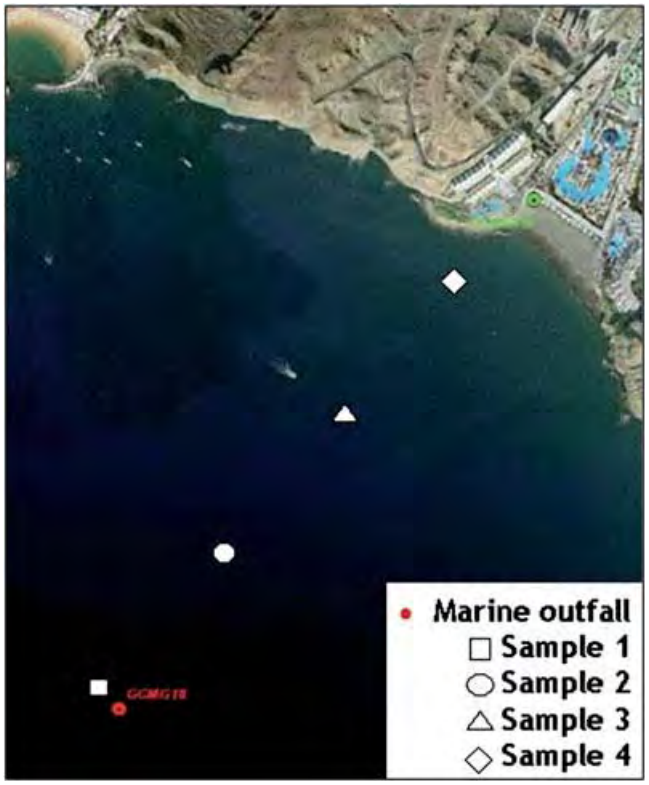


Figure 1. Location of coastal sediments close to marine outfall.

Results and discussion

Optimization of extraction conditions

In a MAME procedure, there are several experimental variables that affect efficiency, and all of them must be evaluated to achieve maximum recoveries of the target analytes. To simplify and decrease the cost of the optimization process, we employed LC with fluorescence detection in this step of the study.

To evaluate the influence of each variable on the extraction process and the variable correlations to each other, an experimental design of 2^4 was used that consisted of two levels: power (100–500 W), extraction time (2–10 min), surfactant volume (5–15 mL) and surfactant concentration (0.5–5% v/v). This experimental design was tested with four surfactants to determine the better extractant: two non-ionic surfactants (POLE and $C_{12}E_6$), one cationic surfactant (HTAB) and one anionic surfactant (NaLS). In the first experiments, we observed that the amount of analyte extracted using NaLS was very low, so this surfactant was not included in subsequent studies.

The highest signal responses were obtained with HTAB, so this cationic surfactant was used in the subsequent optimization of the experimental conditions. Table 3 shows the different correlations between the variables and their influence on the recoveries. We can draw some conclusions from this preliminary study. For example, a high power value (and consequently a high temperature) seemed to provide good extraction efficiency because an increase in temperature facilitates the desorption of analytes from the matrix and improves their solvent solubility and sample penetration. However, longer extraction times, which also favour higher temperatures inside the vessel, provided better results than short extraction times. The variables that have the greatest impact on recoveries are power and surfactant concentration, while those that show the strongest correlation are power/time and power/surfactant concentration. Thus, we investigated each pair of variables separately, using two 3^2 factorial designs (Table 4). The surfactant volume seems to have little influence and was maintained at 15 mL based on the results of the preliminary study.

In the surface response obtained from the first experiment (power/time, Fig. 2), the fluorescence signal was observed to improve with an increase in both power and time. The same trend was observed in the second experiment (surfactant concentration/power, Fig. 3), in which the maximum recoveries

Table 3. Relevant variables and correlation between variables using HTAB as extractant[a]

Correlation	Levofloxacin	Norfloxacin	Ciprofloxacin	Enrofloxacin	Sarafloxacin
Power	0.463	0.577	0.553	0.472	0.532
Time	0.157	0.339	0.346	0.024	0.045
Volume	−0.392	−0.251	−0.088	−0.308	−0.364
Concentration	0.449	0.968	0.331	0.521	0.465
Power/concentration	−0.263	−0.265	−0.233	−0.327	−0.330
Time/concentration	−0.080	−0.136	−0.129	−0.015	−0.024
Power/time	−0.083	−0.255	−0.245	−0.013	−0.028
Volume/concentration	0.214	0.097	0.031	0.198	0.205
Volume/time	0.068	0.094	0.033	0.008	0.018
Volume/power	0.0223	0.183	0.059	0.173	0.245

[a]The maxima correlations are +1 and −1.

Table 4. Factorial experimental designs 3^2 for the optimization of extraction time/power and concentration surfactant/power

Run	Power (V)	Time (min)	Run	Concentration (%)	Power (V)
1	300	10	1	5	500
2	100	10	2	5	300
3	100	15	3	3	100
4	300	5	4	1	300
5	500	15	5	3	300
6	100	5	6	1	100
7	500	10	7	1	500
8	300	15	8	3	500
9	500	5	9	5	100

Fixed parameters:

- surfactant → HTAB;
- surfactant concentration → 5%;
- surfactant volume → 15 mL.

Fixed parameters:

- surfactant → HTAB;
- surfactant volume → 15 mL;
- time → 15 min.

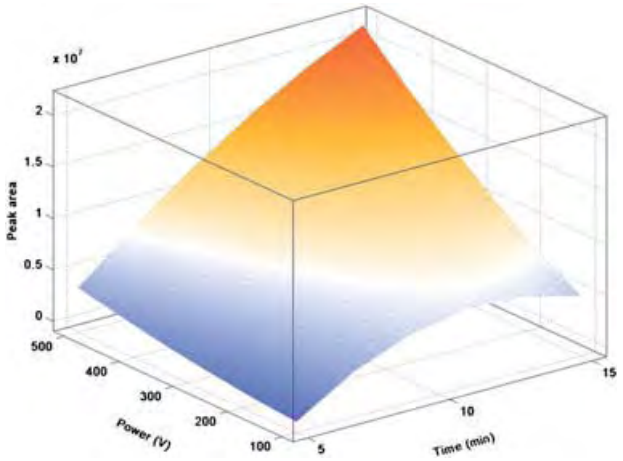


Figure 2. Response surface for the effect of power and extraction time on the extraction of enrofloxacin (2 µg g^{-1}) using MAME-LC-FD procedure.

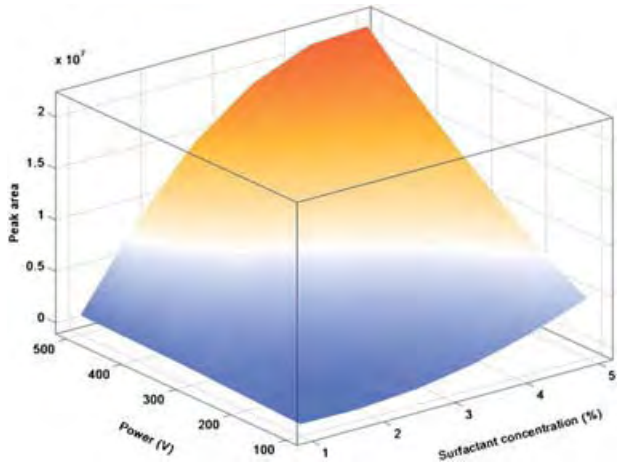


Figure 3. Response surface for the effect of concentration and power on the extraction of enrofloxacin (2 µg g^{-1}) using MAME-LC-FD procedure.

were achieved with higher values of the indicated parameters. In both cases, an attempt was made to extend the experiment to test higher values of power, time and surfactant concentration to determine whether the fluorescence signal would improve. However, when powers higher than 500 W or extraction times exceeding 15 min were applied, the response decreased because the compounds degraded. Conversely, if surfactant concentrations higher than 5% (v/v) were used, the filtration of the extract was very tedious. Based on these results, we chose the following conditions for sample extraction: 500 W power, 15 min extraction time, 15 mL surfactant volume and 5% (v/v) surfactant concentration prepared in Milli-Q water.

To evaluate extraction efficiency and precision, six replicate samples spiked with 2 ng g^{-1} of each analyte were subjected to the optimized MAME-LC-FD procedure. The recoveries were an average of the six determinations and ranged from 73.2% for norfloxacin to 95.6% for enrofloxacin. Reproducibility was expressed as a relative standard deviation (RSD) in a percentage and was satisfactory, with RSDs less than or equal to 8% for all FQs.

MAME procedure coupled with LC-MS/MS

In environmental samples, the concentrations of FQs are very low, and they can be difficult to quantify using optical detectors like fluorescence, which usually has quantification limits higher than mass spectrometry detectors. Therefore, when the extraction process was optimized, we studied the combination with LC-ESI-MS/MS for the determination of target analytes in solid samples.

We were uncertain whether the surfactant was compatible with this analytical system, but direct injection of HTAB determined that no degradation fragments interfered with the quantification or confirmation of the target analyte ions.

Analytical parameters

To determine the analytical quality of the proposed method using MAME and LC-MS/MS, linearity, precision, limits of detection (LOD) and quantification (LOQ) and recoveries were studied. Each point in the calibration curves corresponds to the mean values obtained from three area measurements. Linearity was evaluated for each compound from 0.5 to 500 ng g^{-1}, over

which all compounds had correlation coefficients higher than 0.992. LODs (0.19–0.55 ng g^{-1}) and LOQs (0.64–1.85 ng g^{-1}) were calculated from the signal-to-noise ratio of the individual peaks, assuming minimum detectable signal-to-noise levels of 3 and 10, respectively (Table 5).

Determination of fluoroquinolones in coastal marine sediments and sludge samples

Once the extraction conditions of the method were optimized and the surfactants were shown to be effective as extractants with excellent analytical characteristics using LC-MS/MS, we applied the method to the analysis of solid samples of environmental interest, specifically, coastal marine sediments and sewage sludge samples.

First, we analysed the samples from the marine outfall. Four of the five FQs under investigation were detected in all of the sediment samples, and almost all could be quantified. As seen in Table 6(a), the concentrations were higher in sample 1 (next to the outfall) and lower in sample 4 (closer to the beach), which is a logical and expected trend. The concentrations ranged from 0.69 ng g^{-1} (ciprofloxacin) to 34.3 ng g^{-1} (norfloxacin). Figure 4 shows the chromatogram and mass spectra corresponding to sample 1, which was prepared using the MAME- LC-MS/MS procedure.

Two sewage sludge samples were procured directly from a wastewater treatment plant for analysis. The FQ concentrations found in these samples were relatively high, between 3.43 and 206.1 ng g^{-1}. The samples were taken on two consecutive days, and we observed a slightly lower concentration in the sample from the second day (Table 6a), perhaps due to bacterial degradation or dilution from the incursion of other waters.

Table 5. Detection and quantification limits, and reproducibility obtained for each FQ using MAME-LC-MS/MS procedure

	LOD[a] (ng g^{-1})	LOQ[b] (ng g^{-1})	RSD[c] (%)
LEVO	0.19	0.64	4.06
NOR	0.24	0.79	7.19
CIPRO	0.15	0.49	6.92
ENRO	0.36	1.19	8.00
SARA	0.55	1.85	4.40

[a]Detection limits are calculated as signal-to-noise ratio of 3.
[b]Quantification limits are calculated as signal-to-noise ratio of 10.
[c]Relative standard deviation ($n = 6$).

Matrix effect

When using the LC-MS/MS system, ESI mode was used as ionization source because it allows accurate and sensitive analysis. ESI, a low-energy ionization source, generally does not cause the fragmentation of molecular ions and is therefore recommended for such substances like pharmaceuticals (Al-Odaini *et al.*, 2010). However, the matrix effect caused by co-elution compounds during chromatographic separation is the common drawback of the ESI source that could to lead to relatively high detection limits and decreased reproducibility. Therefore, the quantification of pharmaceuticals in complex matrices requires the evaluation of matrix effect.

Because we developed the MAME-LC-FD procedure, we also used it to analyse the samples to confirm the MS results. The calibration curves were made in the same manner as in LC-MS/MS, in which each point corresponds to the mean value obtained from three area measurements for concentrations of each FQ between 0.1 and 500 ng g^{-1}. The LODs (signal-to-noise ratio of the individual peaks, assuming a minimum detectable signal-to-noise level of 3) ranged from 0.39 to 0.85 ng g^{-1}, and the LOQs (signal-to-noise level of 10) were between 1.31 and 2.83 ng g^{-1}.

Similar to the MS analysis, four of the five FQs under study were detected in all of the samples, but some were below the quantification limits and could not be measured (Table 6b). Analysis of the marine sediments yielded FQs concentrations between 1.73 and 27.9 ng g^{-1}, while those found in the sludge samples were in the range of 2.98–195.6 ng g^{-1}.

Table 6. FQs concentrations measured in marine sediments and sludge samples using (a) MAME-LC-MS/MS and (b) MAME-LC-FD procedure[a]

	Marine sediments (ng g^{-1})				Sludges (ng g^{-1})	
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2
(a)						
LEVO	8.12	2.39	0.69	<LOQ	24.2	20.4
NOR	10.62	2.27	1.30	0.81	13.4	9.57
CIPRO	34.3	14.5	4.45	1.96	206.1	200.8
ENRO	13.7	2.85	<LOQ	<LOQ	5.90	3.43
SARA	ND[b]	ND	ND	ND	ND	ND
(b)						
LEVO	6.11	2.90	<LOQ	<LOQ	19.5	16.9
NOR	8.26	1.73	<LOQ	<LOQ	10.7	8.33
CIPRO	27.9	11.2	2.73	<LOQ	195.6	193.8
ENRO	10.4	2.18	<LOQ	<LOQ	4.94	2.98
SARA	ND	ND	ND	ND	ND	ND

[a]Mean of three determinations.
[b]ND, not detected.

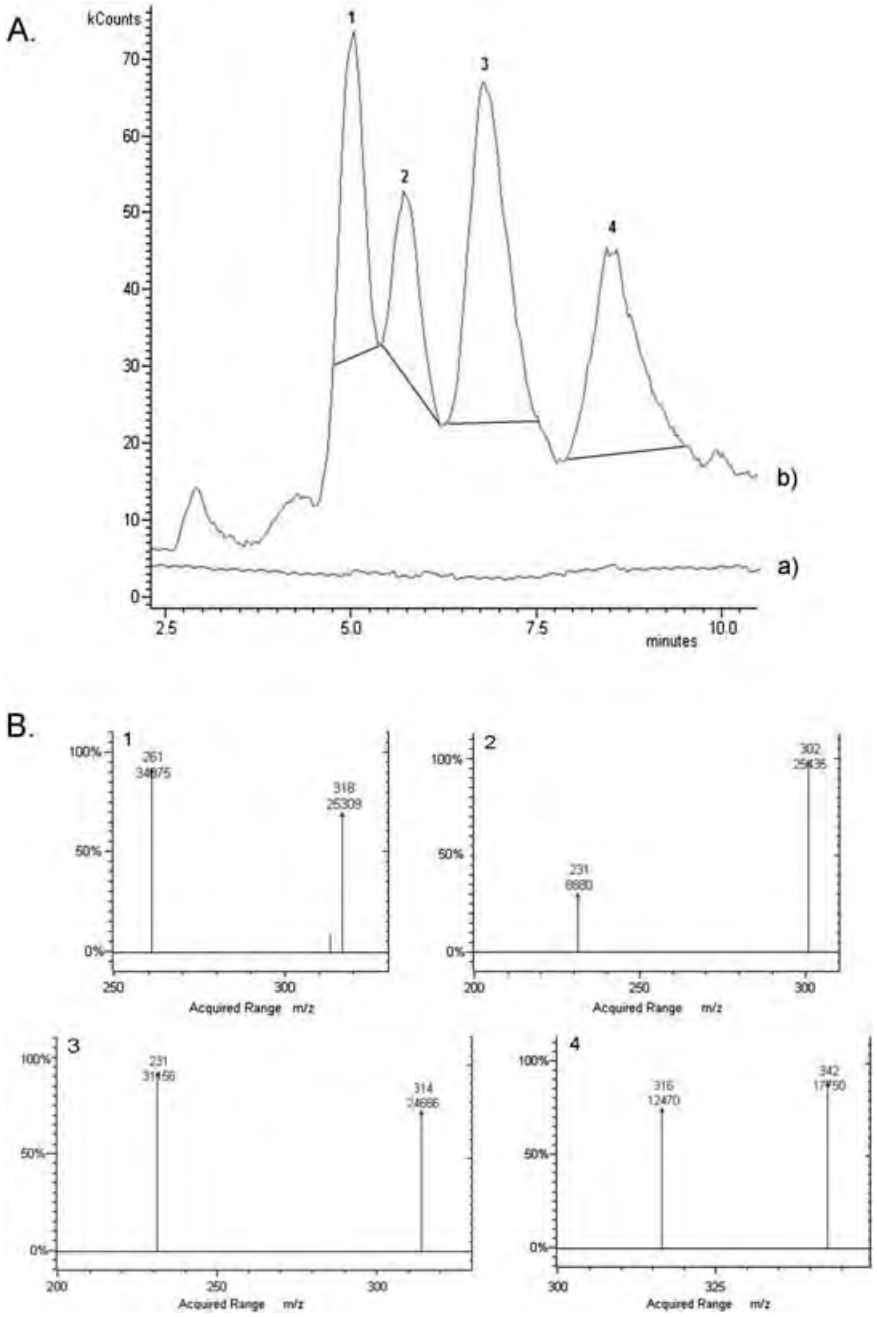


Figure 4. Blank run (Aa), chromatogram (Ab) and mass spectra (B) corresponding to FQs found in marine sediment (sample1) using MAME-LC-MS/MS procedure. Numbers refer to Table 1 and detection parameters are specified in Table 2.

The RSDs between these confirmation measurements and those obtained with LC-MS/MS were consistently lower than 20%, except for ciprofloxacin in sample 3 of the coastal sediments, which had an RSD of 34%.

Conclusions

The use of surfactants as extractants instead of the conventional organic solvents was successfully employed with microwave-assisted extraction and LC-MS/MS for the determination of FQs in coastal marine sediments and sewage sludge samples. In addition to providing the sensitivity needed for the determination of these compounds in real samples, this method is an environmentally friendly process because a biodegradable extractant is used. The concentrations measured in the sludge samples (e.g. approximately 200 ng g^{-1} for ciprofloxacin) indicate that an appreciable concentration of the FQs that enter wastewater treatment plants accumulate in these solid products. The marine coastal sediment samples were found to absorb the antibiotics dissolved in the effluent of the marine outfall; we also observed that their accumulation was higher closer to the outfall and lower nearer to the coast.

Given that the differences between the measurements obtained from LC-FD and LC-MS/MS were lower than 20% for

all of the samples except one, we can confirm that both the MAME-LC-FD and MAME-LC-MS/MS methods are valid and reliable for the determination of these antibiotics in solid samples. However, given that the typical low concentrations in environmental samples require detection systems with high sensitivity, we propose the combination of this developed extraction procedure with mass spectrometry detection.

Acknowledgement

This work was supported by funds provided by the Spanish Ministry of Education of Science, Research Project CTQ 2010–20554. Sarah Montesdeoca Esponda thanks the Canary Agency of Investigation, Innovation and Information Society for her PhD Student Grant.

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III.2. Productos de cuidado personal: Benzotriazoles estabilizadores de luz UV

III.2.1 Extracción en fase sólida en modo "en línea" acoplada a cromatografía liquida de ultra resolución con detector de espectrometría de masas en tándem para la determinación de benzotriazoles estabilizadores de luz UV, en muestras marinas costeras y aguas residuales.

En este primer trabajo que presentamos para la determinación de BUVSs en muestras medioambientales, se empleó la SPE en modo "en línea", acoplada esta vez a una de las técnicas más avanzadas en cromatografía líquida, la UHPLC, con detección por espectrometría de masas.

Además de la optimización, desarrollo y aplicación de la metodología, realizamos una comparación con la SPE convencional (no "en línea"), pudiendo destacarse numerosas ventajas en el proceso "en línea", tales como mayor preconcentración, mayor repetibilidad, menor tiempo empleado y prácticamente ninguna manipulación de la muestra, lo que la convierte en una técnica muy sencilla desde el punto de vista del analista. Además, el uso de UHPLC permite obtener un cromatograma para los siete analitos estudiados de menos de un minuto.

El método proporcionó excelentes recuperaciones y repetibilidades para casi todos los BUVSs, existiendo algunos problemas de extracción para el compuesto más apolar. Los límites de detección conseguidos también fueron muy satisfactorios, entre 0.6 and 4.5 ng· L^{-1}.

El análisis de muestras de agua de mar tomadas alrededor de la isla de Gran Canaria, reveló la presencia de dos de los compuestos (UV P y UV 360) en concentraciones entre 2.8 y 5.2 $ng \cdot L^{-1}$.

Por otro lado, seis de los siete BUVSs analizados fueron detectados en muestras de aguas residuales, en el rango de 4.0-13 $ng \cdot L^{-1}$.

Este trabajo ha sido publicado en la revista *Analytical and Bioanalytical Chemistry 403 (2012) 867-876.*

Anal Bioanal Chem (2012) 403:867–876
DOI 10.1007/s00216-012-5906-x

ORIGINAL PAPER

On-line solid-phase extraction coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection for the determination of benzotriazole UV stabilizers in coastal marine and wastewater samples

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Received: 19 December 2011 / Revised: 17 February 2012 / Accepted: 24 February 2012 / Published online: 13 March 2012

Abstract Benzotriazoles are a group of UV absorbing compounds considered emerging contaminants that are used in different personal care products, and therefore, it is of high interest to develop sensitive and fast methods for investigating their presence in the environment. In this work, we present the development and application of a novel method based on on-line solid-phase extraction coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection (SPE-UPLC-MS/MS) for the determination of seven benzotriazole UV stabilizers (BUVSs) in coastal marine and wastewater samples. This process is compared with a conventional off-line SPE procedure followed by UPLC-MS/MS. The parameters affecting the performance of the sample preparation and determination processes were evaluated. The results indicate that the on-line procedure provides for better sensitivity and reproducibility and is faster and easier than the off-line procedure. The detection limits and quantification limits achieved were in the range of 0.6–4.1 ng L^{-1} and 2.1–14 ng L^{-1} and relative standard deviation between 6.2 and 10 %. The developed method was applied to coastal marine and wastewater samples from Gran Canaria Island (Spain). All of the BUVSs studied were detected in the samples from wastewater treatment plants and two were found in the seawater samples (UV P in the range of 2.8–4.4 ng L^{-1} and UV 360 between 3.6 and 5.2 ng L^{-1}).

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Keywords Benzotriazole UV stabilizers · On-line SPE · UPLC-MS/MS · Water analysis

Introduction

One of the rising areas of research on environmental samples is the determination of so-called emerging contaminants. Researchers often find these contaminants in environmental water samples because they are not completely removed by wastewater treatment plants (WWTPs). Therefore, the identification and quantification of these products is important [1, 2].

The continuous generation of emerging pollutants through anthropogenic activities allows them to pseudo-persist in the environment [3]. Trace analysis of emerging contaminants is achieved with advanced analytical techniques such as liquid chromatography coupled with mass spectrometry (LC-MS). A recent development in LC is the use of the ultra-performance liquid chromatography (UPLC) that allows for higher pressures. In addition, UPLC provides narrow peaks, improves chromatographic separation and leads to shorter analysis time [4–7].

However, in very dilute water samples, such as seawater, a preconcentration procedure is indispensable. Solid-phase extraction (SPE) is widely used in the environmental analytical field because it extracts and pre-concentrates in a single step. Nevertheless, this technique presents some disadvantages such as an increased chance of losses during sample handling and the requirement of large volume samples.

On-line SPE strategies coupled to an LC system appear to solve these drawbacks. The benefits of this on-line extraction system versus the conventional SPE methodology are numerous and important. First, the tedious conventional procedure is automated, and the operator only needs to place

the sample vial in the apparatus. This automation minimises sample loss or contamination during handling and improves the reproducibility of the analysis. Another advantage is the reduction of both the required sample volume and time of the analysis.

When this on-line SPE methodology is coupled with UPLC-MS/MS, the benefits are even greater because the advantages of the on-line SPE are combined with the speed and effectiveness of the chromatographic separation under ultra-pressure conditions and the precision and selectivity of mass spectrometry detection. This combination has been recently used by several authors to determine different types of compounds [8–14]. Compared to the conventional off-line SPE and LC separation systems, these improvements are considerably valuable for analysing emerging compounds in complex environmental samples.

Among the extensive group of the emerging compounds, UV filters used in sunscreens, cosmetics and other personal care products (PCPs) have increased in interest due to their presence in environmental waters. A majority of these products are lipophilic compounds, and due to their use in many PCPs, these compounds can enter aquatic environments.

Table 1 list the seven benzotriazole UV stabilizers (BUVSs) selected for this study. BUVSs are UV stabilizers because they have a phenolic group attached to the benzotriazole structure that absorbs the full spectrum of UV light, UV-A (320–400 nm) and UV-B (280–320 nm) [15].

Fortunately, the negative effects of these BUVSs have been previously studied because they also are used for several other purposes, including additives in food-contact plastics and stabilizers in a variety of building products. For example, in 1989, a contact allergy to UV P (Tinuvin P) was discovered in a patient with wrist dermatitis caused by a plastic watch strap [16]. Although these compounds are considered of low toxicity and health hazard to humans and some of them have no estrogenic activity [17, 18], it has been demonstrated that benzotriazole derivatives are mutagenic in bacterial systems and toxic in plants [19].

The compounds studied in this paper were approved in the Cosmetics Regulation of the European Community [20] and contemplated by the International Nomenclature of Cosmetic Ingredients [21] and the International Fragrance Organization [22]. However, some of these compounds have also been identified by the EPA List of Inert Pesticide Ingredients as inert ingredients of unknown toxicity [23].

However, little information is available regarding the extent of contamination and the concentrations of these organic UV filters, especially concerning the most lipophilic compounds. The BUVSs have a potential for bioaccumulation in aquatic ecosystems because their log K_{ow} values range from 7.0 to 10.0 [24]. Relevant concentrations of some of these seven BUVSs have been found in different marine organisms like fish [25] lugworms, oysters, clams, crabs, ducks, sharks [15], and even in birds and mammals [24, 26]. The presence of some BUVSs has also been reported in sediments samples [15, 27, 28], sewage sludges [29], wastewater [28, 30], and surface waters [28].

As far as we could investigate, the direct contributions due to, for example, beach users have not been measured. In order to determine and to control the persistence of these emerging pollutants in different waters samples, we optimised a method using on-line SPE-UPLC-MS/MS for the determination of seven BUVS compounds in different kinds of samples. After optimisation, the proposed method was applied to seawater samples and wastewater samples from seven wastewater treatment plants from Gran Canaria Island (Canary Island, Spain). Additionally, in order to emphasise the advantages of on-line SPE-UPLC-MS/MS method, we compared this method with an off-line procedure that was developed and optimised.

Experimental

Reagents

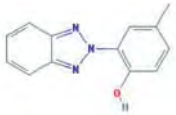
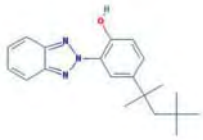
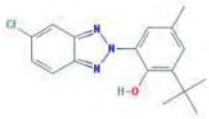
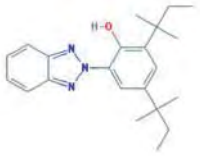
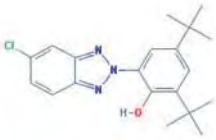
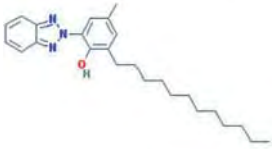
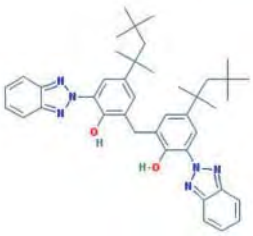
The benzotriazoles (Table 1) were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions (500 $\mu g \cdot mL^{-1}$) were prepared in methanol and stored in glass-stoppered bottles at 4 °C prior to use. Working solutions were prepared daily in ultrapure water provided by a Milli-Q system (Millipore, Bedford, MA, USA). The mobile phase was prepared with LC-MS grade methanol and formic acid obtained from Panreac Química (Barcelona, Spain). The solvents used for the on-line SPE were LC-MS-grade methanol, LC-MS-grade water, hexane and acetone, and were purchased from Panreac Química.

Instrumentation

The preparation and analysis system included an ACQUITY Quaternary Solvent Manager to load samples and wash and recondition the extraction column, an ACQUITY Binary Solvent Manager for the elution of the analytes, a column manager, a 2777 autosampler with 25 and 5,000 μl syringes and trays for 2 and 20 mL vials, and a ACQUITY tandem triple quadrupole mass spectrometer with an electrospray ionisation interface. All components (from Waters, Madrid, Spain) are controlled by MassLinx Mass Spectrometry Software. Both on- and off-line extract detections were made with this instrumentation because when the on-line SPE is not being used, it can operate as a regular UPLC-MS system.

For the SPE, we used a 200-mg Oasis HLB cartridge for the off-line process and an Oasis HLB Direct Connect HP Column (2.1×30 mm, 20 μm) for the on-line process from Waters (Madrid, Spain).

Table 1 List of BUVSs, IUPAC name, chemical structure and pK_a and log K_{ow} values

Compound	IUPAC name	Chemical structure	pk_a	Log K_{ow}
UV P	2-(benzotriazol-2-yl)-4-methylphenol		8.15 ± 0.43	2.99
UV 329	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl) phenol		8.07 ± 0.45	6.21
UV 326	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol		9.31 ± 0.48	5.55
UV 328	2-(benzotriazol-2-yl)-4,6-bis(2-methybutan-2-yl) phenol		8.85 ± 0.50	7.25
UV 327	2,4-ditert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol		9.23 ± 0.48	6.91
UV 571	2-(benzotriazol-2-yl)-6-dodecyl-4-methyl phenol		-	8.95
UV 360	2-(benzotriazol-2-yl)-6-[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl)phenyl]methyl]-4-(2,4,4-trimethylpentan-2-yl)phenol		7.62 ± 0.50	12.46

pK_a values obtained from [32] and log K_{ow} values obtained from [33]

Chromatographic conditions and mass detection

An ACQUITY UPLC BEH Waters C_{18} column (50 mm× 2.1 mm, 1.7 μm particle size, Waters Chromatography, Barcelona, Spain) at 40 °C under a 100 % methanol isocratic mobile phase adjusted to pH 2.5 with 0.1 % (*v*/*v*) formic acid at a flow rate of 0.9 mL min^{-1} was used for the elution of BUVSs using UPLC-MS/MS detection. The sample volumes injected were 10 μL and 5 mL in off-line SPE and on-line SPE, respectively. The electrospray ionisation parameters were fixed as follows: capillary voltage at 3 kV, cone voltage at 50 V, source temperature at 120 °C, desolvation temperature at 450 °C, and desolvation gas at 800 L/h. Nitrogen was used as the desolvation gas, and argon was employed as the collision gas. The detailed MS/MS detection parameters for each BUVS are presented in Table 2 and were optimised by direct injection of a 1 mg L^{-1} standard solution of each analyte into the detector at a flow rate of 10 μl/min. Under these conditions, UPLC-MS/MS allows for the chromatographic elution of the seven compounds studied in less than a minute.

Table 2 Mass spectrometer parameters for benzotriazoles detection

Compound	Precursor ion (*m/z*)	Cone voltage (V)	Quantification ion (collision potential, V)	Confirmation ion (collision potential, V)
UV P	226.2	40	107.1 (20)	120.1 (20)
UV 329	324.2	50	57 (25)	212.2 (25)
UV326	316.3	40	260.2 (20)	107.1 (25)
UV 328	352.3	50	71 (30)	282.2 (20)
UV 327	358.3	60	302.3 (20)	246.1 (30)
UV 571	394.3	50	226.2 (20)	120.1 (30)
UV 360	658.6	40	336.3 (25)	224.2 (35)

Extraction process

For the optimisation of the on-line and off-line extraction process, ultrapure water was spiked with a mixture of 1 ng·mL^{-1} of each BUVS. It has been reported that the most lipophilic compounds can be lost during the sample pre-treatment due to adsorption on the glass flask [30]. Therefore, a 10 % (*v*/*v*) of methanol was added to avoid this loss.

Blanks were previously run to confirm the absence of carryover, and three replicates of each water sample were prepared and analysed with the UPLC-MS/MS system. Once all the parameters that affect both on- and off-line extractions were optimised, the corresponding extraction efficiencies were calculated. Statistical tests were performed with SPSS 11.0 (Chicago, IL, USA).

On-line SPE

The on-line SPE system can utilise two extraction columns in a parallel manner. When a sample is being eluted from one of the extraction column, the next sample can be loaded onto the other column. Therefore, a quaternary pump is necessary to load the sample and carry out the clean-up and a binary pump or chromatographic pump elutes the compounds retained on the extraction column using the same mobile phase employed in the chromatographic elution. This quaternary pump works at a flow rate of 2 mL·min^{-1} for 4 min using water (0.1 % formic acid; phase A) to load the sample. Once the sample is loaded, the time needed to run and the clean-up of impurities is between 0 and 3.8 min using a water/methanol (70:30, *v*/*v*) mixture (phase B and C, respectively) at flow rate of 0.01 mL·min^{-1}. Finally, while the sample is being eluted by the binary pump, the extraction column is washed at 2 mL·min^{-1} with a methanol/acetone/hexane (1:1:1, *v*/*v*) mixture (phase D) to prepare for the next sample. This exhaustive wash at high pressure allows for a better cleaning of the extraction columns than in the off-line SPE and reduces carryover. A Waters Corporation application note [31] demonstrated that the extraction columns gave good results after 500 injections.

Off-line SPE

Cartridges were conditioned before each run with 5 mL of methanol and 5 mL of Milli-Q water at a flow rate of 5 mL min^{-1}. Then, the sample was passed through the cartridge at a flow rate of 10 mL min^{-1}. A wash step was carried out using 2 mL of Milli-Q water with 5 % (*v*/*v*) methanol to remove impurities retained on the cartridge. Subsequently, the cartridge was dried under vacuum for 10 min, and the retained analytes were eluted with 5 mL of methanol at a low flow rate (~1 mL min^{-1}).

Sample collection

The seawater samples were collected from six beaches around the Gran Canaria Island (Spain). Two samples were collected from each beach, the east and west margin, at a depth of approximately 1 meter. Moreover, another sample was collected from a different beach to use it as blank for determining recoveries.

Additionally, wastewater samples were collected from seven wastewater treatment plant effluents located on the same island. Both seawater and wastewater samples were taken using 1 L amber glass bottles containing 10 % (*v*/*v*) of methanol to avoid the adsorption of compounds to the glass flask, filtered through a 0.45-μm cellulose acetate filter and analysed immediately after sampling.

Results and discussion

On- and off-line SPE optimisation

In an SPE procedure, several experimental variables, including sample volume, sample pH, the wash step and elution must be evaluated to achieve the maximum extraction efficiency of the target analytes. We used an initial sample volume of 5 mL for on-line SPE and 250 mL for off-line SPE containing 1 ng·mL^{-1} of each BUVS. The off-line elution was achieved with 2 mL of methanol. The elution

step in the on-line SPE is carried out with the chromatographic mobile phase.

pH

The extraction efficiency is strongly determined by the pH and the polarity of the analytes. While the Oasis HLB is a hydrophilic–hydrophobic balance cartridge that is more influenced by the polarity of the analytes, the analyte pH must also be considered.

Thus, four sample pH values were studied: 3.0, 6.0, 9.0 and 12.0. Retention of the compounds is enhanced when they are in the neutral form because they will be lost in the wash if they are ionised. Since their pK_a values range between 7 and 10 (see Table 1), the neutral form will be present at acidic pH values. In fact, we observed that pH 3.0 yielded the best extraction efficiencies (Fig. 1).

In the on-line SPE, the pH of the solvent used to load the sample, i.e. the mobile phase that accompanies the sample through the column extraction must be also optimised. We tested Milli-Q water solutions at different pH values, and the best response was achieved in acidic media.

Sample volume

The volume of sample that passes through the cartridge is another important variable to be optimised. For this study, we choose volumes between 25 and 500 mL for off-line SPE. Logically, a larger sample volume passing through the cartridges results in a larger response signal. However, if we normalise the data obtained from the off-line SPE to consider the preconcentration step, we obtained similar responses over the 25 to 100 mL sample volume range. After 100 mL, a decrease in signal response occurs, probably because the breakthrough volume had been reached, from which the cartridge was not able to retain anymore analyte. Using a sample volume of 100 mL, we obtained a greater chromatographic signal and a preconcentration factor of 50.

For on-line SPE, the maximum injection volume is 20 mL, running four injection cycles with the 5 mL syringe. Thus, we studied 5, 10, 15 and 20 mL (1, 2, 3 and 4 cycles, respectively). When we increase the number of cycles, the analyte signal is enhanced, but the noise is also intensified. Therefore, it is unnecessary to extend the procedure because each cycle takes an additional 4 min and requires more sample volume. Thus, for the optimum sample volume, we choose one cycle of 5 mL.

Clean-up

A wash step prior the elution is necessary to remove the impurities present in the matrix. We performed the extract clean-up using water containing different percentages of an organic solvent, methanol. We needed to find the maximum percentage of methanol that could be passed through the cartridge without analyte loss for the maximum removal of interferences. We observed the loss of analyte at percentages of methanol up to 5 and 30 % in off-line and on-line SPE, respectively. This step is essential when complex matrices like wastewater are analysed.

Elution

After testing 1, 2 and 5 mL of methanol, we choose 2 mL as the optimum volume in off-line SPE because it allows for the elution of all analytes with the maximum preconcentration factor. We also studied the performance of the elution of these 2 mL in two steps of 1 mL each, but no differences were found. In on-line SPE, after the wash step, a valve movement allows for the passage of chromatographic mobile phase through the cartridge to elute the analytes.

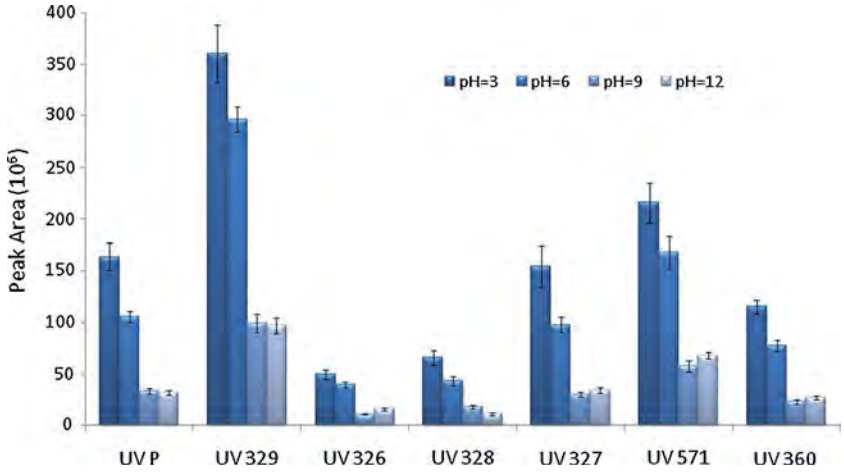


Fig. 1 Relative extraction efficiencies for different sample pH values (Milli-Q water spiked with 1 ng·mL^{-1} of each BUVSs employing on-line SPE-UPLC-MS/MS)

Table 3 Analytical parameters for on-line SPE procedure coupled to UPLC-MS/MS detection

	LOD[a] (ng·L^{-1})			LOQ[b] (ng·L^{-1})			RSD[c] (%)					
							0.001 µg·L^{-1}			1 µg·L^{-1}		
	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater
UV P	0.6	0.9	1.1	2.1	3.0	3.7	7.4	6.6	7.2	6.3	6.5	6.2
UV 329	0.9	1.0	1.0	3.1	3.4	3.4	7.8	8.1	8.3	7.6	7.1	8.3
UV 326	2.3	2.4	2.6	7.8	8.1	8.7	9.5	9.0	10	9.3	6.1	9.4
UV 328	1.3	1.1	1.3	4.4	3.7	4.4	6.5	6.4	6.5	7.3	7.9	8.2
UV 327	1.0	1.3	1.3	3.3	4.5	4.6	9.8	7.9	10	9.8	8.1	10
UV 571	4.1	4.4	4.5	14	15	15	7.9	7.8	8.1	8.3	9.0	7.9
UV 360	1.0	1.1	1.4	3.4	3.8	4.8	8.1	8.0	9.2	7.0	7.7	9.3

	Recovery[d] (%)								
	0.05 ng			0.25 ng			0.5 ng		
	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater
UV P	88±6.1	88±6.5	86±7.0	90±7.3	87±8.8	90±9.0	94±5.7	94±7.1	89±6.9
UV 329	89±6.9	90±6.7	88±7.0	92±7.1	90±7.8	89±8.0	89±7.5	90±7.5	88±8.4
UV 326	86±5.5	85±5.3	83±7.2	91±6.7	88±5.9	90±8.1	91±7.7	90±5.7	90±8.0
UV 328	80±6.7	82±7.3	80±7.2	77±9.1	81±8.8	77±9.0	80±6.1	78±6.6	77±8.1
UV 327	81±8.8	80±10	77±9.7	81±7.7	80±8.3	80±8.5	83±6.0	85±7.1	81±7.2
UV 571	75±6.3	75±7.9	72±7.9	79±8.0	79±9.7	77±9.9	79±6.3	79±5.9	79±7.3
UV 360	60±6.2	61±7.3	58±9.1	68±8.3	65±7.9	65±11	66±9.0	66±9.3	65±9.6

[a] Detection limits are calculated as signal-to-noise ratio of three times

[b] Quantification limits are calculated as signal-to-noise ratio of ten times

[c] Relative standard deviation (n=6)

[d] Recoveries (n=3) obtained comparing the signal response using on-line SPE-UPLC-MS/MS with the response provides by the same amount of analyte using direct injection

Analytical parameters

The performance characteristics of the conventional SPE process and on-line SPE coupled to UPLC-MS/MS were calculated for comparison.

Calibration curves in Milli-Q water for the different compounds were generated from standards ranging from 0.001–1 μg L^{-1} in on-line SPE and from 0.01–1 μg L^{-1} in off-line SPE, and each point corresponds to the mean value obtained from three area measurements. Satisfactory linear ranges were obtained for the three kinds of samples with correlation coefficients greater than 0.990 for all analytes in both on-line and off-line SPE.

The detection (LODs) and quantification limits (LOQs) for the two extraction procedures were calculated from the signal-to-noise ratios of the individual peaks, assuming minimum detectable signal-to-noise levels of 3 and 10, respectively. The LODs obtained were between 0.6 and 4.1 ng·L^{-1} in the on-line SPE system and between 4.9 and 15 ng·L^{-1} in the off-line SPE. LOQs ranged from 2.1–14 ng·L^{-1} (on-line) and 16–51 ng·L^{-1} (off-line; Tables 3 and 4, respectively). LODs and LOQs are lower in on-line SPE than in off-line SPE because the preconcentration factor is higher in on-line SPE. In off-line SPE, the preconcentration is 50 times (sample volume of 100 mL and elution volume of 2 mL), while in on-line SPE, the preconcentration is about 500 times. In this case, the elution cannot be optimised because it is carried out with the mobile phase and the preconcentration factor is calculated by comparing the on-line SPE signal with the signal from a spiked sample that is not passed through the extraction column.

To evaluate the precision, six replicate samples (containing 0.001 and 1 μg L^{-1} for on-line SPE procedure and 0.01 and 1 μg L^{-1} for on-line SPE procedure of the target compounds) were extracted following both procedures, and the reproducibility was expressed as a relative standard deviation (% RSD). Satisfactory results were achieved for all BUVSs with RSDs lower than 10 % and 14 % for on-line and off-line SPE, respectively.

Recoveries of the analytes for off-line procedure (Table 4) were calculated using the calibration curve by spiking Milli-Q water with 0.01 and 1 μg L^{-1} of each BUVS. The results are expressed as the average of three determinations and indicate good extraction efficiencies ranging from 70–88 % with the exception of UV 360. This compound has a very high octanol–water partition coefficient (log K_{ow}=12.46, Table 1), high molecular mass and is the last analyte eluted chromatographically. The lowest recovery (58 %) is probably not due to low retention but incomplete elution from the cartridge.

On the other hand, for the on-line SPE procedure, the recoveries must be calculated in terms of mass rather than concentration. For this purpose, we prepared different

Table 4 Analytical parameters for off-line SPE procedure coupled to UPLC-MS/MS detection

	LOD[a] (ng·L^{-1})			LOQ[b] (ng·L^{-1})			RSD[c] (%)				Recovery[d] (%)							
							0.01 μg·L^{-1}				1 μg·L^{-1}				0.01 μg·L^{-1}			
	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater	Milli-Q water	Seawater	Wastewater		Milli-Q water	Seawater	Wastewater		Milli-Q water	Seawater	Wastewater	
UV P	4.9	5.3	5.4	16	18	18	8.8	9.0	9.1		9.5	9.2	10		86±8.7	84±9.2	82±9.5	
UV 329	9.5	9.4	9.7	32	31	32	8.1	9.0	9.1		8.3	8.1	9.5		85±9.8	85±9.9	81±10	
UV 326	14	15	15	47	50	51	10	10	11		13	12	13		80±7.7	81±7.2	80±8.6	
UV 328	5.1	5.5	5.8	17	18	19	10	9.7	10		9.7	9.5	9.3		77±10	76±10	72±9.9	
UV 327	6.0	6.0	6.1	20	20	20	11	11	12		12	12	12		73±10	73±9.2	70±9.7	
UV 571	15	17	18	51	58	61	10	9.5	10		9.8	8.7	9.9		70±9.8	72±11	70±12	
UV 360	6.2	6.2	6.5	21	21	22	9.7	10	11		12	11	12		60±11	61±10	57±12	
	Milli-Q water	Seawater	Wastewater												Milli-Q water	Seawater	Wastewater	
UV P															87±7.8	86±6.1	81±10	
UV 329															88±8.9	89±7.7	82±9.7	
UV 326															82±9.8	88±6.9	80±8.2	
UV 328															76±9.8	73±11	73±9.6	
UV 327															75±9.3	73±10	69±12	
UV 571															71±8.6	70±7.8	67±11	
UV 360															58±9.7	60±10	56±12	

[a] Detection limits are calculated as signal-to-noise ratio of three times

[b] Quantification limits are calculated as signal-to-noise ratio of ten times

[c] Relative Standard Deviation (n=6)

[d] Mean of three determinations

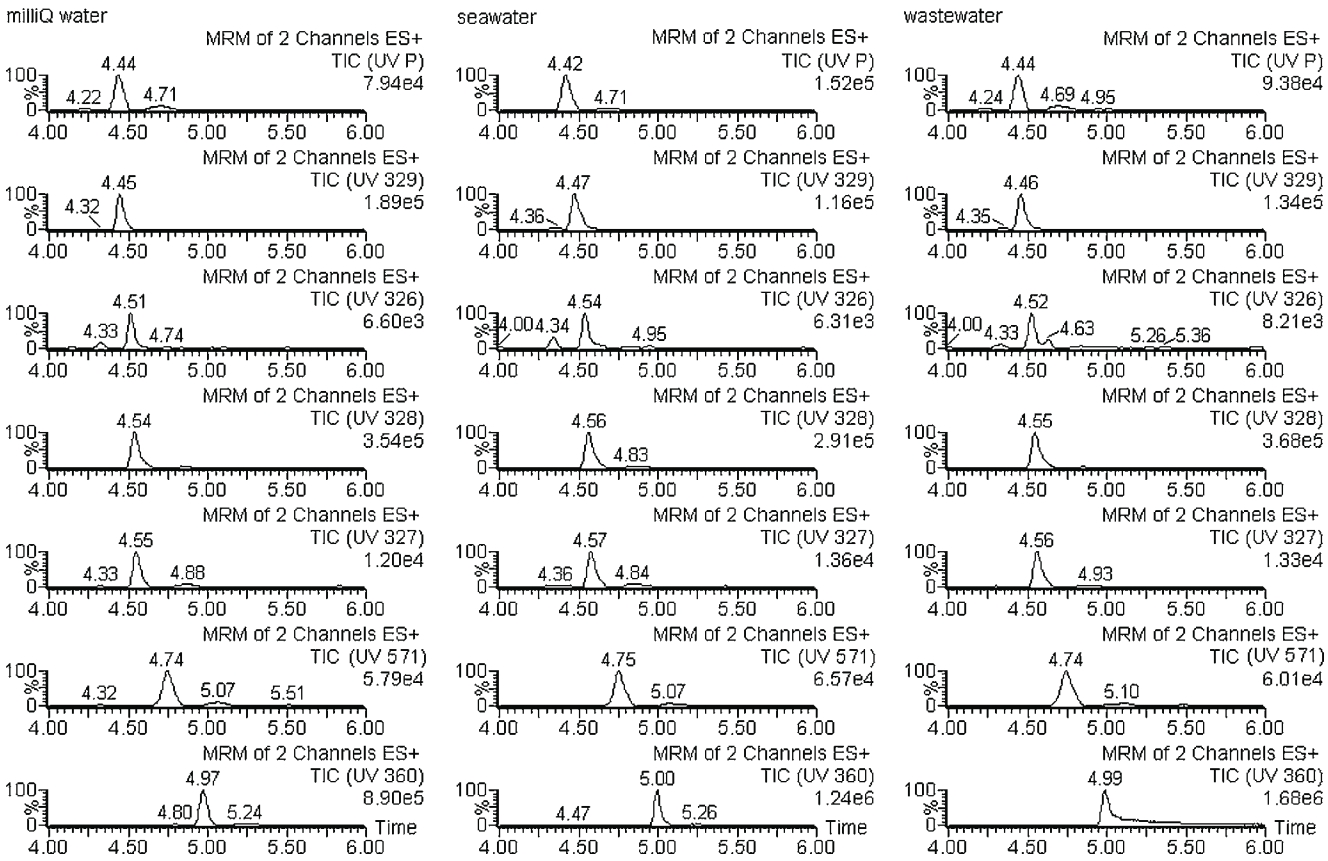


Fig. 2 Chromatograms of Milli-Q water, seawater and wastewater spiked with a 50 ng·L^{-1} of BUVS mixture

Table 5 BUVSs concentrations (ng·L^{-1}; mean of three determinations) measured in seawater and wastewater samples using on-line SPE and UPLC-MS/MS detection

Sample	UVP	UV329	UV326	UV328	UV327	UV571	UV360
Alcaravaneras East	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Alcaravaneras West	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Melenara East	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
MelenaraWest	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anfi del Mar East	3.1±0.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Anfi del Mar West	2.8±0.3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Puerto Rico East	2.8±0.3	<LOD	<LOD	<LOD	<LOD	<LOD	4.2±0.3
Puerto Rico West	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	4.2±.3
Amadores East	2.9±0.1	<LOD	<LOD	<LOD	<LOD	<LOD	4.3±0.3
Amadores West	3.0±0.2	<LOD	<LOD	<LOD	<LOD	<LOD	3.6±0.4
Mogán East	4.4±0.3	<LOD	<LOD	<LOD	<LOD	<LOD	5.2±0.4
Mogán West	4.2±0.2	<LOD	<LOD	<LOD	<LOD	<LOD	4.7±0.3
WWTP 1	7.7±0.6	<LOD	<LOD	7.1±0.5	<LOD	<LOD	5.9 ±0.4
WWTP 2	4.9±0.3	<LOD	<LOD	8.8±0.4	<LOD	<LOD	6.6±0.7
WWTP 3	Nd	<LOD	<LOD	8.7±0.6	<LOQ	<LOD	<LOD
WWTP 4	6.6±0.4	<LOD	<LOQ	6.2±0.7	<LOD	<LOD	<LOD
WWTP 5	<LOQ	<LOD	11±0.9	13±1	4.8±0.5	<LOD	<LOD
WWTP 6	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
WWTP 7	8.8±0.9	4.0±0.2	<LOD	<LOQ	<LOQ	<LOD	<LOD

solutions so that injecting through extraction column or without pass by extraction column, the amount of analyte that reaches the detector is the same. 0.05, 0.25 and 0.5 ng were the amounts of analyte employed and the mean of recoveries calculated are show in Table 3. As can be seen, the recovery values are slightly better than for off-line procedure (between 75 and 94 % for most of BUVSs) observing the same behaviour for UV 360 (65 %).

Matrix effects

Once the effectiveness of the proposed method was verified in spiked Milli-Q water, we calculated all the analytical parameters for the real matrices in order to prove that it is equally valid for the analysis of complex matrices. The samples employed were a seawater sample taken from Arinaga (Gran Canaria Island) that was analysed previously and did not contain BUVSs and an aliquot randomly chosen from the seven WWTPs samples studied (sample B from WWTP 2).

Separate calibration curves for the different compounds in seawater and wastewater samples were generated using the standard addition method (in the range 0.001–1 μg L^{-1} for on-line SPE procedure and 0.01–1 μg L^{-1} for off-line SPE procedure) to correct possible deviations due to matrix effect. Each point corresponds to the mean value obtained from three area measurements and satisfactory linear ranges were obtained with correlation coefficients greater than 0.990 for all analytes in both matrices.

LODs and LOQs calculated for these matrices are somewhat higher than those obtained for Milli-Q water (especially for wastewater samples) because they have a lower signal/noise ratio. Even though the results are also slightly worse for wastewater in terms of recoveries and precision, they are in a satisfactory range to say that the method is applicable. On the other hand, the obtained parameters for seawater matrix demonstrate the absence of a matrix effect.

Figure 2 depicts the chromatograms of Milli-Q water, seawater and wastewater spiked with a 50 ng·L^{-1} of BUVS mixture.

Determination of BUVSs in seawater and wastewater samples

The developed method was used to analyse samples of different origins. First, we analysed 12 seawater samples collected from several points on Gran Canaria Island. We took two samples from each location, one from each margin of the beach (east and west). Using on-line SPE, we detected UV P and UV 360 in most of the beaches sampled. UV P was measured in the range 2.8–4.4 ng L^{-1} and UV 360 was found in concentrations between 3.6 and 5.2 ng L^{-1}. Using off-line SPE, no BUVSs were detected due to the high LODs and LOQs for this procedure. The results are presented in Table 5.

In addition, we analysed seven samples from different wastewater treatment plants (Table 5). The presence of BUVSs was detected in all of these samples using on-line SPE. The maximum values measured were 8.8 ng L^{-1} for UV P, 4.0 ng L^{-1} for UV 329, 11 ng L^{-1} for UV 326, 13 ng L^{-1} for UV 328, 4.8 ng L^{-1} for UV 327 and 6.6 ng L^{-1} for UV 360. Using off-line SPE, only UV P

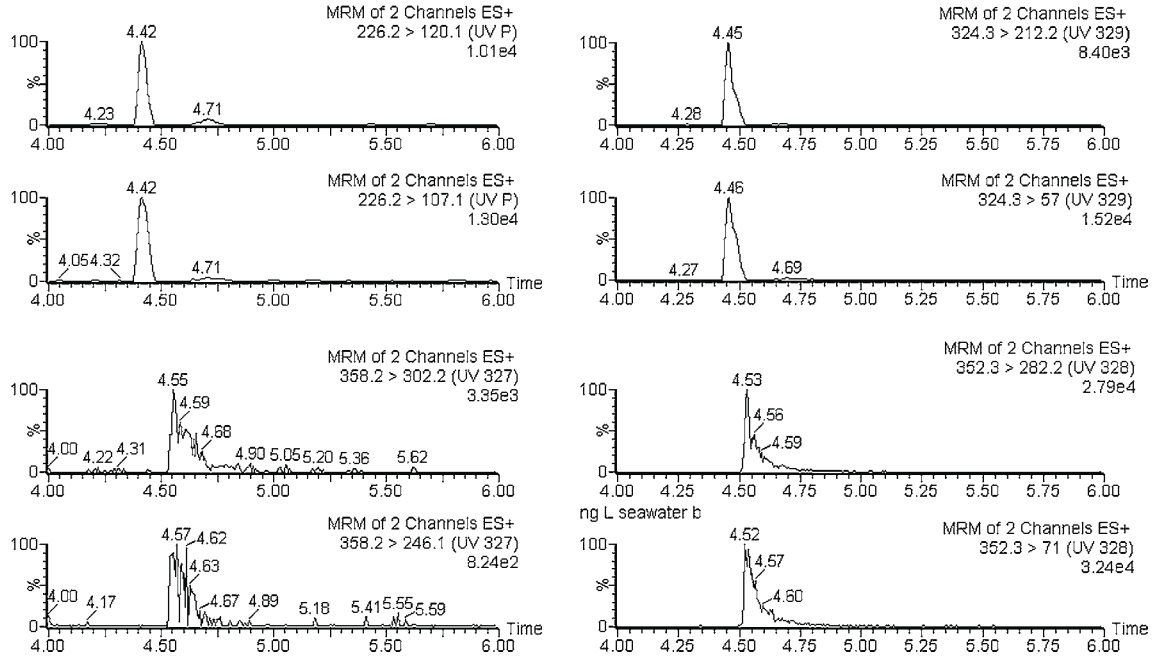


Fig. 3 MRM chromatogram of BUVSs detected in WWTP 7 employing on-line SPE-UPLC-MS/MS

and UV 328 were detected but not quantified. Figure 3 depicts the MRM chromatogram of the compounds detected in WWTP 7.

Conclusions

On-line SPE coupled to UPLC-MS/MS is increasingly used, as it provides high sensitivity and selectivity, more reproducibility and minimum sample preparation. In our case, the on-line SPE-UPLC-MS/MS method developed allows for the simultaneous determination of seven BUVSs compounds in liquid samples with satisfactory recoveries and reproducibility, except for UV 360, which cannot be completely eluted from the cartridge due its high octanol–water partition coefficient and molecular mass. The combination of the preconcentration provided by the SPE procedure and the sensitivity of the UPLC-MS/MS system offers very low detection limits for these BUVSs in different kind of samples, with values between 0.6 and 4.5 ng L^{-1}. Moreover, this method allows for the extraction, preconcentration, clean-up, elution and detection of the studied analytes in only 5 min.

The analysis of coastal seawater samples revealed the presence of UV P and UV 360 in almost all of these samples in the range of 2.8–4.4 ng L^{-1} and 3.6–5.2 ng L^{-1}, respectively. Additionally, in wastewater samples, all BUVSs except UV 571 were detected at concentrations between 4.0 and 13 ng L^{-1}.

This procedure is a viable, fast, selective and easy analytical approach for routine analysis and represents a valuable tool in the analytical characterisation of water samples that may be used on environmental waters or effluents from wastewater treatment plants.

Acknowledgments This work was supported by funds provided by the Spanish Ministry of Science and Innovation, Research Project CTQ2010-20554. Sarah Montesdeoca-Esponda would like to thank the Canary Agency of Investigation, Innovation and Information Society for her PhD Student Grant.

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III.2.2. Extracción asistida por microondas combinada con extracción en fase sólida en modo "en línea" seguida por cromatografía liquida de ultra resolución acoplada a espectrometría de masas en tándem de benzotriazoles estabilizadores de luz UV en sedimentos marinos y lodos de depuradora.

En este trabajo para la determinación de BUVSs en muestras sólidas, se empleó la técnica de SPE "en línea" desarrollada en el articulo anterior (III.2.1.) como paso de limpieza y preconcentración del extracto obtenido después de aplicar la técnica de extracción asistida por microondas.

La optimización de la MAE se realizó de forma similar a la empleada en los apartados III.1.4 y III.1.5., pero usando un diseño factorial con tres variables en lugar de cuatro, ya que en esta ocasión se empleó un disolvente orgánico y por tanto la concentración del extractante no era una variable a optimizar. El cálculo de las correlaciones entre variables mostró que en este caso la potencia y el tiempo fueron las dominantes en el proceso de extracción, por lo que se utilizó un nuevo diseño experimental 2^3 para estudiar estos parámetros en profundidad.

Una vez aplicada la técnica de extracción desde la matriz sólida, el extracto fue sometido a la SPE "en línea" con UHPLC-MS/MS, ofreciendo el método completo unos límites de detección entre 53.3 y 146 $ng \cdot kg^{-1}$ (algo menores para sedimentos que para lodos). El resto de parámetros analíticos también fueron calculados por separado para los dos tipos de muestra a analizar, ofreciendo recuperaciones y RSDs entre 46.1-83.9 y 8.57-17.4 % (lodos) y 50.1-87.1% y 8.16-13.6 % (sedimentos), respectivamente.

Las muestras de sedimentos marinos (tomadas cerca de un emisario submarino) y las de lodos procedentes de una EDAR, mostraron la presencia de dos analitos, el UV 328 y el UV 360, mientras que en los sedimentos costeros no se detectó ningún compuesto.

El trabajo ha sido publicado en la revista *Journal of Separation Science 36 (2013) 781-787.*

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Received July 11, 2012
Revised October 31, 2012
Accepted November 01, 2012

Research Article

Microwave-assisted extraction combined with on-line solid phase extraction followed by ultra-high-performance liquid chromatography with tandem mass spectrometric determination of benzotriazole UV stabilizers in marine sediments and sewage sludges

Benzotriazole ultra-violet stabilisers are compounds widely used in personal care products, which can reach the environment after passing through wastewater treatment plants. In this work, we develop a novel method to evaluate the presence of seven compounds in marine sediments and sewage sludges using microwave-assisted extraction followed by a clean-up step based in on-line solid phase extraction coupled to ultra-high-performance liquid chromatography with MS/MS detection. This method allows for fast and efficient extraction from the solid matrix, subsequent automatic on-line purification and preconcentration, and analysis. For the optimised method, LOD were from 53.3 to 146 ng/kg and LOQ were in the range of 176–486 ng/kg. The method was validated for different environmental solid samples with satisfactory recoveries and relative standard deviations, between 46.1 and 83.9 and 7.8 and 15.5% (sludges) and 50.1 and 87.1% and 8.83 and 16.3% (sediments), respectively. Finally, the studied analytes were quantified in concentrations between 0.18 and 24.0 ng/g in real samples of marine sediments and sewage sludges from Gran Canaria Island (Spain).

Keywords: Benzotriazole / Microwave-assisted extraction / Sludges / Solid phase extraction / Ultra-violet stabilizers
DOI 10.1002/jssc.201200664

1 Introduction

Benzotriazole ultra-violet stabilisers (BUVSs) belong to a group of UV absorbing compounds that are considered to be emerging contaminants. These compounds are employed in different personal care products. The growing concern about the link between sunlight exposure and skin cancer has led to an increased use of BUVSs in sunscreens, soaps, shampoos, lip gloss, hair dyes and makeup, due to the presence of a phenolic group and benzotriazole structure that reflect and absorb the solar radiation (UVA 320–400 nm and UVB 290–320 nm) [1]. The usual concentration of UV filters in cosmetics is between 0.1 and 10% [2].

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Abbreviations: ASE, accelerated solvent extraction; **BUVS**, benzotriazole ultra-violet stabilizer; **MAE**, microwave-assisted extraction; **UHPLC**, ultra-high-performance liquid chromatography; **WWTP**, wastewater treatment plant

It is necessary to develop methods for investigating the presence of BUVSs in the environment because they are not completely removed by wastewater treatment plants (WWTPs) and their continuous introduction through effluents allows them to pseudo-persist [3]. The BUVSs compounds can also reach the environment from recreational activities such as swimming and bathing in oceans, lakes or rivers (direct inputs) [4]. Little information is available regarding the contamination of these organic UV filters in environmental samples, especially in solid samples where the more lipophilic compounds are accumulated. Relevant concentrations of BUVSs have been found in different sediment samples [5,6] and sewage sludges [7]. The fact that the compounds that enter the environment in dissolved form can be accumulated in sediments implies that they can also be bioaccumulated in living organisms. Thus, some BUVSs have been detected in several marine organisms [8,9] and even in birds and mammals [10,11]. It has been demonstrated that their derivatives are mutagenic in bacterial systems and toxic in plants [12], and adverse effects on the fecundity and reproduction of fish have also been reported [13,14]. Some of these compounds have been identified by the United States Environmental Protection Agency as inert ingredients of unknown

toxicity (www.epa.gov/opprd001/inerts/oldlists.html). Recent papers have carried out toxicity studies of these substances in aquatic species such as arthropods [15–17], chordates [18] and fishes [17].

The analysis of emerging contaminants in environmental samples is characterized by the difficulty in the determination of low concentrations in complex matrices. The extraction of analytes from solid samples in environmental applications presents added complications, because the solute-matrix interactions are very difficult to predict and overcome [19]. In the literature we can find several extraction methods employed for different compounds in solid samples: ultrasonic extraction, USE (acidic pharmaceuticals and phenolic endocrine disrupting chemicals in sewage sludge) [20], soxhlet extraction (4-nonylphenols, phthalates and polychlorinated biphenyls in soils and biosolids) [21], supercritical fluid extraction (microbial phospholipid fatty acids in activated sludge) [22], accelerated solvent extraction (ASE), (synthetic musks in lake sediment) [23], pressurised fluid extraction (polycyclic aromatic hydrocarbons in urban street dust) [24] and focussed microwave-assisted extraction (theobromine and caffeine in cacao) [25].

We chose microwave-assisted extraction (MAE) for the extraction of seven BUVSs (UV P, UV 329, UV 326, UV 328, UV 327, UV 571 and UV 360, Table 1) from different solid samples (sewage sludges and marine sediments taken close to shore and near to a marine outfall) prior to their determination by ultra-high-performance liquid chromatography (UHPLC) with MS/MS. This method represents a viable alternative to other methodologies, because it requires much lower volumes of organic solvents, reduces extraction time and allows preparation of multiple samples in one single step [26]. Other methods employed for BUVSs in solid samples require large volume of strong solvents, such as ultrasonication (10 mL of dichloromethane and 10 mL of acetone) [6], successive shaken (25 mL of ethylacetate/dichloromethane) [7] or Soxhlet apparatus (200 mL of dichloromethane/hexane) [9].

On the other hand, the extraction of the selected solid samples requires the use of a clean-up step for purification, and this often causes the method becomes even more tedious and time consuming. This occurs in the study of Ruan et al., where 12 BUVSs are determined in sewage sludges using three cycles of ASE. Then the extract is concentrated by evaporation, fractionated on a gel permeation chromatographic column, anew concentrated, passed through Florisil column and concentrate once again before injecting [27]. Evaporation steps were needed also employing other extraction methods [6, 7, 9].

To solve this problem, in this work an on-line SPE procedure coupled to a UHPLC system was selected as the cleaning and preconcentration protocol to be used prior to the determination by mass spectrometry. This automated SPE system minimises sample loss or contamination during handling and improves the repeatability when compared with the conventional off-line SPE procedure. Evaporation/concentration is not necessary. Moreover, the UHPLC technology provides high separation efficiency, enhances chromatographic resolution and leads to shorter analysis time [28–31]. The reduction of the total time employed in the preparation and analysis of the complex matrix is a very important aspect when a large number of samples are involved.

In this work, we optimised the parameters affecting the microwave extraction process (i.e., extraction time, power and extractant volume) by means of an experimental design to achieve the maximum extraction efficiency for seven selected analytes. We then investigated the best conditions for purifying and analysing the MAE extract using an on-line SPE-UHLPC-MS/MS. Once each step of the procedure was optimised and validated, we evaluated the concentrations of the BUVSs in sediments close to shore and the sediments around a marine outfall from several coastal points around Gran Canaria Island (Spain) that had not been studied previously. Sludge samples from different WWTPs were also analysed.

2 Experimental

2.1 Reagents

The benzotriazoles were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions (500 μg/mL) were prepared in methanol and stored in glass-stoppered bottles at 4°C prior to use. The mobile phase was prepared with LC-MS grade methanol and formic acid obtained from Panreac Química (Barcelona, Spain). The solvents used for the on-line SPE were LC-MS grade methanol, LC-MS grade water, hexane and acetone and were purchased from Panreac Química. The 0.45-μm syringe-driven filter used for filtration of the MAE extract was provided by Scharlau Chemie (Barcelona, Spain). For the on-line SPE an Oasis HLB Direct Connect HP Column (2.1 × 30 mm, 20 μm) from Waters (Madrid, Spain) was used.

2.2 Instrumentation

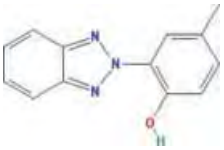
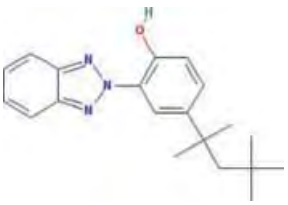
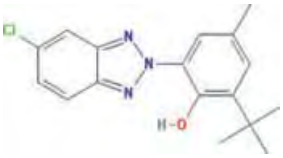
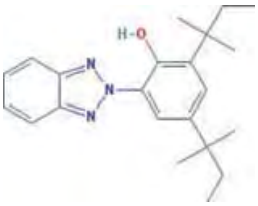
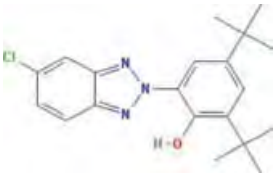
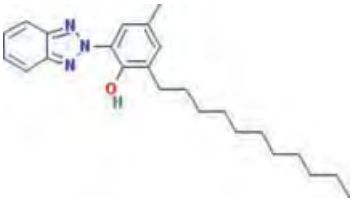
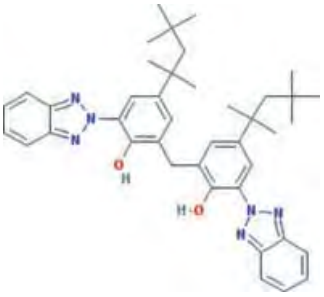
The microwave oven used for the extraction was a multiwave with a 6 EVAP rotor and 6 MF100 vessels (Anton Paar, Graz, Austria).

The purification and analysis system included an ACQUITY quaternary solvent manager to load samples, wash and recondition the extraction column, an ACQUITY Binary Solvent Manager for the elution of the analytes, a column manager, a 2777 autosampler with 25 and 5000 μL syringes and trays for 2 and 20 mL vials, and an ACQUITY tandem triple quadrupole mass spectrometer with an ESI interface. All components from Waters (Madrid, Spain) were controlled by MassLinx Mass Spectrometry Software.

2.3 Chromatographic conditions and mass detection

An ACQUITY UHPLC BEH Waters C_{18} column (50 × 2.1 mm, 1.7 μm particle size) at 40°C under a 100% methanol isocratic mobile phase adjusted to pH 2.5 with 0.1%, v/v, formic acid at a flow rate of 0.9 mL/min was used for the

Table 1. List of BUVSs, IUPAC name, chemical structure and pk_a and $logK_{ow}$ values

Compound	IUPAC name	Chemical structure	pk_a	$logK_{ow}$
UV P	2-(Benzotriazol-2-yl)-4-methylphenol		8.15 ± 0.43	2.99
UV 329	2-(Benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol		8.07 ± 0.45	6.21
UV 326	2-tert-Butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol		9.31 ± 0.48	5.55
UV 328	2-(Benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol		8.85 ± 0.50	7.25
UV 327	2,4-ditert-Butyl-6-(5-chlorobenzotriazol-2-yl)phenol		9.23 ± 0.48	6.91
UV 571	2-(Benzotriazol-2-yl)-6-dodecyl-4-methylphenol		–	8.95
UV 360	2-(Benzotriazol-2-yl)-6-[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl)phenyl]methyl]-4-(2,4,4-trimethylpentan-2-yl)phenol		7.62 ± 0.50	12.46

pk_a values obtained from https://scifinder.cas.org/ and $logK_{ow}$ values obtained from [32].

elution of BUVSs using UHPLC-MS/MS detection. The ESI parameters were fixed as follows: capillary voltage at 3 kV, cone voltage at 50 V, source temperature at 120°C, desolvation temperature at 450°C, and desolvation gas at 800 L/h. Nitrogen was used as the desolvation gas, and argon was employed as the collision gas. The detailed MS/MS detection parameters for each BUVS are presented in Table 2 and were optimised by direct injection of a 1 mg/L standard solution

Table 2. Mass spectrometer parameters for BUVSs detection

Compound	Precursor ion *(m/z)*	Cone voltage (V)	Quantification ion *(m/z)*	Collision potential (V)	Confirmation ion *(m/z)*	Collision potential (V)
UV P	226.2	40	107.1	20	120.1	20
UV 329	324.2	50	57	25	212.2	25
UV326	316.3	40	260.2	20	107.1	25
UV 328	352.3	50	71	30	282.2	20
UV 327	358.3	60	302.3	20	246.1	30
UV 571	394.3	50	226.2	20	120.1	30
UV 360	658.6	40	336.3	25	224.2	35

of each analyte into the detector at a flow rate of 10 μL/min. Under these conditions, UHPLC-MS/MS allows for the chromatographic elution of the seven compounds studied in <1 min.

2.4 Preparation of spiked samples

The marine sediment samples used for the optimisation of the extraction and purification methods were sifted to a particle size of <300 μm and spiked with a solution of BUVSs prepared in methanol to obtain a final concentration of 500 ng/g for each analyte. The samples were stirred and air-dried for 24 h in the dark at room temperature to obtain dry and homogeneous samples. Prior to spiking, analysis of the blank marine sediments showed that they did not contain any signals in the chromatogram for the BUVSs under investigation.

2.5 MAE procedure

One gram of the solid samples was transferred to the MAE vessels. The extractant agent, acetonitrile, was added to the mixture and the closed vessels were subjected to the MAE process. An experimental design obtained using Statgraphics Plus software 5.1 was employed for optimisation of the all parameters that affect the extraction process. Once the extraction time had elapsed, the vessels were allowed to cool for 10 min in the presence of the microwave fan and then for an additional 10 min at room temperature outside of the microwave oven before they were opened. The extracted solution was filtered through a 0.45-μm syringe filter.

2.6 On-line SPE as a clean-up step

The purifying step for the MAE extract based in an on-line SPE procedure was carried out using two extraction columns in a parallel manner. The quaternary pump worked at an initial flow rate of 2 mL/min using water (0.1% formic acid) (phase A) to load the sample. Once the sample was loaded, the time began to run and the clean-up of impurities was performed between 0 and 3.8 min using a water/methanol (70:30, v/v) mixture (phase B and C, respectively) at a flow rate of 0.01 mL/min. The analytes were eluted in less than a minute by the binary pump using the same mobile phase employed in the chromatographic elution. At the same time, the extraction column was washed using the quaternary pump at 2 mL/min with a methanol/acetone/hexane (1:1:1, v/v/v) mixture (phase D) to prepare for the next sample.

2.7 Sample collection

Three kinds of solid samples were analysed in order to demonstrate the applicability of the method and to determine their contamination with the analysed BUVSs. The sampled sediments were taken close to the shore of three tourist beaches of Gran Canaria Island (Spain). We also selected a marine outfall that discharges the depurated waters from a sewage treatment plant, and four sediment samples taken at different distances from the coast (sample 1 is the closest to the marine outfall and sample 4 is the farthest). This marine outfall is located in the southern region of Gran Canaria Island. Additionally, sludges from three WWTP effluents were analysed.

3 Results and discussion

3.1 MAE optimisation

Variables that could affect the efficiency of an MAE procedure must be optimised to achieve maximum recoveries of the target analytes. An initial experimental design of 2^3 was used to study the influence of each variable on the extraction process and the variable correlations to each other. Two levels per parameter were evaluated: power (100 and 500 W), extraction time (2 and 15 min) and extractant volume (1 and 10 mL of methanol). We can draw some conclusions from this preliminary study. For example, a power value of 500 W compared with 100 W (and consequently a high temperature) seemed to provide good extraction efficiency because an increase in temperature facilitates the desorption of analytes from the matrix and improves their solvent solubility and sample penetration. The same behaviour is observed for longer extraction times. No significant differences were observed for the extractant volume.

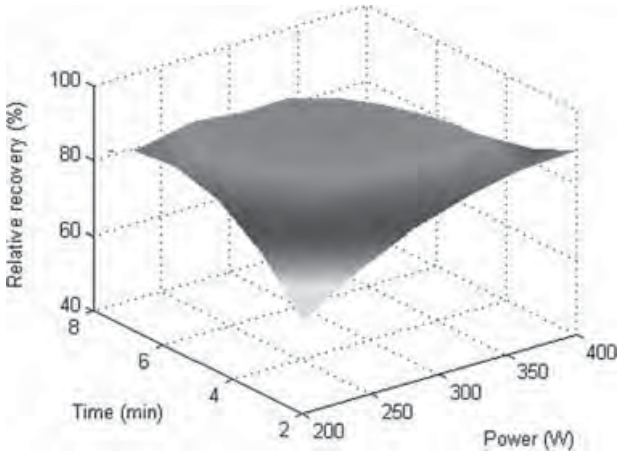


Figure 1. Response surface for the effect of power and extraction time on the UV P extraction (500 ng/g) using MAE-SPE-UHPLC-MS/MS (extraction efficiency, percentages normalised regard to the highest signal response).

We calculate the different correlations between the variables (bivariate correlations) and their influence on the recoveries (partial correlations). The variables that have the greatest impact on recoveries are power and extraction time. These variables also show the strongest correlation between them. The power and the extraction time have an influence on the recoveries between 0.684 and 0.711 and 0.539 and 0.0632, respectively, while the values for the effect of volume were in the range of 0.178–0.342 (partial correlations, being maxima correlations +1 and −1). Since the extractant volume has lower influence, we chose 2 mL in order to facilitate the subsequent on-line SPE process. Then, we more thoroughly studied the most determinant variables (power and time) using a 3^2 factorial design employing two solvents: methanol and acetonitrile. In this second design we studied three levels per parameter (200, 300 and 400 W of power and 2, 5 and 8 min of extraction time).

Figure 1 shows the surface response obtained with the different power and time values for UV P. The signal response is expressed in percentages, which have been normalised with regard to the highest signal obtained. The extraction efficiency improves with an increase in both power and time up to a point at which an excess of temperature inside the vessels generates a loss of analytes. The same trend was observed for the rest of compounds. On the other hand, an improvement in the extraction was observed using acetonitrile. Based on these results, we chose the following conditions as the best for sample extraction: 300 W power, 5 min extraction time and 2 mL of acetonitrile as the extractant.

3.2 On-line SPE optimisation

To pass the acetonitrile MAE extract onto the on-line SPE system, we diluted it to 20 mL of Milli-Q water. We injected these 20 mL of sample running four injection cycles with the 5 mL syringe. One of the main advantages of the on-line SPE compared to conventional off-line SPE is that, regardless of the extract concentration, all of the analyte mass injected comes to the detector, while only a fraction of the extract is injected in off-line SPE.

Several experimental variables like ionic strength, sample pH, sample loading, wash step and elution have been previously optimised to achieve the maximum extraction efficiency of the target analytes [31]. The optimum conditions of sample are 0%, w/v, of ionic strength and pH 3. The loading phase, i.e. the mobile phase that accompanies the sample through the column extraction, consists of an acidified Milli-Q water solution (0.1% formic acid). A wash step prior to the elution is necessary to obtain the maximum removal of the impurities without analyte loss. This is achieved using a solution of water/methanol (70:30, v/v). Finally, the elution of the analytes from the extraction columns is carried out with the chromatographic mobile phase.

3.3 Analytical parameters

The performance characterisation of the MAE process followed by on-line SPE coupled to UHPLC-MS/MS, was carried out for sediment and sludge samples.

Separate matrix-matched calibration curves for each of the different compounds in the sediments and sludges were generated using six concentration levels to correct for possible deviations due to matrix effect. The range of concentrations used for the calibration curves in sediments was 0.1–100 ng/g for UV P, UV 329 and UV 360 and 0.2–100 ng/g for UV 326, UV 328, UV 327 and UV 571. For sludges, concentrations between 0.2 and 100 ng/g for UV 326, 0.3 and 100 ng/g for UV P and UV 360 and 0.4 and 100 ng/g for UV 329, UV 328, UV 327 and UV 571 were employed. Each point corresponds to the mean value obtained from three different samples subjected to entire process. Satisfactory linear ranges were obtained with determination coefficients >0.990 for all analytes in both matrices. Figure 2 shows the chromatogram obtained for a sludge sample spiked with 1 ng/g of the BUVSs mixture.

LODs and LOQs were calculated from the S/N of the individual peaks, assuming minimum detectable signal-to-noise levels of 3 and 10, respectively. As can be seen in Table 2, the LODs and LOQs are lower for sediment samples than for sludge samples. To evaluate the precision, six replicate samples containing 1, 25 and 50 ng/g of the target compounds were subjected to the optimised MAE-SPE-UHPLC-MS/MS procedure, and the repeatability was expressed as a relative standard deviation (%RSD). Satisfactory results were achieved for all BUVSs with RSDs < 14%, except for sludges where the last three compounds of the chromatogram have RSDs between 15.3 and 17.4% (Table 3). The higher detection limits and lower repeatability in sludges are likely due to major interferences caused by the co-elution of compounds.

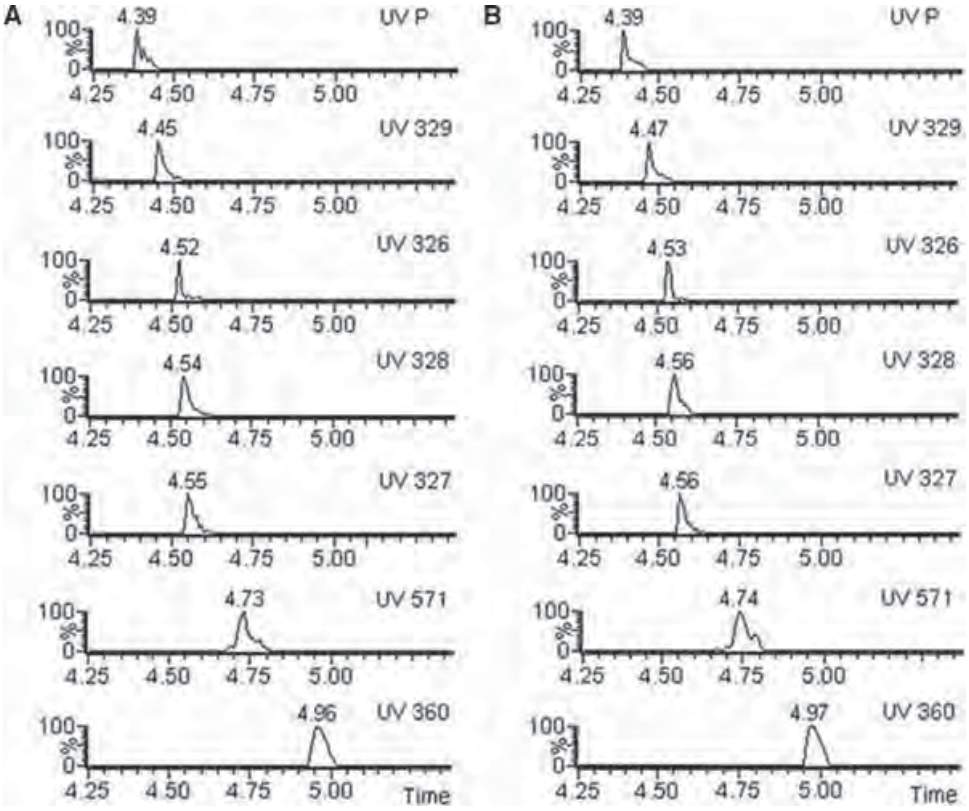


Figure 2. Total ion current chromatogram of (a) sediment sample and (b) sludge sample spiked with a 1 ng/g of BUVSs mixture applying MAE-SPE-UHPLC-MS/MS.

Table 3. Analytical parameters for MAE-SPE-UHPLC-MS/MS, (a) LODs, LOQs and calibration points, (b) RSDs and recoveries

(a)	LOD [a] (ng/kg)		LOQ[b] (ng/kg)		Calibration concentration (ng/g)	
	Sediment	Sludge	Sediment	Sludge	Sediment	Sludge
UV P	55.1	69.9	183	230	0.1, 1, 10, 25, 50, 100	0.3, 1, 10, 25, 50, 100
UV 329	73.8	98.2	243	326	0.1, 1, 10, 25, 50, 100	0.4, 1, 10, 25, 50, 100
UV 326	99.3	146	327	486	0.2, 1, 10, 25, 50, 100	0.2, 1, 10, 25, 50, 100
UV 328	78.4	108	260	360	0.2, 1, 10, 25, 50, 100	0.4, 1, 10, 25, 50, 100
UV 327	84.1	106	280	353	0.2, 1, 10, 25, 50, 100	0.4, 1, 10, 25, 50, 100
UV 571	106	108	353	360	0.2, 1, 10, 25, 50, 100	0.4, 1, 10, 25, 50, 100
UV 360	53.3	70.7	176	233	0.1, 1, 10, 25, 50, 100	0.3, 1, 10, 25, 50, 100

(b)	RSD[c] (%)						Recovery[d] (%)					
	1 ng/g		25 ng/g[1]		50 ng/g		0.05 ng		0.25 ng		0.5 ng	
	Sediment	Sludge	Sediment	Sludge	Sediment	Sludge	Sediment	Sludge	Sediment	Sludge	Sediment	Sludge
UV P	8.77	10.9	8.16	8.57	8.33	8.87	84.5 ± 9.72	82.4 ± 10.1	84.6 ± 8.4	82.4 ± 8.83	87.1 ± 7.80	83.9 ± 9.18
UV 329	9.13	11.2	9.01	10.7	9.13	10.4	85.2 ± 10.4	80.6 ± 10.2	84.0 ± 8.3	81.2 ± 10.7	86.2 ± 8.13	81.1 ± 10.6
UV 326	11.6	13.5	9.86	11.3	9.19	11.8	77. 6 ± 10.4	75.8 ± 12.6	77.9 ± 10.9	75.2 ± 11.2	79.1± 9.72	77.1 ± 11.5
UV 328	9.92	12.2	9.91	12.4	9.36	11.6	70 .8 ± 11.2	65.6 ± 12.1	71.1 ± 11.1	67.1 ± 12.3	70.8 ± 10.7	70.6 ± 12.2
UV 327	11.6	15.3	11.3	12.8	10.6	12.3	65.3 ± 12.8	61.0 ± 14.0	65.1 ± 12.4	70.8 ± 13.7	69.7 ± 11.5	68.7 ± 12.2
UV 571	13.6	17.4	12.4	13.5	11.7	13.8	66.2 ± 13.3	61.5 ± 14.8	66 .2 ± 11.6	61.1 ± 14.9	68.5 ± 10.8	63.2 ± 13.8
UV 360	12.0	16.3	10.2	15.1	11.1	13.5	50.1 ± 15.5	46.1 ± 16.3	51.6 ± 12.1	48.9 ± 15.0	55.7 ± 10.6	51.5 ± 14.1

a) Detection limits are calculated as S/N = 3.
b) Quantification limits are calculated as S/N = 10.
c) RSD ($n = 6$).
d) Recoveries ($n = 3$) obtained comparing the signal response using on-line SPE-UHPLC-MS/MS with the response provides by the same amount of analyte using direct injection.

Table 4. BUVSs concentrations (ng/g) measured in marine sediments and sewage sludges using MAE-SPE-UHPLC-MS/MS (mean of three determinations)

Sample	UV P	UV 329	UV 326	UV 328	UV 327	UV 571	UV 360
Beach 1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Beach 2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Beach 3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Marine outfall 1	<LOQ	<LOQ	<LOD	24.0 ± 2.43	<LOD	<LOD	0.33 ± 0.04
Marine outfall 2	<LOQ	<LOQ	<LOD	22.0 ± 2.33	<LOD	<LOD	0.19 ± 0.02
Marine outfall 3	<LOD	<LOD	<LOD	20.7 ± 1.95	<LOD	<LOD	0.18 ± 0.02
Marine outfall 4	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ
Sewage sludge 1	<LOQ	<LOD	<LOD	12.2 ± 1.49	<LOD	<LOD	6.32 ± 0.92
Sewage sludge 2	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	2.30 ± 0.31
Sewage sludge 3	<LOD	<LOQ	<LOD	0.94 ± 0.11	<LOD	<LOD	<LOQ

These LODs and LOQs are lower than the found in the literature for these compounds in similar matrices. In a work in which UV 326, UV 327 and UV 328 were extracted and analysed from sediments [6], the LODs were 0.05, 1 and 10 ng/g, respectively, while our values are 99.3, 84.1 and 78.4 ng/kg, one lower order of magnitude. Although in this paper the extracts were concentred using off-line SPE, the use of on-line SPE step offers better preconcentration factors. The same compounds (UV 326, UV 327 and UV 328) in sediments samples were studied in other papers achieving also higher LODs, for example between 0.1 and 0.5 ng/g [7] and values of 20 ng/g for all compounds and UV P [5]. For sludges samples, we found a work employing ASE and SPE concentration with LOQ comparable with ours results (in the range of 0.15–0.77 and 20 ng/g for the different BUVSs analysed) but the procedure is more tedious and time consuming because involves additional evaporation and separation of different fractions [27].

Recoveries were also studied for both matrices. Since the procedure includes an on-line SPE step, the recoveries must be calculated in terms of mass rather than concentration. To calculate the recoveries, we added 0.05, 0.25 and 0.5 ng of analyte to 1 g of solid sample and the complete procedure (MAE-SPE-UHPLC-MS/MS) was employed. The obtained signal was then compared with the signal of a direct injection of a standard solution that contains the same amount of analyte. Thus, the amount of analyte that reaches the detector should be the same. The recoveries are expressed as the average of three determinations (Table 3) and indicate relatively good extraction efficiencies taking into account the complex matrices under study. For the sediment samples, the recoveries were between 87.1 and 50.1%, corresponding to the first and last compound chromatographically eluted. This difference in the behaviour of the compounds is probably due to the progressive increase of the octanol-water partition coefficient ($\log K_{ow}$ between 2.99 and 12.46, see Table 1). The more lipophilic compounds (higher $\log K_{ow}$) have stronger analyte-matrix interactions and the extraction from the solid is more complicated. Moreover, when the on-line SPE step is carried out, this lipophilic character causes a huge retention in the extraction column and incomplete elution from the cartridge can occur. The same trend is observed for sludge samples (Table 3) due to the higher complexity and organic matter content.

3.4 Determination of BUVSs in coastal and marine sediment samples and sludges from wastewater samples

Once we determined the analytical characteristics of the procedure for different kind of samples and given that the recoveries obtained are satisfactory except for UV 360, the developed method was applied to samples of different origins and characteristics. Two groups of sediment samples were analysed: three samples from tourist beaches' sand and four samples taken around a marine outfall that discharges the depurated waters from a WWTP. Three samples of sludges from three WWTP effluents were also analysed. We quantified several BUVSs in sediments around the marine outfall and sludge samples, while no compounds were detected in the beach sand samples. The results obtained are presented in Table 4. UV P and UV 329 were detected in both outfall marine sediments and sludges but were not quantified because their concentrations were below the LOQs. UV 326, UV 327 and UV 571 were not detected. The concentrations of UV 328 and UV 360 measured in marine sediment samples were between 20.7 and 24.0 ng/g and between 0.18 and 0.33 ng/g, respectively. In the sewage sludge samples, UV 328 was found in a range of concentrations between 0.94 and 12.2 ng/g and UV 360 between 2.30 and 6.32 ng/g.

The concentrations of BUVSs measured in the outfall marine sediments are higher closer to the outfall than in the farthest sample, which suggests that there is a dispersion of analytes from wastewater discharged.

4 Concluding remarks

The MAE-SPE-UHPLC-MS/MS method developed allows for the simultaneous determination of seven BUVS compounds in solid samples with satisfactory results for six of them. The validity of the method has been tested for solid

samples of different origin and physical characteristics, and its application to the analysis of environmental sediments and sewage sludges reveals the presence of BUVSs. Although the extraction method shows some limitations for the most apolar compounds, the combination of the preconcentration provided by the SPE procedure and the efficiency and sensitivity of the UHPLC-MS/MS system allows the achievement of very low detection limits, between 53.3 and 146 ng/kg for this kind of analytes. In addition to sensitivity, on-line SPE coupled to UHPLC-MS/MS provides high selectivity, more repeatability, minimum sample preparation and automation of the process.

The concentrations measured in the sludge samples indicate that, due to the physico-chemical characteristics, an appreciable amount of the studied compounds that enters into WWTPs is accumulated in this kind of solid matrices. An accumulation of BUVSs in the sediments around the marine outfall also occurs.

This procedure is a viable, selective and easy analytical tool that can be used on environmental samples or solid products from WWTPs.

This work was supported by funds provided by the Spanish Ministry of Science and Innovation, Research Project CTQ2010–20554. Sarah Montesdeoca Esponda would like to thank the Canary Agency of Investigation, Innovation and Information Society for her PhD Student Grant.

The authors have declared no conflict of interest.

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III.2.3. Desarrollo de un método basado en la extracción por adsorción con barras agitadoras y desorción liquida para la determinación de benzotriazoles estabilizadores de luz UV en muestras de agua por cromatografía liquida de ultra resolución con detector de espectrometría de masas en tándem.

Este trabajo propone una metodología de extracción, basada en la SBSE, para la extracción miniaturizada de BUVSs desde muestras de aguas residuales y agua de mar. La determinación fue nuevamente llevada cabo con UHPLC-MS/MS.

En la extracción usando barras agitadoras, las variables que deben ser optimizadas son el volumen de muestra, el tiempo de extracción, la temperatura, el pH y la fuerza iónica del medio. Manteniendo fijo el volumen de muestra, se aplicó un diseño experimental 2^4 para las otras cuatro variables, resultando las más determinantes la fuerza iónica (reduciendo notablemente la eficiencia de extracción) y la temperatura. Dado que esta última estaba fuertemente influenciada por el tiempo de extracción, estas dos variables fueron estudiadas más detenidamente en un nuevo diseño factorial 3^2.

Por otro lado, para el tipo de desorción empleada en este caso, la desorción líquida, deben estudiarse la naturaleza del desorbente, el tiempo y la temperatura. El agente desorbente seleccionado fue el acetonitrilo, que puede ser inyectado directamente en el sistema de UHPLC.

El procedimiento fue aplicado tanto en agua Milli-Q como en muestras de agua de mar y agua residual, obteniéndose límites de detección en el rango 14.8-41.7 $ng \cdot L^{-1}$, 18.4-51.1 $ng \cdot L^{-1}$ y 19.1-55.1 $ng \cdot L^{-1}$, respectivamente.

A pesar de que se detectó ningún compuesto en las muestras de agua residual y agua de mar analizadas, los estudios de recuperación realizados en las distintas matrices mostraron buenas eficiencias para cinco de los siete BUVSs (entre 68 y 92%). Para los dos compuestos con carácter más apolar, el UV 571 y el UV 360, los resultados obtenidos fueron más bajos, probablemente porque quedan fuertemente retenidos en la fase extractante de PDMS.

Este trabajo ha sido aceptado para su publicación en *Journal of Separation Science* y se encuentra actualmente en proceso de producción.

UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Editor of *Journal of Separation Science*

Dear Editor,

I´m enclosing you the manuscript entitled: "**DEVELOPMENT OF SENSITIVE DETERMINATION METHOD FOR BENZOTRIAZOLE UV STABILIZERS IN ENVIROMENTAL WATER SAMPLES WITH STIR BAR SORPTION EXTRACTION AND LIQUID DESORPTION PRIOR TO ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY"** for your consideration for publication in *Journal of Separation Science.*

In this paper we optimise, develop and apply a method based on stir bar sorptive extraction (SBSE) followed to ultra-performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS) for the determination of a group of UV absorbing compounds, the benzotriazole UV stabilizers (BUVSs), used in different personal care products (PCPs).

BUVSs have been described as mutagenic, toxics, bioaccumulative, and also pose a significant estrogenic activity. The emissions sources are often focused in "hot-spots" of discharge such as Wastewater Treatment Plants (WWTPs) besides of discharges from swimming poll waters or from or recreational activities such as swimming and bathing in beaches, lakes or rivers (direct inputs). Its continuous introduction in the environment allows them to pseudo-persist. Taking account these sources of contamination, we applied satisfactorily our developed method to seawater and wastewater samples from Gran Canaria Island, Spain.

To our knowledge, no other research group has published a method for the determination of BUVSs using SBSE followed by UHPLC-MS/MS. In fact, only our group had published a prior paper (in this Journal) analysing BUVS in liquid environmental samples using liquid chromatography.

Las Palmas de Gran Canaria, 21 February 2013.

Sincerely yours,

Prof. Dr. José Juan Santana Rodríguez

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Body: 03-Apr-2013

Dear Prof. Santana Rodriguez:

Thank you for submitting your revised manuscript entitled "Development of sensitive determination method for benzotriazole UV stabilizers in environmental water samples with stir bar sorption extraction and liquid desorption prior to ultra-high performance liquid chromatography with tandem mass spectrometry" to the Journal of Separation Science. It is a pleasure to accept your manuscript in its current form for publication.

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DEVELOPMENT OF SENSITIVE DETERMINATION METHOD FOR BENZOTRIAZOLE UV STABILIZERS IN ENVIROMENTAL WATER SAMPLES WITH STIR BAR SORPTION EXTRACTION AND LIQUID DESORPTION PRIOR TO ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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Abstract

Benzotriazole ultra-violet stabilizers are emerging compounds used in personal care products and can enter surface water after passing through wastewater treatment plants without be removed. Because these analytes are strongly hydrophobic, there is an environmental risk of accumulation in solid matrices and magnification through the trophic chain. In this work, a method based on stir bar sorption extraction with liquid desorption is presented for the extraction of Benzotriazole ultra-violet stabilizers from water samples. Stir bar sorptive extraction was combined with ultra-high performance liquid chromatography with tandem mass spectrometry detection. All important factors affecting the stir bar sorptive extraction procedure are discussed, and the optimised method was applied to seawater and wastewater samples from Gran Canaria Island, providing good selectivity and sensitivity with limits of detection and limits of quantification in the range of 18.4-55.1 and 61.5-184 $ng \cdot L^{-1}$, respectively. Recoveries between 68.4-92.2 % were achieved for the more polar compounds, whereas the recoveries were lower for the two less polar compounds, most likely due to their strong absorption into the polydimethylsiloxane stir bar phase that does not allows the complete desorption. The repeatability studies gave relative standard deviations of between 6.45 and 12.6 % for all compounds in the real samples.

Keywords: Benzotriazols; Liquid desorption; Stir Bar Sorption Extraction; Ultra-high performance liquid chromatography; Tandem mass spectrometry

1. Introduction

Benzotriazole UV stabilizers (BUVSs) are compounds added to cosmetic products employed as UV filters and personal care products (PCPs), such as sunscreen, soap, shampoo, hair dye, makeup and lip gloss [1]. Benzotriazole has a heterocyclic structure with three N atoms and the molecular formula $C_6H_5N_3$. Its derivatives, the UV stabilizers, have a phenolic group attached to the benzotriazole structure that absorbs the full UV spectrum, both UV-A (320-400 nm) and UV-B (280-320 nm) [2]. Thus, due in part to its wide range of uses, BUVSs is an emerging contaminant, a term which has gained environmental relevance because these contaminants are not completely removed by wastewater treatment plants (WWTPs) and can enter surface waters.

Because of their numerous applications, the hazards of these compounds in the environment and their measureable concentrations must be determined. In contrast to the polar benzotriazole specie, its phenolic derivatives are hydrophobic and may accumulate in solid environmental matrices; they may even be magnified through the trophic chain [3]. Several studies have revealed significant BUVSs concentrations in different matrices, such as seawater [4], river water [5], raw wastewater [3], treated wastewater [4], river sediments [6], marine sediments [7], sludges from wastewater treatment plants [7, 8] and organisms, including fish [9] and even mammals [10].

Usually, a preconcentration step is necessary for dilute environmental water samples, such as seawater. Solid phase extraction (SPE) is the most common procedure employed for this purpose in the environmental analytical studies [11]. However, in the last several decades, several microextraction techniques have appeared and developments in analytical chemistry have led to the simplification of extraction procedures and the minimisation of volumes used [12]. One miniaturised technique is stir bar sorption extraction (SBSE), a relatively new procedure developed by Baltussen et al. in 1999 [13].

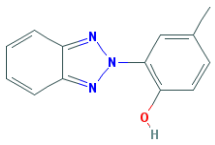
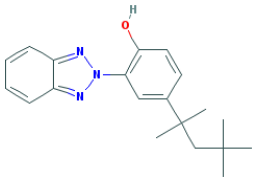
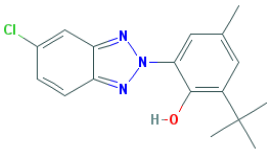
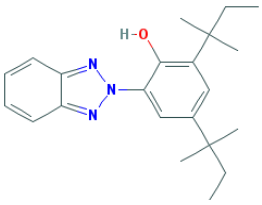
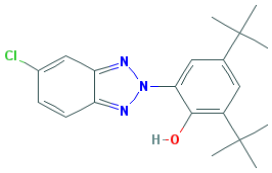
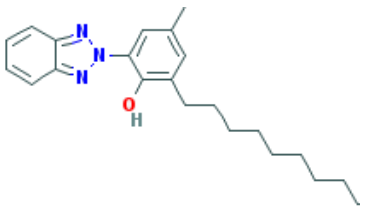
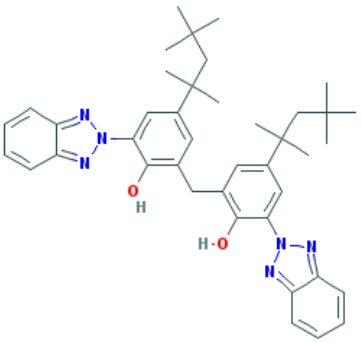
SBSE involves the extraction of the analytes from the matrix using a magnetic stir bar with a coating of polydimethylsiloxane (PDMS), a non-polar polymeric phase with a large affinity for low-polarity species [14]. Although other coatings for polar compounds have been introduced recently, their use is not yet widespread. The stir bar is then removed from the aqueous sample and subjected to a desorption process, namely, thermal desorption (TD) or liquid desorption (LD), before chromatographic separation and detection [15]. TD presents the advantage of allowing the complete introduction of the extract in the

chromatographic system but is a "one-shot" process; once the sample had been desorbed, the sample cannot be re-injected [16]. However, although TD is the most straightforward desorption mode, it is limited to thermally stable volatile and semi-volatile solutes and gas chromatography. Liquid desorption (LD) is an alternative to TD that allows the analysis of thermally labile solutes and the use of liquid chromatography separation. In LD mode, the polymer-coated stir bar is immersed in a stripping solvent for the chemical desorption of the extracted solutes. LD is also more suitable than TD for minimising contamination from PDMS phase. Moreover, it is compatible with liquid chromatography employing any auxiliary equipment because it does not require expensive desorption units or interfaces [17]. SBSE is based on the same principles as solid phase microextraction (SPME) but with a higher volume of the PDMS phase, resulting in a better sample capacity and extraction efficiency [18]. Thus, besides providing efficient extraction without high solvent consumption [19], SBSE offers additional advantages, such as cost-effectiveness, more effective separation from the matrix and the preconcentration of the analytes [20] as well as avoiding further clean-up steps [21]. The stir bar device is more robust and cheaper than SPME fibres [14], being able to be reused at least for 100 times [20]. SBSE is adequate for on-site or passive air sampling because the loaded stir bars can be stored at 4ºC for 1 week without loss of solutes [21]. This technique has been applied successfully to a wide variety of matrices (wastewater [22], food and drink [23], human serum [24]) and for the determination of completely different compounds, such as endocrine-disrupting substances [25], antibiotics [26] or PAHs [27]. It is most effective for the extraction of non-polar compounds, such as the BUVSs studied herein.

To develop an analytical procedure that allows the selective and precise quantification of benzotriazole UV filters at very low concentration in environmental liquid samples, we employed ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) for the determination of BUVSs in coastal seawater samples and wastewater samples from WWTPs after SBSE with liquid desorption (LD). To the best of our knowledge, this study is the first to use SBSE-LD-UHPLC-MS/MS for the determination of this type of compounds.

Although the compounds studied in this work (UV P, UV 329, UV 326, UV 328, UV 327, UV 571 AND UV 360, see Table 1) have been approved for the European community [28] and studied by the International Nomenclature of

Table 1. List of BUVSs, IUPAC name, chemical structure and pk_a and log K_{ow} values.

Compound	IUPAC name	Chemical structure	pK_a	Log K_{ow}
UV P	2-(benzotriazol-2-yl)-4-methylphenol		8.2	4.3
UV 329	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl) phenol		8.1	7.4
UV 326	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol		9.5	6.6
UV 328	2-(benzotriazol-2-yl)-4,6-bis(2-methybutan-2-yl) phenol		8.9	7.5
UV 327	2,4-ditert-butyl-6-(5-chlorobenzotriazol-2-yl)phenol		9.4	7.8
UV 571	2-(benzotriazol-2-yl)-6-dodecyl-4-methyl phenol		8.4	10.3
UV 360	2-(benzotriazol-2-yl)-6-[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl)phenyl]methyl]-4-(2,4,4-trimethylpentan-2-yl)phenol		7.6	14.5

pk_a and log K_{ow} values obtained from SciFinder Scholar Database (Temp: 25 °C)

Cosmetic Ingredients (INCI) [29] and the International Fragrance Organization (IFRA) [30], some of them have also been identified by the USEPA (United States Environmental Protection Agency) as compounds of unknown toxicity [31].

2. Experimental

2.1 Reagents and materials

The analysed BUVSs (Table 1) were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions (250 $\mu g \cdot mL^{-1}$) were prepared in methanol and stored in glass-stoppered bottles at 4ºC prior to use. Working solutions were prepared daily in ultrapure water provided by a Milli-Q system (Millipore, Bedford, MA, USA). The mobile phase was prepared with LC-MS grade methanol and formic acid obtained from Panreac Química (Barcelona, Spain). For the pH measure we employed a Crison micro-pH 2002 that provides a accuracy of ±0.01. The acetonitrile employed in the desorption and the dichloromethane used to remove impurities from the PDMS stir bars was purchased from Panreac Química. The 20 mm x 0.5 mm, 47 µL PDMS stir bars employed were commercialised by Gerstel (Mulheim and der Ruhr, Germany).

2.2 Chromatography and mass detection

The analytical system included an ACQUITY UHPLC BEH C_{18} column (50 mm x 2.1 mm, 1.7 µm particle size), an ACQUITY Binary Solvent Manager (BSM), a 2777 autosampler and a triple quadrupole detector (TQD) with an electrospray ionisation (ESI) interface controlled by MassLinx Mass Spectrometry software (Waters Chromatography, Barcelona, Spain). Isocratic mobile phase consisting in methanol adjusted to pH 2.5 with 0.1 % (v/v) formic acid was used for the separation of BUVSs at a flow rate of 0.8 $mL \cdot min^{-1}$. The sample volume injected was 10 µL, and the electrospray ionisation parameters were fixed as follows: capillary voltage of 3 kV, source temperature of 120ºC, desolvation temperature of 450ºC and desolvation gas flow rate of 800 L/hr. Nitrogen was used as the desolvation gas, and argon was employed as the collision gas. The detailed MS/MS detection parameters for each BUVS are presented in Table 2 and were optimised by the direct injection of a 1 $mg \cdot L^{-1}$ standard solution of each analyte into the detector at a flow rate of 10 µL/min. Under these conditions, UHPLC-MS/MS allows for the chromatographic elution of the seven compounds studied in less than one minute.

2.3 Extraction technique

A stir bar is introduced into a glass vessel containing 25 mL of the sample with 30 % (v/v)

Table 2. Mass spectrometer parameters for BUVSs detection.

Compound	Precursor ion *(m/z)*	Cone voltage (V)	Quantification ion *(m/z)*	Collision potential (V)	Confirmation ion *(m/z)*	Collision potential (V)
UV P	226.2	40	107.1	20	120.1	20
UV 329	324.2	50	57.0	25	212.2	25
UV326	316.3	40	260.2	20	107.1	25
UV 328	352.3	50	71.0	30	282.2	20
UV 327	358.3	60	302.3	20	246.1	30
UV 571	394.3	50	226.2	20	120.1	30
UV 360	658.6	40	336.3	25	224.2	35

of methanol as an organic modifier and stirred at 700 rpm for 120 min at 40ºC. The stir bar is then removed from the sample using a magnetic rod, rinsed with ultrapure water, dried with a lint-free tissue and immersed in a glass vial with 1.5 mL of acetonitrile for the desorption of the analytes. This vial is ultrasonicated for 20 min at 60ºC.

A clean-up step is necessary between each extraction to avoid carryover effects. For this purpose, the stir bar is inserted in 20 mL of acetonitrile/dichloromethane (1:1, v/v) and stirred at 700 rpm for 15 min. After this clean-up step, we verified that no amount of any analyte was recovered in a second desorption.

2.4 Sample collection

Three seawater samples were collected from different tourist beaches around the coast of Gran Canaria Island (Spain) at a depth of approximately 1 m. Additionally, three wastewater samples were collected from different WWTP effluents located on the same island. Both the seawater and wastewater samples were taken using 1 L amber glass bottles containing 30 % (v/v) methanol to avoid the adsorption of the compounds to the glass flask. Next, the samples were filtered through a 0.45 μm cellulose acetate filter, acidified at pH = 3 with formic acid and analysed immediately.

2.5 Preparation of spiked samples

The seawater and wastewater samples used for the application of the optimised SBSE procedure to real samples were spiked with a solution prepared in methanol at two concentration levels, 250 and 10000 $ng \cdot L^{-1}$, for each analyte. Prior to spiking, an analysis of the samples showed that they did not contain any signals in the chromatogram for the BUVSs under investigation.

3. Results and discussion

3.1 Extraction process

To optimise the conditions for the extraction of the target analytes using SBSE, different experimental assays were carried out in ultrapure water spiked with a BUVSs mixture in methanol to give a final concentration of 20000 $ng \cdot L^{-1}$ for each analyte. For the extraction process we must consider and optimise several parameters, such as sample volume, extraction time, temperature, pH and ionic strength, which will govern the partition coefficients of the analytes between both phases, PDMS and water ($K_{PDMS,W}$). These $K_{PDMS,W}$ coefficients are correlated with the octanol-water distribution coefficients (log K_{ow}) [32], which theoretically favours the extraction of our target analytes because they are rather non-polar.

Regarding the ionic strength of the sample, the literature generally indicates that the addition of salts reduces the extraction of the non-polar compounds (log K_{ow} >3.5), favouring the more polar compounds [14,16,17,33,34]. There are several explanations for the decreased response of hydrophobic compounds, such as BUVSs, with increasing ionic strength. One of these explanations is that the salt causes an "oil effect" that promotes the movement of the analytes to the water surface and minimises the interaction with the PDMS phase [35]. Other factors that can explain the low interaction between the analyte and the PDMS phase are the increase of the viscosity [36] or the occupation of the superficial area of PDMS with salt ions [37].

Another important parameter is the sample pH. When we apply SBSE to analytes with acidic or basic characters, the sample pH must be adjusted such that the solute is partially or totally in its non-ionic form to improve the extraction efficiency [17]. Because the BUVSs under study have pK_a values of 7.6-9.5, the best sample pH for this purpose will most likely be in the range 5-7. In compounds with strong non-polar character, the distribution between the aqueous phase and the PDMS phase will be more strongly influenced by the polarity than the pH.

Temperature and extraction time are strongly linked because high temperatures allow the system to reach equilibrium more quickly. However, although PDMS is thermally stable and can be used in a wide range of temperatures [17], high temperatures can reduce the lifetime of the stir bar [32]. For this reason, temperatures above 70ºC are not usually employed. Although the optimum time for the extraction process is described as the time necessary to reach the equilibrium conditions, sometimes analysts choose a shorter time near the equilibrium to reduce the analysis time. Even if this process is quite complex, involving thermodynamic and

kinetic aspects, the equilibrium time increases with the sample volume [21].

To optimise all of these experimental variables, we have applied an experimental design that has enabled the identification of the interactions among the different variables over the SBSE process. Thus, we established a 2^4 factorial design with two levels per parameter for the extraction time (30 and 240 min) temperature (25 and 40ºC), pH (5 and 7) and ionic strength (0 and 30 % of NaCl). We fixed some conditions, such as the stirring speed (we chose a stirring of 700 rpm because the use of a higher stirring speed can jeopardise the integrity of the stir bar) and sample volume (25 mL, because 47 µL of PDMS can ensure enough sorptive power for water samples in the range of 10-50 mL [16]). Although a more sensitive response is achieved by increasing the sample volume because more analyte is available, this increase in the signal is not proportional to the increment of volume because the recovery efficiency is reduced when the sample volume increases [34]. Moreover, we added some methanol to the sample to improve the extraction efficiency. Although the addition of an organic modifier such as methanol has been used to avoid losing the most lipophilic compounds during the sample pre-treatment due to adsorption onto the glass flask [21], the methanol also increases the solubility of the compounds in the solution, therefore decreasing the partitioning into the PDMS phase [32]. Different percentages of organic modifier were tested, and 30 % methanol was chosen as a compromise between the two behaviours. The desorption was initially carried out with 1.5 mL of methanol at 25ºC during 30 min.

Using the results obtained in this first screening phase, we calculated the different correlations between the variables (bivariate correlations) and their influence on the recoveries (partial correlations) for maxima correlations of +1 and -1. Figure 1 shows the partial correlation coefficients, which indicated that the ionic strength is the most influential parameter, in a negative way, due to the non-polar character of the analytes (log K_{ow} from 4.3 to 14.5, see Table 1). The second most influential factor is the temperature, with a high temperature being more favourable for almost all the compounds. Only UV P seems to be unaffected as the temperature is increased. The responses generated for the pH changes are slightly better for pH = 5 than for pH = 7, but as we can expect, no significant effect was observed. The extraction time is strongly correlated with the temperature (both parameters have the higher bivariate correlation) and provides better results when the upper-end value of the experimental design is employed. This trend is clearer for the analytes with intermediate

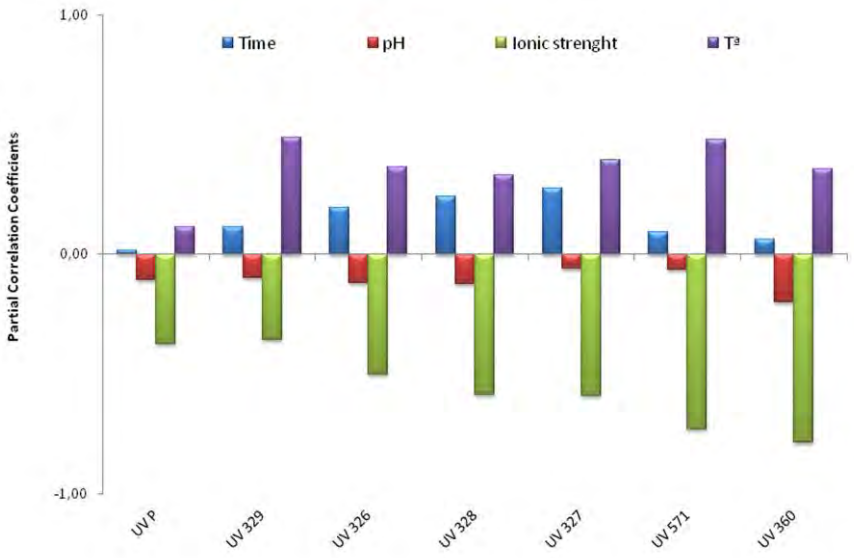


Figure 1. Partial Correlations Coefficients for the effect of extraction time, sample pH, ionic strength and temperature (being maxima correlations +1 and -1).

log K_{ow} values, given that UV P is not affected and the two more non-polar analytes do not improve greatly.

Due to the negative effect of inert salts, we chose to omit salt addition in further assays. We selected the time and temperature for a second 2^3 (2 variables, 3 levels) screening phase with intermediate values. We observed that an increase in the extraction time yields greater responses but only until a certain time, after which the extraction efficiency cannot be improved. To avoid unnecessary analytical time, we chose 120 min as the optimum extraction time. Meanwhile, an intermediate temperature value does not provide the best results, so we decided to maintain 40ºC as a suitable condition for the extraction. Figure 2 shows the response surface for the effect of both parameters on the UV 328 extraction. The same trend was observed for the other compounds.

3.2 Desorption process

The variables that can affect the liquid desorption (LD) process are solvent type, desorption time and temperature. Polar solvents such as acetonitrile and methanol are the most commonly employed in LD [17], whereas non-polar solvents can have a strong partition into the

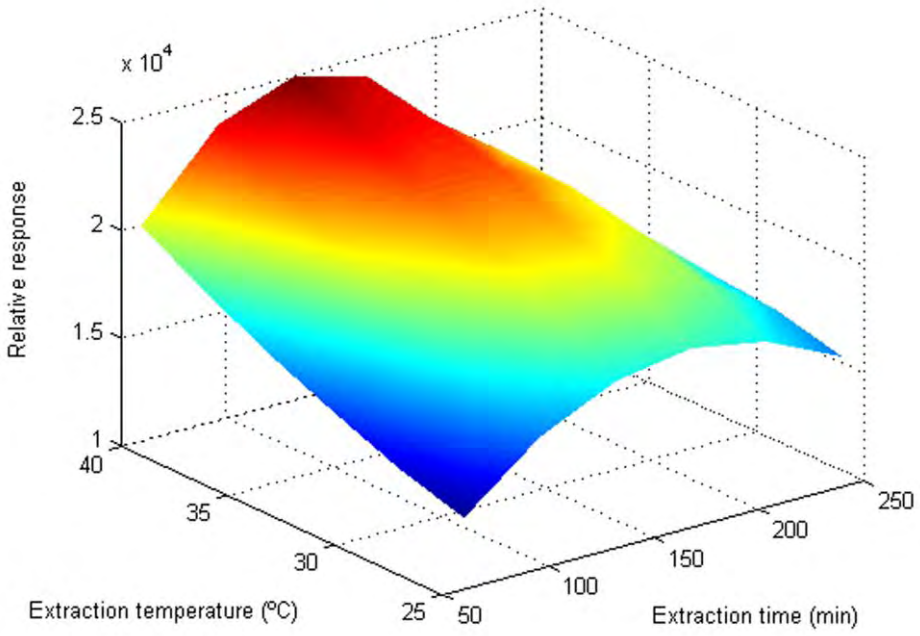


Figure 2. Response surface for the effect of temperature and extraction time on the UV 328 extraction (20 ng·mL^{-1}) using SBSE-LD-UHPLC-MS/MS.

PDMS phase, resulting in the undesirable effect of a substantial increase in the weight of the stir-bar, leading to low recoveries [38]. The desorption times are lower than extraction times and the process can be accelerated by mechanical shaking, temperature increases or sonication [17]. Thus, the nature of the solvent, the time and the temperature were studied under sonication because it is adequate for accelerating the stripping of the analytes from the stir bar [16]. Among the selected solvents, namely, acetonitrile, methanol and the mixture thereof, acetonitrile offered the best results as desorption solvent. Regarding the temperature, temperatures higher than 60ºC did not have a significant effect on the desorption. Thus, we chose 60ºC to increase the lifetime of the stir bar.

Finally, desorption times between 5 and 30 min were studied, yielding an increase of the signal until 20 min. After this point, the response decreases (Figure 3).

In summary, the optimised SBSE conditions are as follows: 120 min at 40ºC and a sample with 0 % NaCl and pH = 5 for the extraction step; 20 min at 60ºC using 1.5 mL of acetonitrile for the desorption step. Given that the sample volume is 25 mL and the final volume of the extract is 1.5

mL, the theoretical preconcentration factor is 16.6.

3.3 Analytical parameters

Separate matrix-matched calibration curves for Milli Q water, seawater and wastewater were generated using six concentration levels (between 30-250 ng·L^{-1} for UV P, UV 329, UV 328, UV 571 and UV 360 and 60-250 ng·L^{-1} for UV 326 and UV 327), where each point corresponds to the mean value obtained from three area measurements. Satisfactory linear ranges were obtained with correlation coefficients greater than 0.990 for all analytes (regression equations are showed in Table 3). The detection and quantification limits (LODs and LOQs, respectively) were calculated from the signal-to-noise ratios of the individual peaks, assuming minimum detectable signal-to-noise levels of 3 and 10, respectively. The LODs obtained were in the range 14.8-41.7 ng·L^{-1} for Milli Q water, 18.4-51.1 ng·L^{-1} for seawater and 19.1-55.1 ng·L^{-1} for wastewater. The LOQs were in the range 49.4-139 ng·L^{-1} for Milli Q water, 61.5-170 ng·L^{-1} for seawater and 63.7-184 ng·L^{-1} for wastewater (Table 3). These LODs and LOQs are slightly higher in seawater and wastewater than those obtained for Milli Q water, most likely because the extraction efficiency is poorest in real water due to the presence of salt and organic matter.

To evaluate the precision, expressed as relative standard deviation (% RSD), six replicate samples at two concentration levels (250 and 10000 ng·L^{-1}) were employed, obtaining repeatabilities below 13 % in all cases (see Table 3). In general, the repeatability is comparable for Milli Q water and seawater, whereas a slightly higher variability is observed for the wastewater samples, which were the dirtiest. Figure 4 shows the chromatograms obtained for seawater and wastewater samples spiked with 250 ng·L^{-1} of the BUVS mixture.

3.4 Analysis of seawater and wastewater samples

Once the analytical parameters of the SBSE-LC-UHPLC-MS/MS method were obtained, it was used for the analysis of spiked and non-spiked seawater samples from three different beaches of Gran Canaria Island and three wastewater samples from WWTPs with different treatments to demonstrate its applicability. The results for the non-spiked samples showed no detection of the studied BUVSs. Concerning the fortified samples, two concentration levels (250 and 10000 ng·L^{-1}) were spiked for each sample type and the recoveries were also compared with those obtained for Milli Q water (Table 4). The results are expressed as the average of three determinations and indicate similar results, with good extraction efficiencies

Table 3. Analytical parameters for SBSE-LD-UHPLC-MS/MS.

	LODs[a] ($ng \cdot L^{-1}$)			LOQs[b] ($ng \cdot L^{-1}$)			RSDs (%) n=6						Regression equations
							250 $ng \cdot L^{-1}$			10000 $ng \cdot L^{-1}$			
	Milli Q water	Seawater	Waste-water	Milli Q water	Seawater	Waste-water	Milli Q water	Seawater	Waste-water	Milli Q water	Seawater	Waste-water	
UV P	22.5	28.3	30.4	75.3	94.4	102	7.22	7.48	9.03	6.32	6.99	9.76	y = 210.46 x + 910.96
UV 329	14.8	18.4	19.1	49.4	61.5	63.7	7.14	7.44	9.44	6.77	6.45	9.10	y = 1355.6 x + 2232.9
UV 326	41.7	51.1	55.1	139	170	184	7.24	10.43	12.6	8.31	8.65	10.5	y = 3.8201 x + 60.675
UV 328	18.6	20.1	25.7	62.3	63.0	85.7	7.99	9.42	11.3	6.33	7.04	8.22	y = 361.04 x – 299.59
UV 327	33.0	38.9	43.8	111	130	146	8.22	8.21	11.1	7.43	8.43	11.3	y = 16.465 x – 14.572
UV 571	19.3	26.9	22.7	64.4	89.6	75.7	6.47	9.01	11.8	7.31	7.88	10.4	y = 24.362 x – 101.51
UV 360	19.2	22.9	23.2	64.1	76.3	77.4	7.31	8.92	10.3	5.43	7.00	9.34	y = 252.42 x – 496.73

[a] Detection limits are calculated as signal to noise ratio of three times; [b] Quantification limits are calculated as signal to noise ratio of ten times

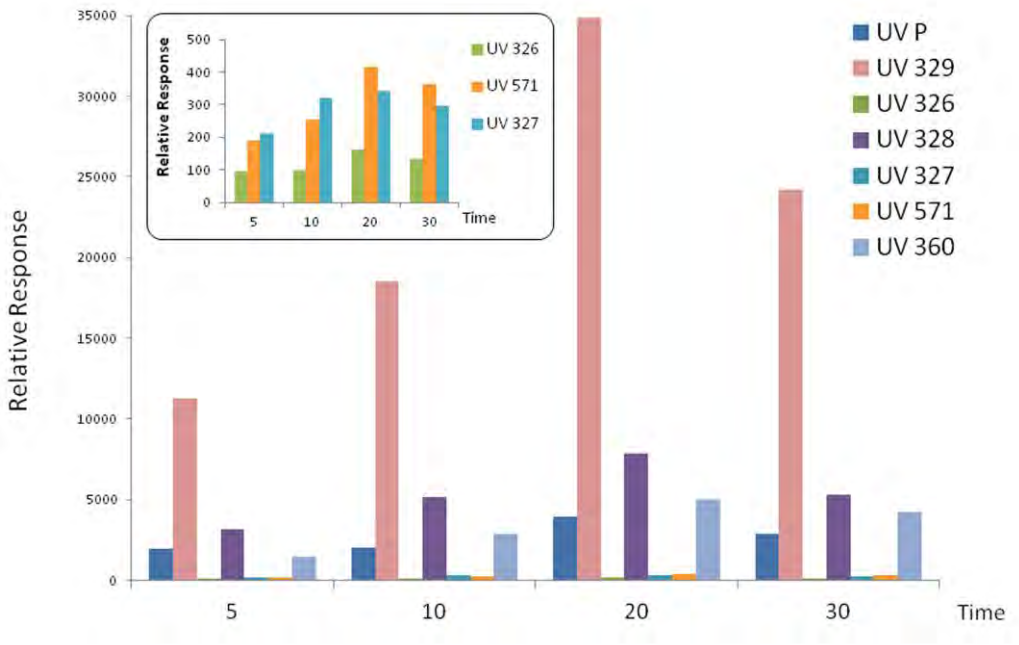


Figure 3. Effect of the desorption time in the SBSE-LD-UHPLC-MS/MS method (Milli-Q water containing a final concentration of 20 ng·mL^{-1} of each BUVS).

Table 4. Recoveries (n=3) obtained for the application of SBSE-LD-UHPLC-MS/MS to Milli Q water, seawater and wastewater samples.

	250 ng·L^{-1}			**10000 ng·L^{-1}**		
	Milli Q water	**Seawater**	**Wastewater**	**Milli Q water**	**Seawater**	**Wastewater**
UV P	82.2 ± 9.37	83.7 ± 10.4	75.1 ± 14.4	84.8 ± 7.42	86.0 ± 9.21	75.8 ± 10.0
UV 329	87.3 ± 9.85	85.0 ± 10.4	77.4± 12.3	91.1 ± 7.33	85.0 ± 9.37	79.2± 9.7
UV 326	90.2 ± 10.1	90.4 ± 10.3	80.9 ± 14.0	91.0 ± 7.27	92.2 ± 8.44	83.3 ± 9.9
UV 328	77.3 ± 7.42	76.1 ± 10.8	76.2 ± 12.8	77.8 ± 8.91	77.9 ± 9.65	75.7 ± 10.2
UV 327	71.4 ± 8.99	68.4 ± 9.61	69.6 ± 14.7	73.8 ± 6.31	69.6 ± 8.24	70.6 ± 11.4
UV 571	45.2 ± 13.6	45.7 ± 12.6	40 .4 ± 15.1	47.0 ± 9.16	45.5 ± 10.4	41 .3 ± 12.2
UV 360	20.6 ± 12.7	18.4 ± 10.7	18.3 ± 14.4	22.1 ± 9.74	19.9 ± 11.4	19.8 ± 11.2

ranging from 68.40-92.2 % for five of the seven BUVSs under study in the three types of samples. For two of the compounds, UV 571 and UV 360, lower recoveries were obtained, with values between 40.4 and 45.7 % for UV 571 and 18.3 and 22.1 % for UV 360. High RSDs are also obtained, with values in the range of 9.16-15.1 %. Given that these compounds are the less polar substances under study and that SBSE using PDMS favours the extraction of non-polar compounds, the low recoveries are most likely due to an incomplete desorption rather than a problem of extraction in the stir bar. The log K_{ow} values for UV 571 and UV 360 are 10.3 and 14.5, respectively, which mean that these compounds have a great capability for leaving the aqueous media and being absorbed into the organic phase. Once absorbed, the acetonitrile most likely lacks a sufficient capacity to achieve complete desorption despite the use of high temperature and ultrasonication. To overcome this problem, we could use stronger solvents, such as n-pentane or diethyl ether, but we prefer avoid very dangerous and contaminating products. Moreover, the use of very non-polar solvents involves an extra step or evaporation and reconstitution prior to injection in the chromatographic system, which is laborious and can introduce errors as well as increasing the analysis time.

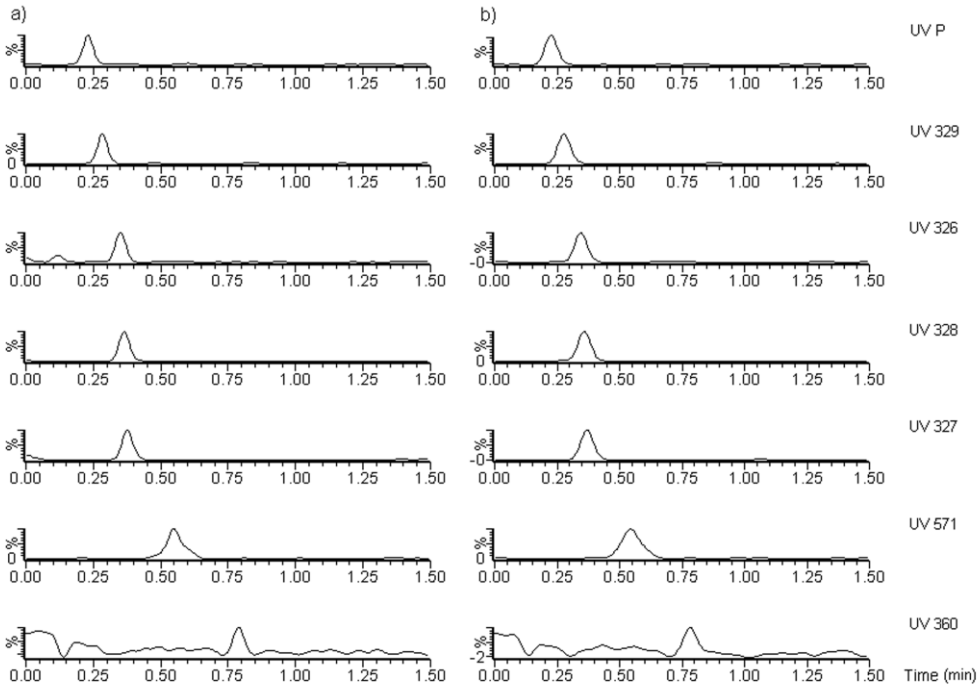


Figure 4. Chromatogram obtained for a) seawater and b) wastewater sample spiked with 0.25 ng·mL^{-1} of the BUVSs mixture using SBSE-LD-UHPLC-MS/MS.

4. Concluding remarks

A new miniaturised technique based on a stir bar coated with polydimethylsiloxane has been optimised for the extraction of the benzotriazole UV stabilizers used in personal care products. The optimised methodology coupled to ultra-high performance liquid chromatography with tandem mass spectrometry detection has allowed the efficient extraction and preconcentration of the majority of the compounds under study from seawater and wastewater samples with good repeatability and sensitivity (RSDs lower than 13 % and LODs between 18.4-55.1 $ng \cdot L^{-1}$). This extraction procedure using liquid desorption allows the minimum volume of organic solvent to be used and can be coupled with liquid chromatography, obtaining a chromatographic separation in less than one minute, without additional expensive desorption units or interfaces.

Moreover, satisfactory extraction efficiencies (68.4-92.2 %) were achieved for the less non-polar compounds. For the more non-polar analytes, with log K_{ow} 10.3 and 14.5, recoveries of approximately 20 and 40 % were obtained, respectively. These lower efficiencies are most likely due to an incomplete desorption in acetonitrile once the compounds were strongly absorbed into the polydimethylsiloxane phase.

SBSE is a relatively low cost extraction technique that can be implemented in modest laboratories that not posses very sophisticated equipments. Moreover, it is a very promising procedure due to its suitability for *in situ* sampling which applicability could be improved in the future developing new coated for the extraction of analytes of different nature.

Acknowledgments

This work was supported by funds provided by the Spanish Ministry of Science Education, Research Project CTQ2010-20554. Sarah Montesdeoca Esponda would like to thank the Canary Agency of Investigation, Innovation and Information Society for her PhD Student Grant.

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IV. Conclusiones

Del conjunto de trabajos que conforman la presente Tesis Doctoral se pueden extraer las siguientes conclusiones;

a) De la optimización y desarrollo de las técnicas de extracción:

a.1. La metodología basada en la microextracción en fase sólida (SPME) optimizada ofreció excelente linealidad y repetibilidad para las cinco fluoroquinolonas estudiadas, además de buenas eficiencias de extracción (recuperaciones mayores del 80%) para las distintas matrices líquidas estudiadas. El volumen de muestra necesario para llevar a cabo la extracción fue de sólo cuatro mililitros.

a.2. La metodología basada en la extracción en fase sólida (SPE) optimizada se mostró como una herramienta rápida y sencilla para la extracción de las cinco fluoroquinolonas estudiadas, proporcionando parámetros analíticos muy satisfactorios que permitieron su aplicación en aguas residuales procedentes de una estación depuradora y de un complejo hospitalario. Las recuperaciones calculadas para este tipo de agua estuvieron entre 82 y 94%, por lo que se puede concluir que es una técnica de extracción adecuada para fluoroquinolonas a pesar de la complejidad de la matriz.

a.3. La comparación entre la desorción/elución micelar y convencional, realizada para las técnicas de SPME y SPE en la determinación de fluoroquinolonas, mostró que los disolventes orgánicos pueden ser sustituidos por los medios micelares en ambos casos. Los medios micelares son compatibles con la

cromatografía líquida, son más baratos, no son tóxicos para el analista y son biodegradables, además de proporcionar límites de detección y cuantificación menores que los calculados empleando disolventes orgánicos.

a.4. La optimización de un procedimiento para fluoroquinolonas basado en la extracción asistida por microondas (MAE) usando medios micelares como extractantes, resultó en un procedimiento rápido, muy sencillo, de bajo coste, y altamente efectivo y reproducible (recuperaciones entre 73-96% con RSDs entre 4 y 8%) para extraer estos contaminantes desde sedimentos costeros y lodos de depuradora.

a.5. El acoplamiento de un sistema de extracción en fase sólida "en línea" con cromatografía líquida de ultra resolución (On-line SPE), permitió desarrollar con éxito un procedimiento analítico automatizado y muy simple para la determinación de benzotriazoles estabilizadores de luz UV en agua de mar y aguas residuales. Dicho procedimiento proporcionó buena repetibilidad, derivada de la escasa manipulación de la muestra, así como una preconcentración de la muestra de 500 veces.

a.6. La comparación de la extracción en fase sólida "en línea" con la extracción en fase sólida convencional (no "en línea") para benzotriazoles estabilizadores de luz UV en agua de mar y aguas residuales, demostró que el método "en línea" ofrece mayor preconcentración de la muestra ya que toda la masa de analito

retenida en el cartucho llega al detector. Con el procedimiento convencional se obtiene un factor de preconcentración de solo 50 veces (100 veces menor que usando el procedimiento "en línea"), lo que se traduce en una menor sensibilidad. Además, la extracción "en línea" muestra otras ventajas, como mayor repetibilidad, sencillez y rapidez.

a.7. La técnica basada en la extracción asistida por microondas seguida por un paso de purificación por extracción en fase sólida "en línea" (MAE-On-line SPE) optimizada para benzotriazoles estabilizadores de luz UV en sedimentos marinos y lodos de depuradora, ofreció resultados satisfactorios para casi todos los compuestos estudiados en términos de eficiencia de extracción así como de limpieza del extracto. Las recuperaciones obtenidas estuvieron entre un 61 y un 87% para seis de los siete BUVSs, mientras que sólo fue posible recuperar en torno a un 50% del UV 360, el compuesto más apolar. A pesar de esta limitada eficiencia de extracción, el procedimiento permitió detectar y cuantificar dicho compuesto tanto en sedimentos marinos como en lodos procedentes de depuradora.

a.8. La extracción basada en la absorción con barras agitadoras (SBSE) para benzotriazoles estabilizadores de luz UV optimizada proporcionó buena repetibilidad y eficiencia para casi todos los analitos, a excepción de los dos más apolares, cuyas recuperaciones fueron menores. Por otro lado, a pesar de ser una técnica miniaturizada muy robusta y sencilla y de que se ha

demostrado su aplicabilidad en términos de selectividad en las distintas matrices líquidas estudiadas, los bajos factores de preconcentración conseguidos hacen que sea algo limitada para el análisis de muestras muy diluidas como el agua de mar o cuando la presencia de los contaminantes se produce a muy bajos niveles, como es el caso de benzotriazoles estabilizadores de luz UV.

b) De las técnicas de separación y detección empleadas:

b.1. Las técnicas basadas en LC o en UHPLC tanto con detección por fluorescencia (FD) como con detección por espectrometría de masas en tándem (MS/MS) trabajando en la modalidad "*multiple reaction monitoring*" (MRM), proporcionaron el poder de identificación necesario para determinar la presencia de fluoroquinolonas y benzotriazoles estabilizadores de luz UV en distintas matrices de diferente complejidad.

b.2. El uso de la cromatografía líquida de ultra resolución (UHPLC) aportó ventajas sobre la técnica de cromatografía liquida de alta resolución (LC). La UHPLC permite separar los analitos más eficientemente (picos más estrechos y mejor definidos) y en mucho menor tiempo que la LC. En el caso de los benzotriazoles estabilizadores de luz UV, se consiguió eluir siete compuestos en menos de un minuto. Además, se usan caudales de fase móvil mucho más bajos, lo que supone un importante ahorro en disolventes orgánicos.

b.3. Las técnicas basadas en detección por fluorescencia combinadas con SPE, SPME y MAE ofrecieron una respuesta bastante competitiva en términos de sensibilidad en comparación con la detección por espectrometría de masas, alcanzándose LODs de hasta 0.01 $ng \cdot mL^{-1}$ para algunas de las fluoroquinolonas estudiadas con las técnicas SPE y SPME.

b.4. La detección por fluorescencia se vio favorecida por el uso de medios micelares, ya que estas sustancias son capaces de realzar la señal de respuesta de compuestos con propiedades fluorescentes como las fluoroquinolonas. Así, en los trabajos de SPE y SPME donde se compararon los parámetros analíticos usando disolventes orgánicos frente a surfactantes, éstos últimos proporcionaron mayor sensibilidad (con LODs en el rango de 0.07-0.20 $ng \cdot mL^{-1}$ usando MeOH y 0.01-0.03 $ng \cdot mL^{-1}$ usando POLE en SPE, y en el rango 0.02- 0.23 $ng \cdot mL^{-1}$ usando MeOH y 0.01-0.19 $ng \cdot mL^{-1}$ usando POLE en SPME).

c) De la aplicación de las metodologías a muestras reales:

c.1. Se ha estudiado por primera vez la presencia de fluoroquinolonas en muestras medioambientales de la isla de Gran Canaria. Los niveles de concentración detectados en cada una de las muestras analizadas se hallaron entre los siguientes intervalos:

- Aguas residuales procedentes de un hospital de Las Palmas de Gran Canaria, entre 46.01 y 698.8 $ng \cdot mL^{-1}$.

- Aguas residuales procedentes del tratamiento primario una EDAR de Las Palmas de Gran Canaria, entre 5.391 y 46.71 $ng \cdot mL^{-1}$.
- Aguas residuales procedentes del tratamiento secundario de una EDAR de Las Palmas de Gran Canaria, entre 0.831 y 14.08 $ng \cdot mL^{-1}$.
- Aguas residuales del efluente final de una EDAR de Las Palmas de Gran Canaria, entre 0.062 y 13.62 $ng \cdot mL^{-1}$.
- Lodos procedentes de una EDAR de Las Palmas de Gran Canaria, entre 2.98 y 206.1 $ng \cdot g^{-1}$.
- Sedimentos marinos tomados cerca de un emisario submarino de aguas residuales, entre 0.690 y 34.30 $ng \cdot g^{-1}$.

c.2. De las concentraciones encontradas tras los distintos procesos de tratamiento de la EDAR en las muestras estudiadas, se puede deducir, en una primera estimación, que la mayor reducción en las concentraciones de fluoroquinolonas en la fase disuelta se produce durante el tratamiento secundario (entre un 62 y un 72% para la levofloxacina, entre un 89 y un 96% para la norfloxacina y entre el 70 y el 74% para la ciprofloxacina). Por el contrario, entre la concentración de las aguas procedentes del tratamiento secundario y las del efluente final, la reducción es muy pequeña. La reducción total de las concentraciones de fluoroquinolonas para las muestras analizadas en la EDAR estudiada fueron de 72-76% para la levofloxacina, 98-99% para la norfloxacina y 70-79% para la ciprofloxacina. Por tanto, y aunque esta eliminación es bastante eficaz para algunos analitos, como por ejemplo la norfloxacina, queda patente la incapacidad de la EDAR para generar aguas totalmente libres de estos

contaminantes y la necesidad de tratamientos terciarios específicos para alcanzar una eliminación completa.

c.3. Se ha estudiado por primera vez la presencia de benzotriazoles estabilizadores de luz UV en distintas muestras medioambientales de la isla de Gran Canaria. Los niveles de concentración encontrados en cada una de las muestras analizadas resultaron estar en los siguientes intervalos:

- Agua de mar tomada en distintos puntos de la costa de la isla de Gran Canaria, 0.003-0.005 $ng \cdot mL^{-1}$.
- Aguas residuales procedentes de distintas EDARs de la isla de Gran Canaria, 0.004-0.013 $ng \cdot mL^{-1}$.
- Lodos procedentes de una EDAR de Las Palmas de Gran Canaria, 0.940-12.20 $ng \cdot g^{-1}$.
- Sedimentos marinos tomados cerca de un emisario submarino de aguas residuales, 0.180-24.00 $ng \cdot g^{-1}$.

Conclusions

The following conclusions can be obtained from the whole of the work presented in this Doctoral Thesis;

a) About the optimization and development of the extraction techniques:

a.1. The optimized methodology based in solid phase microextraction (SPME) provided excellent linearity and repeatability for the five studied fluoroquinolones, besides good extraction efficiencies (recoveries higher than 80%) for the different studied liquid matrices. The required sample volume for the extraction was only four millilitres.

a.2. The optimized methodology based in solid phase extraction (SPE) was found to be a quick and simple tool for the extraction of the five studied fluoroquinolones, providing very satisfactory analytical parameters that allowed its application in WWTP and hospital wastewater samples. The calculated recoveries for this kind of matrices was between 82 and 94%, therefore can be concluded that it is an adequate extraction technique for fluoroquinolone despite the complexity of the matrices.

a.3. The comparison between micellar and conventional desorption/elution steps, carried out for the developed SPME and SPE methodologies in the determination of fluoroquinolones, showed that the conventional organic solvents can be replaced by surfactants in both cases. Micellar media are compatible with liquid chromatography, being cheaper than organic solvents,

biodegradable and not harmful for the analysts. Moreover, they provide lower limits of detection and quantification than those estimated when organic solvents are employed.

a.4. The optimisation of an analytical procedure for fluoroquinolones in solid samples based on microwave assisted extraction (MAE) with micelar media as extractants, resulted in a very fast and simple procedure, with a low cost, and highly effective and reproducible (recoveries between 73-96% with RSDs ranging from 4 to 8%) for the extraction of these pollutants from coastal sediments and sewage sludge samples.

a.5. The coupling of a solid phase extraction technique with ultra high performance liquid chromatography (On-line SPE), allowed develop successfully an analytical procedure for the automated and simple determination of benzotriazole UV stabilizers in seawater and wastewaters samples. This procedure provided good repeatability, mainly because of the lack of sample manipulation, as well as an important preconcentration of the sample (500 times).

a.6. The comparison of both On-Line and Off-Line SPE techniques for benzotriazole UV stabilizers in seawater and wastewaters, has demonstrated that the On-Line SPE system offers higher preconcentration factors because all of the mass of the analytes retained in the SPE column reach the detector. Conventional procedure provide a preconcentration factor of just 50 (100 times less than

those obtained in the On-Line procedure), which means lower LODs and LOQs. In addition, On-Line showed other advantages such as better repeatability, simplicity and quickness.

a.7. The optimised methodology based on microwave assisted extraction followed by a purification step with On-Line solid phase extraction (MAE-On-Line SPE) for the determination of benzotriazole UV stabilizers in marine sediments and sewage sludges, offered satisfactory results for almost all of the studied analytes in terms of extraction efficiency and cleaning up. The recoveries obtained ranged from 61 to 87% for six of the seven BUVSs, whereas it was only possible to recover about 50% of UV 360, which is the most non polar compound. However, in spite of this limited extraction efficiency, the developed procedure allowed to detect and quantify this compound in both marine sediments and sewage sludge samples.

a.8. The stir bar sorptive extraction (SBSE) technique, optimised for benzotriazole UV stabilizers, provided good repeatability and efficiency for almost all of the analytes, with the exception of two most non polar compounds, which recoveries were lower. On the other hand, in spite of SBSE is considered as a very robust and simple miniaturised technique and having being demonstrated its applicability in the different liquid matrices studied in terms of selectivity, the considerably low preconcentration factors achieved became it into an slightly limited technique for the analysis of very diluted samples such as

seawater, or in those cases where the pollutants are present in very low concentrations, as occur with benzotriazole UV stabilizers.

b) About the employed separation and detection techniques:

b.1. LC and UHPLC based techniques with both fluorescence detection (FD) and with tandem mass spectrometry detection (MS/MS) working in the mode "multiple reaction monitoring" (MRM), provided the identification power required to determine the presence of fluoroquinolones and benzotriazole UV stabilizers in various matrices of different complexity.

b.2. The use of ultra high performance liquid chromatography (UHPLC) provided advantages over conventional liquid chromatography (LC). UHPLC enables a more efficient separation of the analytes (narrower peaks and better defined) and in a considerably less time than in LC. In the case of benzotriazoles UV stabilizers, the complete elution of the seven selected analytes was achieved in less than one minute. In addition, in UHPLC the mobile phase flow rates employed are much lower than in LC, which means a significant savings in organic solvents.

b.3. The techniques based on fluorescence detection combined with SPE, SPME and MAE, offered a quite competitive response in terms of sensitivity when they were compared to the techniques based on mass spectrometry detection,

achieving detection limits as low as 0.01 $ng \cdot mL^{-1}$ for some of the fluoroquinolones studied with the SPE and SPME methodologies.

b.4. Fluorescence detection was facilitated by the use of micellar media, because these substances are able to enhance the signal response of compounds with fluorescent properties such as fluoroquinolones. Therefore, in SPE and SPME works, which analytical parameters obtained by using organic solvents and surfactants were compared, the latter provided higher sensitivity (with LODs ranging from 0.07 to 0.20 $ng \cdot mL^{-1}$ and from 0.01 to 0.03 $ng \cdot mL^{-1}$ when methanol and POLE were employed in SPE, respectively, and from 0.02 to 0.23 $ng \cdot mL^{-1}$ and from 0.01 to 0.19 $ng \cdot mL^{-1}$ when methanol and POLE were employed in SPME, respectively)

c) About the application of the developed methodologies to real samples:

c.1. The presence of fluoroquinolones in environmental samples from the Gran Canaria island has been studied for first time. The levels of concentration detected in each analysed sample were in the following ranges:

- Wastewaters from a hospital of Las Palmas de Gran Canaria, between 46.01 and 698.8 $ng \cdot mL^{-1}$.
- Wastewaters from primary treatment of a WWTP of Las Palmas de Gran Canaria, between 5.391 and 46.71 $ng \cdot mL^{-1}$.
- Wastewaters from secondary treatment of a WWTP of Las Palmas de Gran Canaria, between 0.831 and 14.08 $ng \cdot mL^{-1}$.

- Wastewaters from final effluent of a WWTP of Las Palmas de Gran Canaria, between 0.062 and 13.62 $ng \cdot mL^{-1}$.
- Sewage sludges from a WWTP of Las Palmas de Gran Canaria, between 2.98 and 206.1 $ng \cdot g^{-1}$.
- Marine sediments collected near to a wastewater submarine outfall, between 0.690 and 34.30 $ng \cdot g^{-1}$.

c.2. From the measured concentrations after the different treatments of the WWTP studied, we can deduce, in a first approach, that the greatest reduction in the fluoroquinolone concentrations in the dissolve phase occurs during the secondary treatment (between 62 and 72% for the levofloxacin, between 89 and 96% for the norfloxacin and between 70 and 74% for the ciprofloxacin). Contrary, the difference between the concentration measured in the samples from secondary treatment and final effluent, shows a very small reduction. The total reduction in the fluoroquinolone concentration of analysed samples in the WWTP studied was between 72-76% for the levofloxacin, 98-99% for the norfloxacin and 70-79% for the ciprofloxacin. Therefore, despite this elimination is rather efficient for some analytes, such as the norfloxacin, is evident that the WWTP is unable to generate waters totally free of these contaminants. Tertiary treatment is required for achieve a total elimination.

c.3. The presence of benzotriazole UV stabilizers in environmental samples from the Gran Canaria island has been studied for first time. The levels of concentration detected in each analysed sample were in the following ranges:

- Seawater collected in different points of the coast of Gran Canaria island, between 0.003 and 0.005 $ng \cdot mL^{-1}$.
- Wastewater from different WWTPs of Gran Canaria island, between 0.004 and 0.013 $ng \cdot mL^{-1}$.
- Sewage sludges from different WWTPs of Las Palmas de Gran Canaria, between 0.940 and 12.20 $ng \cdot g^{-1}$.
- Marine sediments collected near to a wastewater submarine outfall, between 0.180 and 24.00 $ng \cdot g^{-1}$.

Anexos

I. Acrónimos

ACN: *Acetonitrile*

APCI: *Atmospheric pressure chemical ionization*

APMALDI: *Atmospheric pressure matrix-assisted laser desorption ionization*

APPI: *Atmospheric pressure photo ionization*

ASE: *Accelerated solvent extraction*

BSM: *Binary solvent manager*

BUVSs: *Benzotriazole ultra-violet stabilizers*

CAR: *Carboxen*

CI: *Chemical Ionization*

CW*: Carbowax*

$C_{12}E_6$: *Polyoxyethylene 6 lauryl ether / hexaethylene glycol monododecyl ether*

DAD: *Diode-array detector*

DLLME: *Dispersive liquid-liquid microextraction*

DVB: *Divinilbenceno*

ECD: *Electronic capture detector*

EI: *Electronic ionization*

EDARs: *Estaciones depuradoras de aguas residuales*

EDR: *Extended dynamic range*

EPA: *Environmental protection agency*

ESI: *Electrospray ionization*

EU: *European Union*

FD: *Fluorescence detector*

FID: *Flame ionization detector*

FMAE: *Focused microwave assisted extraction*

FQs: *Fluoroquinolonas*

GC: *Gas chromatography*

GENAPOL: *Oligoethyleneglycol monoalkyl ether*

HF-LPME: *Hollow fiber liquid phase microextraction*

HLB: *Hydrophilic-lipophilic balance*

HPLC: *High performance liquid chromatography*

HS: *Head space desorption*

HSSE: *High speed solvent extraction*

HTAB: *Hexadecyltrimethylammonium bromide*

IFRA: *International fragrance organization*

INCI: *International nomenclature of cosmetic ingredients*

IT: *Ion trap*

LC: *Liquid chromatography*

LD: *Liquid desorption*

LLE: *Liquid-liquid extraction*

LOD: *Limit of detection*

log K_{ow}: *Coeficiente de partición octanol-agua*

LOQ: *Limit of quantification*

LVI: *Large volume injection*

MAE: *Microwave assisted extraction*

MALDI: *Matrix-assisted laser desorption ionization*

MAME: *Microwave assisted micellar extraction*

MD: *Micellar desorption*

MeOH: *Methanol*

MIPs: *Moleculary imprinted polymers*

MRL*: Maximum residue limits*

MRM: *Multiple reaction monitoring*

MS: *Mass spectrometry*

MS/MS: *Triple quadrupole mass spectrometry*

MSPD: *Matrix solid phase dispersion*

NPD: *Nitrogen-phosphor detector*

PA: *Polyacrylate*

PB: *Particle beam*

PDMS: *Polydimethylsiloxane*

PFE: *Pressurized fluid extraction*

PI: *Positive ionization mode*

PLE: *Pressurized liquid extraction*

POLE: *Polyoxyethylene 10 lauryl ether*

POLIDOCANOL: *Polyoxyethylene 9 lauryl ether*

PPCPs: *Pharmaceuticals and personal care products*

QTRAP: *Triple quadrupole linear ion trap mass spectrometer*

QuEChERS: *Quick, Easy, Cheap, Effective, Rugged, and Safe*

RAM*: Restricted access materials*

RP: *Reverse phase*

RSD: *Relative standard deviation*

SBSE: *Stir bar sorptive extraction*

SDB: *Styrene divinylbenzene*

SDLPME: *Solid drop liquid phase microextraction*

SDME: *Single drop microextraction*

SDS / NaLS: *Sodium dodecyl sulfate*

SE: *Soxhlet extraction*

SFE: *Supercritical fluid extraction.*

SIM: *Selected ion monitoring*

SPE: *Solid phase extraction*

SPME: *Solid phase microextraction*

TD: *Thermal desorption*

TIC: *Total ion current*

TOF: *Time-of-flight*

TQD: *Triple quadrupole detector*

TSP: *Thermospray interface*

UAE: *Ultrasonic Assisted Extraction*

UHPLC: *Ultra high performance liquid chromatography*

UPLC: *Ultra high pressure liquid chromatography*

UV-vis: *Ultraviolet-visible*

WWTPs: *Wastewater treatment plants*

II. Lista de publicaciones de la Tesis Doctoral

1) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Extraction and determination of fluoroquinolone by coupling solid phase microextraction with micelar desorption and HPLC-fluorescence detection."

   Revista: Luminescence

   Volumen: 23

   Páginas: 250-251

   Fecha: 2008

2) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Solid phase microextraction with micellar desorption and HPLC-fluorescence detection for the analysis of fluoroquinolones residues in water."

   Revista: Analytical and Bionalytical Chemistry

   Volumen: 394

   Páginas: 927-935

   Fecha: 2009

3) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Microwave assisted extraction with micellar media for determination of fluoroquinolones in coastal marine sediments followed by HPLC with fluorescence detection."

   Revista: Luminescence

   Volumen: 25

   Páginas: 239-240

   Fecha: 2010

4) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Combination of microwave assisted micellar extraction with liquid chromatography tandem mass spectrometry to the determination of fluoroquinolone antibiotics in coastal marine sediments and sewage sludges samples."

Revista: Biomedical Chromatography

Volumen: 26

Páginas: 33-40

Fecha: 2012

5) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Comparison of solid phase extraction using micellar desorption combined with LC-FD and LC-MS/MS in the determination of antibiotic fluoroquinolone residues in sewage samples."

Revista: Journal of Liquid Chromatography & Related Technologies

Volumen: 35

Páginas: 2081-2096

Fecha: 2012

6) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "On-line solid-phase extraction coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection for the determination of benzotriazole UV stabilizers in coastal marine and wastewater samples."

Revista: Analytical and Bioanalytical Chemistry

Volumen: 403

Páginas: 867-876

Fecha: 2012

7) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Microwave assisted extraction combined with on-line solid phase extraction followed by ultra-high-performance liquid chromatography with tandem mass spectrometry determination of benzotriazole UV stabilisers in marine sediments and sewage sludges."

Revista: Journal of Separation Science

Volumen: 36

Páginas: 781-787

Fecha: 2013

8) **Autores:** S. Montesdeoca Esponda, A. del Toro Moreno, Z. Sosa Ferrera, J.J. Santana Rodríguez .

Título: "Development of sensitive determination method for benzotriazole UV stabilizers in environmental water samples with stir bar sorption extraction and liquid desorption prior to ultra-high performance liquid chromatography with tandem mass spectrometry."

Revista: Journal of Separation Science (aceptado, en prensa)

Volumen:

Páginas:

Fecha:

9) **Autores:** S. Montesdeoca Esponda, T. Vega Morales, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Benzotriazole UV stabilizers (BUVSs) in personal care products: recent extraction and determination methodologies in environmental and biological samples."

Revista: Trends in Analytical Chemistry (enviado)

Volumen:

Páginas:

Fecha:

III. Comunicaciones presentadas en congresos

1) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Extraction and determination of fluoroquinolone by coupling solid phase microextraction with micelar desorption and HPLC-fluorescence detection."

   Congreso: XIII International Symposium on Luminescence Spectrometry

   Tipo de participación: Comunicación oral

   Lugar de celebración: Bolonia, Italia

   Fecha: Septiembre 2008

2) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Solid phase microextraction with micellar desorption followed by high-performance liquid chromatography as an efficient and sensitive method for simultaneous determination of fluoroquinolones in liquid environmental samples."

   Congreso: II International Symposium in marine sciences

   Tipo de participación: Póster

   Lugar de celebración: Vigo, España

   Fecha: Abril 2009

3) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Optimization of a novel solid-phase extraction process using micellar media coupled to HPLC-FD for simultaneous determination of fluoroquinolones."

   Congreso: 34th International Symposium on HPLC and Related Techniques

   Tipo de participación: Póster

   Lugar de celebración: Dresden, Alemania

   Fecha: Junio 2009

4) **Autores:** S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Implementation of solid phase extraction with micellar desorption and HPLC for determination of fluoroquinolones in natural waters."

   Congreso: EuroAnalysis 2009

   Tipo de participación: Póster

   Lugar de celebración: Innsbruck, Austria

   Fecha: Septiembre 2009

5) **Autores:** M.E. Torres Padrón, C. Mahugo Santana, S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "A novel approach of solid phase microextraction with micellar desorption (SPME-MD) for the extraction of organic contaminants in environmental liquid samples."

   Congreso: XXXII Reunión Bienal de la Real Sociedad Española de Química

   Tipo de participación: Póster

   Lugar de celebración: Oviedo, España

   Fecha: Septiembre 2009

6) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Microwave assisted extraction with micellar media for determination of fluoroquinolones in coastal marine sediments followed by HPLC with fluorescence detection."

   Congreso: XIV International Symposium on Luminescence Spectrometry

   Tipo de participación: Comunicación oral

   Lugar de celebración: Praga, República Checa

   Fecha: Julio 2010

7) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Applicability of solid phase extraction with micellar desorption (SPE-MD) combined with HPLC to the determination of fluoroquinolones in hospital and municipal wastewaters."

Congreso: 28º International Symposium on Chromatography

Tipo de participación: Póster

Lugar de celebración: Valencia, España

Fecha: Septiembre 2010

8) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Optimization of a SPE-HPLC-ESI-MS/MS method for the determination of benzotriazole compounds used in personal care products."

Congreso: 36th International Symposium on HPLC and Related Techniques

Tipo de participación: Póster

Lugar de celebración: Budapest, Hungría

Fecha: Junio 2011

9) **Autores:** A. del Toro Moreno, S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "Optimization of a micellar SBSE-UHPLC-MS/MS method for the determination of benzotriazoles used in personal care products."

Congreso: 13ª Jornadas de Análisis Instrumental

Tipo de participación: Póster

Lugar de celebración: Barcelona, España

Fecha: Noviembre 2011

10) **Autores:** S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez.

Título: "On-line solid-phase extraction coupled to UPLCMS/MS for the determination of benzotriazole UV stabilizers in coastal marine and wastewater samples."

Congreso: Hyphenated techniques in sample preparation and Hyphenated techniques in chromatography

Tipo de participación: Póster

Lugar de celebración: Brujas, Bélgica

Fecha: Enero 2012

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

EXTRACTION AND DETERMINATION OF FLUOROQUINOLONES BY COUPLING SOLID PHASE MICROEXTRACTION WITH MICELLAR DESORPTION AND HPLC - FLUORESCENCE DETECTION

S. Montesdeoca Esponda, M.E. Torres Padrón, Z. Sosa Ferrera and J.J. Santana Rodríguez*

Department of Chemistry, Faculty of Marine Sciences, University of Las Palmas de G.C., 35017 Las Palmas de G.C., Spain. (*) E-mail: jsantana@dqui.ulpgc.es

XIII International Symposium on Luminescence Spectrometry

(Bolonia, Italia, 2008)

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

AQMA

Solid phase microextraction with micellar desorption followed by high-performance liquid chromatography as an efficient and sensitive method for simultaneous determination of fluoroquinolones in liquid environmental samples

Sarah Montesdeoca Esponda, María Esther Torres Padrón , Zoraida Sosa Ferrera, José Juan Santana Rodríguez*

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INTRODUCTION

A sensitive and useful method based on solid phase microextraction with micellar desorption (SPME-MD) coupled to HPLC with fluorescence detection was developed for the determination of five fluoroquinolones: levofloxacin (LEVO), norfloxacin (NOR), ciprofloxacin (CIPRO), enrofloxacin (ENRO) and sarafloxacin (SARA), in environmental water matrices. Fluoroquinolones (FQs) are a new and synthetic generation of quinolones antibacterials family, used in human and veterinary medicine [1]. These compounds are widely used, which may result in health problems [2] because they are rather resistance to microbial degradation and may be persisting within environmental waters [3]. Moreover, their environmental concentrations are very low, for that these analytes need to be extracted and preconcentrated prior to environmental analysis.

In this work the SPME extraction efficiency was optimized and a detailed study about the optimum conditions for micellar desorption were made. Finally, the SPME-MD methodology was applied to the determination of these target analytes in several environmental liquid samples, including seawater, groundwater and wastewater samples.

EXPERIMENTAL PROCEDURE

SPME was carried out by introducing 4 mL of aqueous samples containing 50 ng mL−1 of each fluoroquinolone into glass vials. The fiber was then immersed in the sample. During the extraction, the samples were heated and they were stirred with a magnetic stir bar. After the extraction step, the fiber loaded with the analytes was introduced into a conical glass insert of 100 µL contained in a 4-mL glass vial with 60 µL of selected desorbing solvent.

OPTIMIZATION OF VARIABLES

For the variable optimization was used a multiparametric analysis:

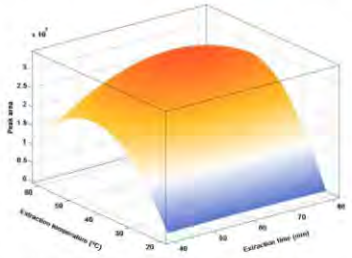

Fig. 1 Response surface for the effect of extraction time and temperature on the extraction on norfloxacin (50 ng mL^{-1}) using SPME procedure.

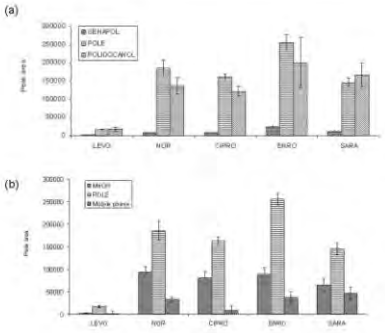

Fig. 2 a) Response of analytes to the presence of different surfactants in the desorption process

b) Response of different mode desorptions in SPME: methanol, mobile phase, and micellar desorption.

OPTIMUM EXTRACTION PROCESS	
FIBER TYPE	CW-TPR
EXTRACTION TIME	60 min.
TEMPERATURE	40ºC
pH	7
IONIC STRENGTH	30% NaCl

OPTIMUM DESORPTION PROCESS	
DESORPTION TIME	15 min.
SURFACTANT	POLE
SURFACTANT CONCENTRATION	7.5 %

RESULTS

	1 ng·mL^{-1}			50 ng·mL^{-1}		
COMPOUND	SEWAGE	SEA WATER	GROUNDWATER	SEWAGE	SEA WATER	GROUNDWATER
LEVO	87 ± 11	100 ± 15	114 ± 7	91 ± 8	104 ± 7	85 ± 9
NOR	89 ± 4	88 ± 7	82 ± 5	92 ± 3	88 ± 7	90 ± 9
CIPRO	90 ± 7	89 ± 9	81 ± 16	81 ± 3	85 ± 6	83 ± 4
ENRO	91 ± 7	112 ± 22	116 ± 17	89 ± 4	93 ± 5	88 ± 4
SARA	90 ± 12	93 ± 11	88 ± 11	86 ± 6	94 ± 3	87 ± 9

Table 1. Recovery percentages and RSD (%) obtained for different real liquid samples spiked with 1 and 50 ng·mL^{-1} of each fluoroquinolone. Mean of three determinations.

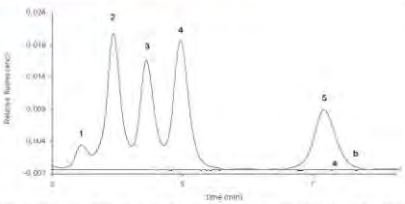

Fig. 3 Chromatogram of blank a) and spiked (b) wastewater sample (1 ng mL^{-1} for each analyte) using SPME-MD procedure: (1)Levofloxacin (2)Norfloxacin (3)Ciprofloxacin (4)Enrofloxacin (5)Sarafloxacin

CONCLUSIONS

This SPME-MD method could represent a viable and easy analytical approach for routine analysis and proved to be a valuable tool in the analytical characterization of water samples like effluents from hospitals or sewage waters of the treatment plants.

It may be used for routine analysis because HPLC-fluorescence detection is an inexpensive analytical technique compared with others one like LC-MS.

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ACKNOWLEDGMENTS

This work was supported by funds provided by the Spanish Ministry of Education and Science. Research Project Nº CTQ 2006-06507.

II International Symposium in Marine Sciences

(Vigo, España, 2009)

Optimization of a novel solid-phase extraction process using micellar media coupled to HPLC-FD for simultaneous determination of fluoroquinolones

Sarah Montesdeoca Esponda, María Esther Torres Padrón , Zoraida Sosa Ferrera, José Juan Santana Rodríguez*

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INTRODUCTION

A method based on solid-phase extraction coupled to HPLC with fluorescence detection was developed for the determination of five fluoroquinolones: levofloxacin (LEVO), norfloxacin (NOR), ciprofloxacin (CIPRO), enrofloxacin (ENRO) and sarafloxacin (SARA), in environmental water matrices. Fluoroquinolones (FQs) are a new and synthetic generation of quinolones antibacterials family, used in human and veterinary medicine [1]. These compounds are widely used, which may result in health problems [2] because they are rather resistance to microbial degradation and may be persisting within environmental waters [3]. Moreover, their environmental concentrations are very low, and therefore need to be extracted and preconcentrated prior to environmental analysis.

We propose a novelty in the solid-phase process: to carry out the elution step with micellar media. Thus, we avoid the use of organic solvents, which can result very dangerous, expensive and polluting for the environment.

EXPERIMENTAL PROCEDURE

Fig. 1 Comparison of the relative extraction efficiencies with different SPE cartridges (deionized water spiked with 5 ng·mL⁻¹ of each FQ and using methanol for the elution)

Fig. 2 Comparison of the relative elution efficiencies with different surfactants (deionized water spiked with 5 ng·mL⁻¹ of each FQ)

Fig. 3 Response surface for the effect of POLE concentration and volumen on the elution on enrofloxacin (deionized water spiked with 5 ng·mL⁻¹)

OPTIMUM EXTRACTION PROCESS	
CARTRIDGE	Oasis HLB
FLOW RATE	5 mL/min
VOLUME SAMPLE	200 mL
pH	3
IONIC STRENGTH	0% NaCl

OPTIMUM ELUTION PROCESS	
FLOW RATE	5 mL/min
SURFACTANT	POLE
SURFACTANT VOLUME	5 mL
SURFACTANT CONCENTRATION	7.5% (v/v)

CHROMATOGRAPHIC CONDITIONS	
MOBILE PHASE	15 % methanol 85 % water at pH=2.5
FLOW RATE	1 mL/min
λ_{exc}	280 nm
λ_{em}	450 nm

RESULTS

		LOD[a] (ng·mL⁻¹)	LOQ[b] (ng·mL⁻¹)	RSD[c] (%)	Recoveries[d] (%)
1	LEVOFLOXACIN	0.07	0.28	8.47	84.12
2	NORFLOXACIN	0.03	0.11	8.62	101.30
3	CIPROFLOXACIN	0.03	0.11	6.87	107.64
4	ENROFLOXACIN	0.02	0.08	7.00	98.02
5	SARAFLOXACIN	0.04	0.12	11.79	90.01

[a] Detection limits are calculated as signal to noise ratio of 3 times
[b] Quantification limits are calculated as signal to noise ratio of 10 times
[c] Relative Standard Deviation (n=6)
[d] Mean of three determinations in deionized water spiked with 5 ng·mL⁻¹

Fig 4. Chromatogram of spiked deionized water (5 ng·mL⁻¹ for each analyte) using SPE with micelar elution

CONCLUSIONS

This optimized method represent an advance in the called "green methods" because replaced the use of organic solvents by a surfactant solution. In this way, we achieve a easy, inexpensive and safe method, which does not pollute the environment. This procedure may be used for routine analysis and proved to be a valuable tool in the analytical characterization of water samples like effluents from hospitals or sewage waters from the treatment plants.

REFERENCES

[1] Kaur, K., Kumar, A., Kumar Malik, A., Singh, B., Rao A.L.J., *Crit. Rev. Anal. Chem.* 2008, 38, 2-18.
[2] Pozo, O.J., Guerrero, C., Sancho, J.V., Ibáñez, M., Pitarch, E., Hogendoorn, E., Hernández, F., *J. Chromatogr. A.* 2006, *1103*, 83-93. [3] Mitani, K., Kataoka, H., *Anal. Chim. Acta.* 2006, *562*, 16-22.

ACKNOWLEDGMENTS

This work was supported by funds provided by the Spanish Ministry of Education and Science. Research Project Nº CTQ 2006-06507.

34th International Symposium on High Performance Liquid Phase Separations and Related Techniques

(Dresden, Alemania, 2009)

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

AQMA

Optimization of a SPE-UPLC-MS/MS method for the determination of Benzotriazole compounds used in Personal Care Products

Sarah Montesdeoca Esponda, María Esther Torres Padrón , Zoraida Sosa Ferrera, José Juan Santana Rodríguez*

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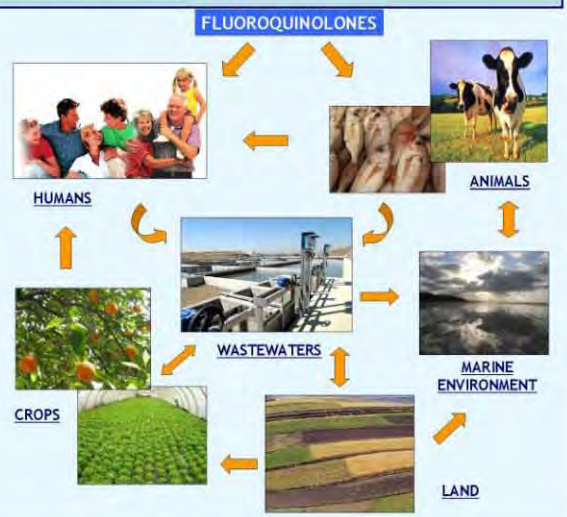
INTRODUCTION

The pharmaceutical drugs used during medical treatment may partly be excreted in an unmetabolized form, enter municipal sewage systems, and can even survive the passage through the sewage treatment plants [1]. Then, the residues can reach sea waters, surface waters or groundwaters.

These substances are among the so-called "emerging" contaminants and can be considered "pseudo" persistent pollutants due to their continual introduction into the environment [2]. For that, in the recent years has increased the number of publications related to the development of new methods for the determination of different analytes in several matrices.

Fluoroquinolones (FQs) are within of the antibiotics compounds group and are a new and synthetic generation of quinolones antibacterials family, used in human and veterinary medicine [3]. The FQs are efficient against a widely range of infections process, but they are resistant to microbial degradation and may be persisting within environmental waters [4]. Moreover, their environmental concentrations are very low, for that these analytes need to be extracted and preconcentrated prior their analysis. For that, we applied a SPE with micellar elution method previously optimized for their validation in real environmental samples.



EXPERIMENTAL PROCEDURE

OPTIMUM EXTRACTION PROCESS	
CARTRIDGE	Oasis HLB
FLOW RATE	5 mL/min
VOLUME SAMPLE	200 mL
pH	3 (mv)
IONIC STRENGTH	0% NaCl (m/v)

OPTIMUM ELUTION PROCESS	
FLOW RATE	1 mL/min
SURFACTANT	POLE
SURFACTANT VOLUME	5 mL
SURFACTANT CONCENTRATION	7.5% (v/v)

CHROMATOGRAPHIC CONDITIONS	
MOBILE PHASE	15 % methanol 85 % water at pH=2.5
FLOW RATE	1 mL/min
λ_{exc}	280 nm
λ_{em}	450 nm

RESULTS

		LOD[a] (ng·mL⁻¹)	LOQ[b] (ng·mL⁻¹)	RSD[c] (%)
1	LEVOFLOXACIN	0.07	0.28	8.47
2	NORFLOXACIN	0.03	0.11	8.62
3	CIPROFLOXACIN	0.03	0.11	6.87
4	ENROFLOXACIN	0.02	0.08	7.00
5	SARAFLOXACIN	0.04	0.12	9.79

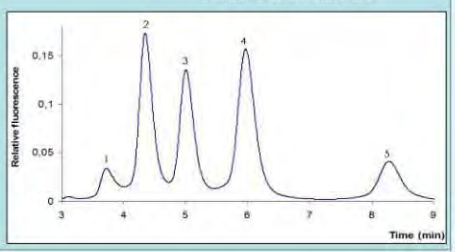
[a] Detection limits are calculated as signal to noise ratio of three times

[b] Quantification limits are calculated as signal to noise ratio of ten times

[c] Relative Standard Deviation (n=6)

	SEA WATER	WASTEWATER	GROUNDWATER
1	88.13 ± 12.33	82.33 ± 13.50	73.25 ± 2.42
2	96.69 ± 9.91	85.19 ± 5.43	83.23 ± 2.97
3	88.00 ± 8.18	86.05 ± 2.41	85.81 ± 0.42
4	82.27 ± 6.14	94.15 ± 6.44	91.85 ± 1.73
5	89.46 ± 9.33	86.06 ± 11.27	84.29 ± 13.66

Recovery percentages and RSD (%) obtained for different liquid samples spiked with 5 ng·mL⁻¹ of each FQ. Mean of three determinations.



Chromatogram of spiked wastewater sample (5 ng·mL⁻¹ for each analyte) using SPE with micellar elution method

CONCLUSIONS

The developed SPE with micellar elution follow of HPLC-FD method has been applied to simultaneous determination of fluoroquinolones in different liquid samples. The recoveries obtained for sea water, wastewater and groundwater are satisfactory and matrix effect has not been observed. This procedure represents an advance in the called "green methods" because replaces the use of organic solvents by a surfactant solution and proves to be a valuable tool in the analytical characterization of water samples like effluents from hospitals or sewage waters from the treatment plants.

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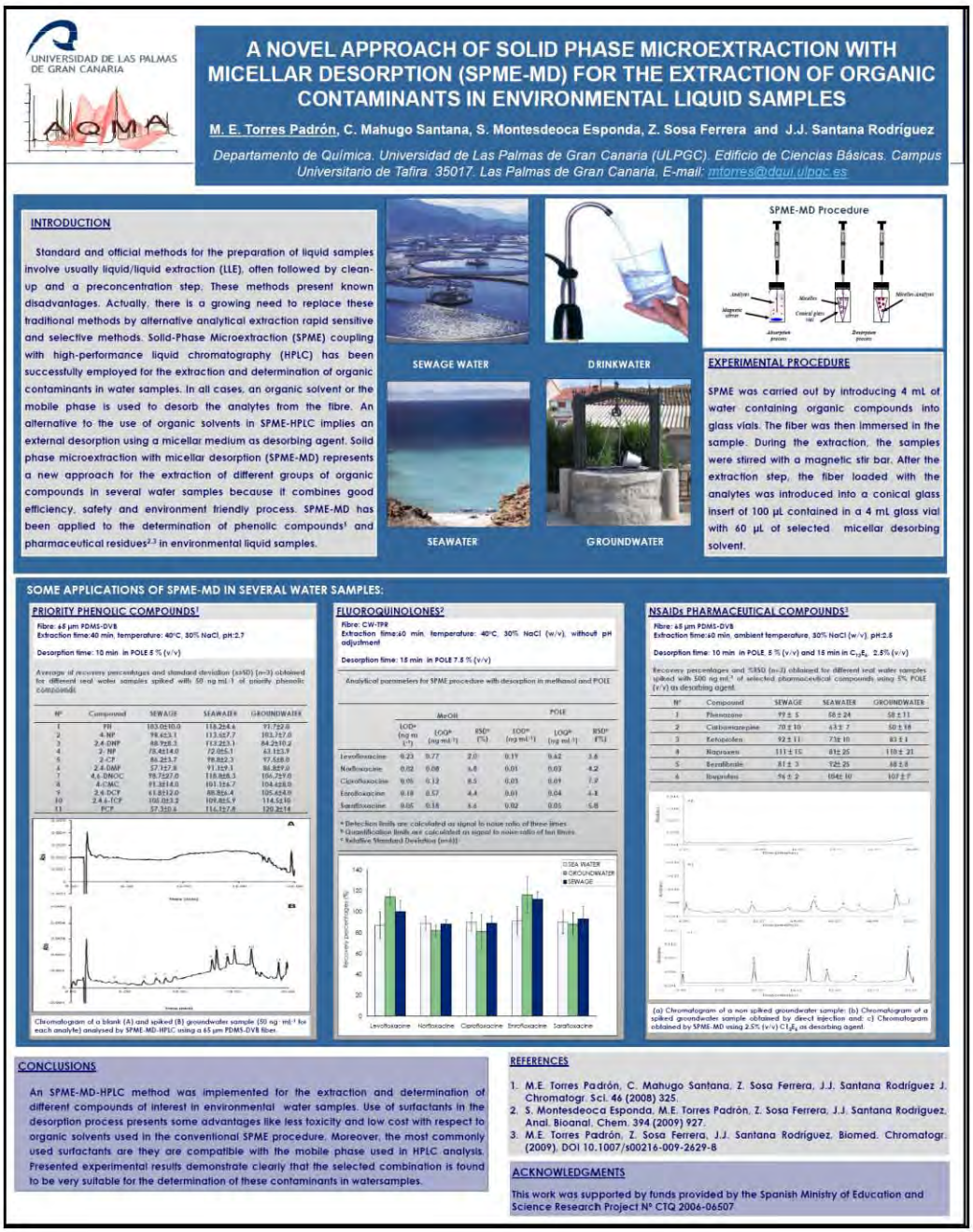
ACKNOWLEDGMENTS

This work was supported by funds provided by the Spanish Ministry of Education and Science. Research Project N° CTQ 2006-06507.

EURO ANALYSIS 2009

EuroAnalysis 2009

(Innsbruck, Austria, 2009)



UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

A NOVEL APPROACH OF SOLID PHASE MICROEXTRACTION WITH MICELLAR DESORPTION (SPME-MD) FOR THE EXTRACTION OF ORGANIC CONTAMINANTS IN ENVIRONMENTAL LIQUID SAMPLES

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INTRODUCTION

Standard and official methods for the preparation of liquid samples involve usually liquid/liquid extraction (LLE), often followed by clean-up and a preconcentration step. These methods present known disadvantages. Actually, there is a growing need to replace these traditional methods by alternative analytical extraction rapid sensitive and selective methods. Solid-Phase Microextraction (SPME) coupling with high-performance liquid chromatography (HPLC) has been successfully employed for the extraction and determination of organic contaminants in water samples. In all cases, an organic solvent or the mobile phase is used to desorb the analytes from the fibre. An alternative to the use of organic solvents in SPME-HPLC implies an external desorption using a micellar medium as desorbing agent. Solid phase microextraction with micellar desorption (SPME-MD) represents a new approach for the extraction of different groups of organic compounds in several water samples because it combines good efficiency, safety and environment friendly process. SPME-MD has been applied to the determination of phenolic compounds[1] and pharmaceutical residues[2,3] in environmental liquid samples.

SEWAGE WATER

DRINKWATER

SEAWATER

GROUNDWATER

SPME-MD Procedure

EXPERIMENTAL PROCEDURE

SPME was carried out by introducing 4 mL of water containing organic compounds into glass vials. The fiber was then immersed in the sample. During the extraction, the samples were stirred with a magnetic stir bar. After the extraction step, the fiber loaded with the analytes was introduced into a conical glass insert of 100 µL contained in a 4 mL glass vial with 60 µL of selected micellar desorbing solvent.

SOME APPLICATIONS OF SPME-MD IN SEVERAL WATER SAMPLES:

PRIORITY PHENOLIC COMPOUNDS[1]

Fibre: 65 µm PDMS-DVB
Extraction time:40 min, temperature: 40°C, 30% NaCl, pH:2.7

Desorption time: 10 min in POLE 5 % (v/v)

Average of recovery percentages and standard deviation (±SD) (n=3) obtained for different real water samples spiked with 50 ng mL-1 of priority phenolic compounds

Nº	Compound	SEWAGE	SEAWATER	GROUNDWATER
1	FN	103.0±10.0	118.2±4.6	91.7±2.0
2	4-NP	98.4±3.1	113.6±7.7	103.7±7.0
3	2,4-DNP	88.9±8.3	112.2±5.1	84.2±10.2
4	2- NP	78.4±14.0	72.0±5.1	63.1±3.9
5	2-CP	86.2±3.7	98.8±2.3	97.6±8.0
6	2,4-DMP	57.1±7.8	91.3±9.1	86.8±9.0
7	4,6-DNOC	98.7±7.0	118.8±8.3	106.7±9.0
8	4-CMC	91.3±14.0	101.1±6.7	104.4±8.0
9	2,4-DCP	61.8±12.0	88.8±6.4	105.4±4.0
10	2,4,6-TCP	100.0±3.2	109.8±5.9	114.5±10
11	PCP	57.3±0.6	116.1±7.8	120.2±14

Chromatogram of a blank (A) and spiked (B) groundwater sample (50 ng · mL-1 for each analyte) analysed by SPME-MD-HPLC using a 65 µm PDMS-DVB fiber.

FLUOROQUINOLONES[2]

Fibre: CW-TPR
Extraction time:60 min, temperature: 40°C, 30% NaCl (w/v), without pH adjustment

Desorption time: 15 min in POLE 7.5 % (v/v)

Analytical parameters for SPME procedure with desorption in methanol and POLE

	MeOH			POLE		
	LOD* (ng mL-1)	LOQ* (ng mL-1)	RSD* (%)	LOD* (ng mL-1)	LOQ* (ng mL-1)	RSD* (%)
Levofloxacine	0.23	0.77	2.0	0.19	0.62	3.6
Norfloxacine	0.02	0.08	6.8	0.01	0.03	4.2
Ciprofloxacine	0.05	0.12	8.5	0.03	0.09	7.2
Enrofloxacine	0.18	0.57	4.4	0.01	0.04	4.8
Sarafloxacine	0.05	0.16	5.6	0.02	0.05	6.8

* Detection limits are calculated as signal to noise ratio of three times
* Quantification limits are calculated as signal to noise ratio of ten times.
* Relative Standard Deviation (n=6)

NSAIDs PHARMACEUTICAL COMPOUNDS[3]

Fibre: 65 µm PDMS-DVB
Extraction time:30 min, ambient temperature, 30% NaCl (w/v), pH:2.5

Desorption time: 10 min in POLE 5 % (v/v) and 15 min in $C_{12}E_6$ 2.5% (v/v)

Recovery percentages and %RSD (n=3) obtained for different real water samples spiked with 500 ng mL-1 of selected pharmaceutical compounds using 5% POLE (v/v) as desorbing agent.

Nº	Compound	SEWAGE	SEAWATER	GROUNDWATER
1	Phenazone	99 ± 5	88 ± 24	58 ± 11
2	Carbamazepine	70 ± 10	63 ± 7	50 ± 10
3	Ketoprofen	92 ± 11	73± 10	83 ± 1
4	Naproxen	111 ± 15	81± 25	110 ± 21
5	Bezafibrate	81 ± 3	72± 25	68 ± 8
6	Ibuprofen	96 ± 2	104± 10	107 ± 7

(a) Chromatogram of a non spiked groundwater sample; (b) Chromatogram of a spiked groundwater sample obtained by direct injection and; c) Chromatogram obtained by SPME-MD using 2.5% (v/v) $C_{12}E_6$ as desorbing agent.

CONCLUSIONS

An SPME-MD-HPLC method was implemented for the extraction and determination of different compounds of interest in environmental water samples. Use of surfactants in the desorption process presents some advantages like less toxicity and low cost with respect to organic solvents used in the conventional SPME procedure. Moreover, the most commonly used surfactants are they are compatible with the mobile phase used in HPLC analysis. Presented experimental results demonstrate clearly that the selected combination is found to be very suitable for the determination of these contaminants in watersamples.

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ACKNOWLEDGMENTS

This work was supported by funds provided by the Spanish Ministry of Education and Science Research Project Nº CTQ 2006-06507

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APPLICABILITY OF SOLID PHASE EXTRACTION WITH MICELLAR DESORPTION (SPE-MD) COMBINED WITH HPLC TO THE DETERMINATION OF FLUOROQUINOLONES IN HOSPITAL AND MUNICIPAL WASTEWATERS

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AQMA

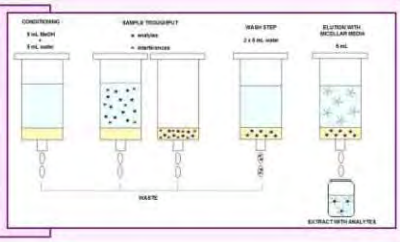
INTRODUCTION

Fluoroquinolones (FQs) are human and veterinary antibiotics considered emerging compounds that enter in the municipal sewage systems before be excreted in an unmetabolized form, and can even survive the passage through the wastewater treatment plants (WWTPs) [1]. Then, the residues can reach sea waters, surface waters or groundwaters. Because their environmental concentrations are very low, is necessary the use of a extraction and preconcentration method of the analytes prior their analysis. We used a solid phase extraction with micellar desorption (SPE-MD) process and HPLC with fluorescence and mass spectrometry detection for the analysis of FQs in hospital and municipal wastewaters. The novelty that we proposed is the replacement of the organic solvents used as eluent by a micellar medium, cheaper, no toxic and biodegradable.

EXPERIMENTAL PROCEDURE

CHROMATOGRAPHIC CONDITIONS	
MOBILE PHASE	15 % (v/v) methanol 85 % (v/v) water at pH=2.5
FLOW RATE	1 mL/min
λ_{exc}	280 nm
λ_{em}	450 nm

OPTIMUM EXTRACTION PROCESS	
CARTRIDGE	Oasis HLB
FLOW RATE	5 mL/min
VOLUME SAMPLE	200 mL
pH	3 (mv)
IONIC STRENGTH	0% NaCl (m/v)
OPTIMUM ELUTION PROCESS	
FLOW RATE	1 mL/min
SURFACTANT	POLE
SURFACTANT VOLUME	5 mL
SURFACTANT CONCENTRATION	7.5% (v/v)



RESULTS: Analytical parameters

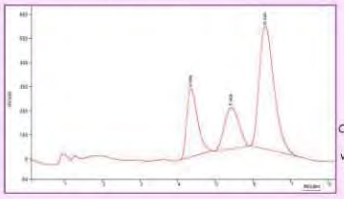
		LOD[a] (ng·mL⁻¹)	LOQ[b] (ng·mL⁻¹)	RSD[c] (%)
1	LEVOFLOXACIN	0.034	0.112	8.47
2	NORFLOXACIN	0.013	0.044	8.52
3	CIPROFLOXACIN	0.013	0.044	6.78
4	ENROFLOXACIN	0.010	0.032	7.00
5	SARAFLOXACIN	0.014	0.048	9.79

[a] Calculated as signal to noise ratio of three times
[b] Calculated as signal to noise ratio of ten times
[c] Relative Standard Deviation (n=6)

	SEA WATER	WASTEWATER	GROUNDWATER
1	88.13 ± 12.33	82.33 ± 13.50	73.25 ± 2.42
2	96.69 ± 9.91	85.19 ± 5.43	83.23 ± 2.97
3	88.00 ± 8.18	86.05 ± 2.41	85.81 ± 0.42
4	82.27 ± 6.14	94.15 ± 6.44	91.85 ± 1.73
5	89.46 ± 9.33	86.06 ± 11.27	84.29 ± 13.66

Recoveries and RSD (%) obtained for different liquid samples spiked with 5 ng·mL⁻¹ of each FQ. Mean of three determinations.

Analysis of hospital and wastewaters



Chromatogram of hospital water analized with HPLC-FD

Confirmation with HPLC-MS/MS

	Retention time (min)	Precursor ion (m/z)	Cone (V)	Quantification ion (collision potential, V)	Confirmation ion (collision potential, V)
1	5.3	362	56	318 (17)	261 (24)
2	5.6	320.1	56	301.9 (19)	230.8 (37)
3	6.1	332.1	52	313.9 (19)	230.8 (36)
4	9.6	360.2	60	342 (19.5)	316 (16.5)
5	13.5	386.1	52	299 (21.5)	342 (15.5)

Mass spectrometer parameters for FQs detection

	Primary treatment WWTP	Secondary Treatment WWTP	Final effluent WWTP	Hospital wastewater
1	11.23 ± 2.33	3.32 ± 0.62	2.93 ± 0.33	100.07 ± 12.21
2	7.56 ± 1.82	0.31 ± .0.08	0.06 ± 0.02	601.20 ± 10.33
3	42.02 ± 3.97	10.95 ± 2.01	10.07 ± 3.34	46.94 ± 4.62
4	ND	ND	ND	ND
5	ND	ND	ND	ND

Concentrations founded in samples analyzed with HPLC-FD (ng·mL⁻¹)
ND: No detected

	LOD[a] (ng·mL⁻¹)	LOQ[b] (ng·mL⁻¹)	Primary treatment WWTP	Secondary Treatment WWTP	Final effluent WWTP	Hospital wastewater
1	0.009	0.030	13.84 ± 3.81	3.91 ± 1.44	3.27 ± 1.09	128.09 ± 9.33
2	0.008	0.027	9.33 ± 2.13	0.92 ± 0.12	0.11 ± 0.03	698.8 ± 13.37
3	0.008	0.027	46.71 ± 6.91	14.08 ± 3.98	13.62 ± 2.95	49.07 ± 3.72
4	0.005	0.017	ND	ND	ND	ND
5	0.011	0.037	ND	ND	ND	ND

LODs, LOQs and concentrations found in samples analyzed with HPLC-MS/MS (ng·mL⁻¹)
ND: No detected

CONCLUSIONS

The developed SPE with micellar elution follow of HPLC-FD method has allowed the simultaneous determination of four FQs in different samples of environmental interest. The confirmation carried out with HPLC-MS/MS shows similar results and provide a unequivocal identification of the compounds. This procedure represents an advance in the called "green methods" because replaces the use of organic solvents by a surfactant and proves to be a valuable tool in the analytical characterization of environmental or sewage waters.

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ACKNOWLEDGMENTS

- Spanish Ministry of Education and Science (Research Project Nº CTQ 2006-06507)
- Canary Agency of Investigation, Innovation and Information Society

28º International Symposium on Chromatography

(Valencia, España, 2010)

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

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Optimization of a SPE-UPLC-MS/MS method for the determination of Benzotriazole compounds used in Personal Care Products

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INTRODUCTION

The growing concern about exposure to sunlight causing skin cancer has incremented the use of Ultraviolet (UV)-absorbing compounds in personal-care products like sunscreens, lipsticks, shampoos and hair sprays [1]. The benzotriazole UV stabilizers, which have a phenolic group attached to the benzotriazole structure, are known to absorb full spectrum of UV light, UV-A (320-400 nm) and UV-B (280-320 nm) [2]. However, little information is available on the contamination and concentrations of these organic UV filters, above all concerning to the most lipophilic. Its $\log_{Kows}$ generally are ranged from 6.0 to 8.5, so have a potential for bioaccumulation in the aquatic ecosystems [3]. Due to the low concentrations of these emerging compounds in real samples, an extraction and preconcentration step previous to the determination is necessary for its analysis.

In order to determine and to control the presence of these emerging pollutants in waters samples, we optimized a method based in Solid Phase Extraction and Ultra Performance Liquid Chromatography with Mass Spectrometry detection (SPE-UPLC-MS/MS) for the determination of seven benzotriazole compounds used in different personal care products. Ultra Performance Liquid Chromatography with Mass Spectrometry detection (ULPC-MS/MS) offers a very fast and selective detection in complex matrices.

EXPERIMENTAL PROCEDURE

CHROMATOGRAPHIC CONDITIONS	
UPLC COLUMN	ACQUITY UPLC BEH C18 1.7 µm 2.1 x 50 mm
MOBILE PHASE	MeOH 0.1% formic acid
FLOW RATE	0.9 mL/min

OPTIMUM SPE PROCESS	
CARTRIDGE	BOND ELUT SCX
VOLUME SAMPLE	50 mL
pH	6
IONIC STRENGTH	30% NaCl (m/v)
ELUYENT	MeOH
ELUYENT VOLUME	5 mL

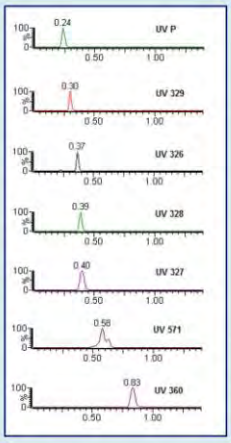
MASS SPECTROMETER PARAMETERS

	RETENTION TIME (MIN)	PRECURSOR ION (M/Z)	CONE (V)	QUANTIFICATION ION (COLLISION POTENTIAL, V)	CONFIRMATION ION (COLLISION POTENTIAL, V)
UV P	0.24	223.2	40	107.1 (20)	120.1 (20)
UV 329	0.30	324.2	50	57 (25)	212.2 (25)
UV 326	0.37	316.3	40	57 (25)	260.2 (20)
UV 328	0.39	352.3	50	71 (30)	282.2 (20)
UV 327	0.40	358.3	60	57 (30)	302.3 (20)
UV 571	0.58	394.3	50	226.2 (20)	120.1 (30)
UV 360	0.83	658.6	40	336.3 (25)	224.2 (35)

RESULTS

	LOD[a] (ng·L⁻¹)	LOQ[b] (ng·L⁻¹)	RSD[c] (%)	Recoveries[d] (%)
UV P	57.1	190.3	6.36	72.3
UV 329	5.4	17.9	7.51	93.5
UV 326	61.5	206.6	6.40	99.5
UV 328	46.7	155.6	3.21	101.5
UV 327	8.5	29.2	14.19	87.3
UV 571	11.3	37.8	14.88	89.5
UV 360	17.0	56.8	1.17	101.7

[a] *Detection limits are calculated as signal to noise ratio of 3 times*
[b] *Quantification limits are calculated as signal to noise ratio of 10 times*
[c] *Relative Standard Deviation (n=6)*
[d] *Mean of three determinations in deionized water spiked with 10 ng·mL⁻¹*



Chromatogram of spiked deionized water (10 ng·mL⁻¹ for each analyte) using SPE-UPLC-MS/MS

CONCLUSIONS

The developed SPE-UPLC-MS/MS method has allowed the simultaneous determination of seven Benzotriazole compounds in liquid samples with satisfactory recoveries and reproducibility (in the range 72.25-101,7 and 1,17-14,88, respectively). The combination of the preconcentration provided by the SPE procedure and the sensitivity of the UPLC-MS/MS detection offer a very low detection limits, between 5.4 and 61.5 ng·L⁻¹.

This procedure is a viable, fast and easy analytical approach for routine analysis and represents a valuable tool in the analytical characterization of water samples that may be used in enviromental waters or effluents from waste water treatment plants.

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ACKNOWLEDGMENTS

- Spanish Ministry of Science and Innovation, Research Project CTQ2010-20554.
- Canary Agency of Investigation, Innovation and Information Society

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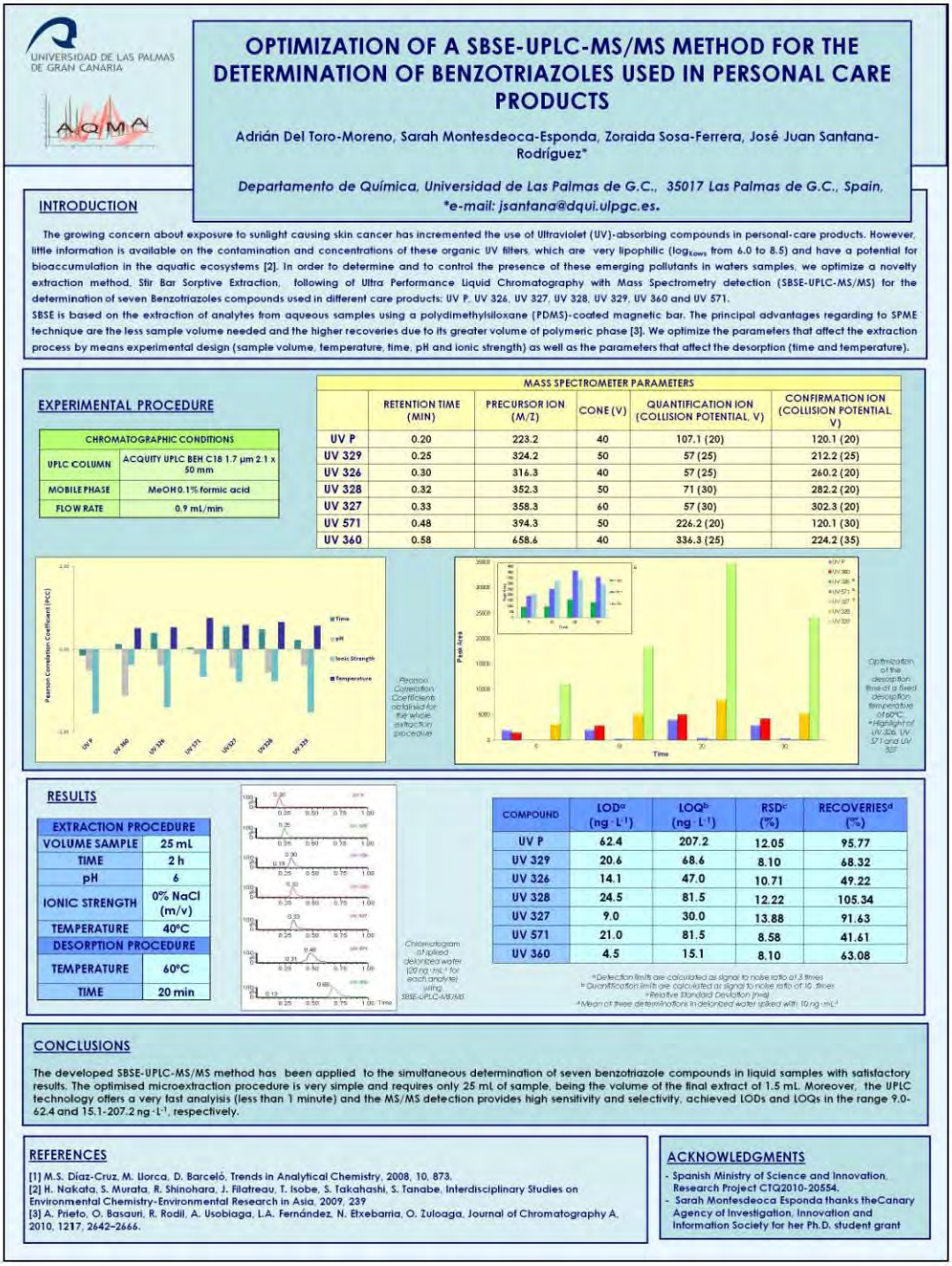

OPTIMIZATION OF A SBSE-UPLC-MS/MS METHOD FOR THE DETERMINATION OF BENZOTRIAZOLES USED IN PERSONAL CARE PRODUCTS

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INTRODUCTION

The growing concern about exposure to sunlight causing skin cancer has incremented the use of Ultraviolet (UV)-absorbing compounds in personal-care products. However, little information is available on the contamination and concentrations of these organic UV filters, which are very lipophilic (log_{Kows} from 6.0 to 8.5) and have a potential for bioaccumulation in the aquatic ecosystems [2]. In order to determine and to control the presence of these emerging pollutants in waters samples, we optimize a novelty extraction method, Stir Bar Sorptive Extraction, following of Ultra Performance Liquid Chromatography with Mass Spectrometry detection (SBSE-UPLC-MS/MS) for the determination of seven Benzotriazoles compounds used in different care products: UV P, UV 326, UV 327, UV 328, UV 329, UV 360 and UV 571.

SBSE is based on the extraction of analytes from aqueous samples using a polydimethylsiloxane (PDMS)-coated magnetic bar. The principal advantages regarding to SPME technique are the less sample volume needed and the higher recoveries due to its greater volume of polymeric phase [3]. We optimize the parameters that affect the extraction process by means experimental design (sample volume, temperature, time, pH and ionic strength) as well as the parameters that affect the desorption (time and temperature).

EXPERIMENTAL PROCEDURE

CHROMATOGRAPHIC CONDITIONS	
UPLC COLUMN	ACQUITY UPLC BEH C18 1.7 µm 2.1 x 50 mm
MOBILE PHASE	MeOH 0.1% formic acid
FLOW RATE	0.9 mL/min

MASS SPECTROMETER PARAMETERS					
	RETENTION TIME (MIN)	PRECURSOR ION (M/Z)	CONE (V)	QUANTIFICATION ION (COLLISION POTENTIAL, V)	CONFIRMATION ION (COLLISION POTENTIAL, V)
UV P	0.20	223.2	40	107.1 (20)	120.1 (20)
UV 329	0.25	324.2	50	57 (25)	212.2 (25)
UV 326	0.30	316.3	40	57 (25)	260.2 (20)
UV 328	0.32	352.3	50	71 (30)	282.2 (20)
UV 327	0.33	358.3	60	57 (30)	302.3 (20)
UV 571	0.48	394.3	50	226.2 (20)	120.1 (30)
UV 360	0.58	658.6	40	336.3 (25)	224.2 (35)

Pearson Correlation Coefficients obtained for the whole extraction procedure

Optimization of the desorption time at a fixed desorption temperature of 60ºC. *Highlight of UV 326, UV 571 and UV 327

RESULTS

EXTRACTION PROCEDURE	
VOLUME SAMPLE	25 mL
TIME	2 h
pH	6
IONIC STRENGTH	0% NaCl (m/v)
TEMPERATURE	40ºC
DESORPTION PROCEDURE	
TEMPERATURE	60ºC
TIME	20 min

Chromatogram of spiked deionized water (20 ng·mL^{-1} for each analyte) using SBSE-UPLC-MS/MS

COMPOUND	LOD[a] (ng·L^{-1})	LOQ[b] (ng·L^{-1})	RSD[c] (%)	RECOVERIES[d] (%)
UV P	62.4	207.2	12.05	95.77
UV 329	20.6	68.6	8.10	68.32
UV 326	14.1	47.0	10.71	49.22
UV 328	24.5	81.5	12.22	105.34
UV 327	9.0	30.0	13.88	91.63
UV 571	21.0	81.5	8.58	41.61
UV 360	4.5	15.1	8.10	63.08

[a] Detection limits are calculated as signal to noise ratio of 3 times
[b] Quantification limits are calculated as signal to noise ratio of 10 times
[c] Relative Standard Deviation (n=6)
[d] Mean of three determinations in deionized water spiked with 10 ng·mL^{-1}

CONCLUSIONS

The developed SBSE-UPLC-MS/MS method has been applied to the simultaneous determination of seven benzotriazole compounds in liquid samples with satisfactory results. The optimised microextraction procedure is very simple and requires only 25 mL of sample, being the volume of the final extract of 1.5 mL. Moreover, the UPLC technology offers a very fast analysis (less than 1 minute) and the MS/MS detection provides high sensitivity and selectivity, achieved LODs and LOQs in the range 9.0-62.4 and 15.1-207.2 ng·L^{-1}, respectively.

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ACKNOWLEDGMENTS

- Spanish Ministry of Science and Innovation, Research Project CTQ2010-20554.
- Sarah Montesdeoca Esponda thanks theCanary Agency of Investigation, Innovation and Information Society for her Ph.D. student grant

13as Jornadas de Análisis Instrumental

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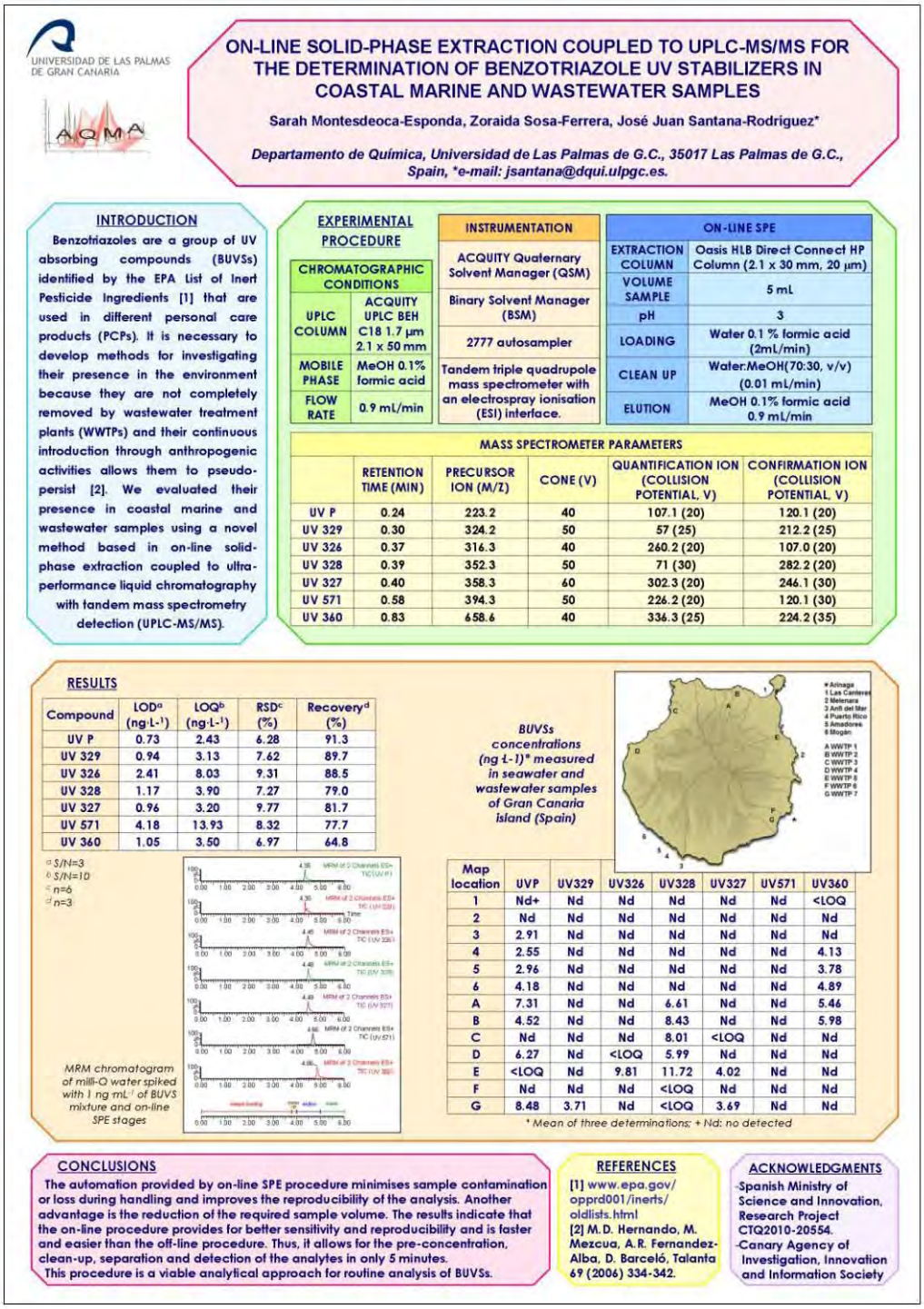

ON-LINE SOLID-PHASE EXTRACTION COUPLED TO UPLC-MS/MS FOR THE DETERMINATION OF BENZOTRIAZOLE UV STABILIZERS IN COASTAL MARINE AND WASTEWATER SAMPLES

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INTRODUCTION

Benzotriazoles are a group of UV absorbing compounds (BUVSs) identified by the EPA List of Inert Pesticide Ingredients [1] that are used in different personal care products (PCPs). It is necessary to develop methods for investigating their presence in the environment because they are not completely removed by wastewater treatment plants (WWTPs) and their continuous introduction through anthropogenic activities allows them to pseudo-persist [2]. We evaluated their presence in coastal marine and wastewater samples using a novel method based in on-line solid-phase extraction coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection (UPLC-MS/MS).

EXPERIMENTAL PROCEDURE

CHROMATOGRAPHIC CONDITIONS	
UPLC COLUMN	ACQUITY UPLC BEH C18 1.7 µm 2.1 x 50 mm
MOBILE PHASE	MeOH 0.1% formic acid
FLOW RATE	0.9 mL/min

INSTRUMENTATION
ACQUITY Quaternary Solvent Manager (QSM)
Binary Solvent Manager (BSM)
2777 autosampler
Tandem triple quadrupole mass spectrometer with an electrospray ionisation (ESI) interface.

ON-LINE SPE	
EXTRACTION COLUMN	Oasis HLB Direct Connect HP Column (2.1 x 30 mm, 20 µm)
VOLUME SAMPLE	5 mL
pH	3
LOADING	Water 0.1 % formic acid (2mL/min)
CLEAN UP	Water:MeOH(70:30, v/v) (0.01 mL/min)
ELUTION	MeOH 0.1% formic acid 0.9 mL/min

MASS SPECTROMETER PARAMETERS

	RETENTION TIME (MIN)	PRECURSOR ION (M/Z)	CONE (V)	QUANTIFICATION ION (COLLISION POTENTIAL, V)	CONFIRMATION ION (COLLISION POTENTIAL, V)
UV P	0.24	223.2	40	107.1 (20)	120.1 (20)
UV 329	0.30	324.2	50	57 (25)	212.2 (25)
UV 326	0.37	316.3	40	260.2 (20)	107.0 (20)
UV 328	0.39	352.3	50	71 (30)	282.2 (20)
UV 327	0.40	358.3	60	302.3 (20)	246.1 (30)
UV 571	0.58	394.3	50	226.2 (20)	120.1 (30)
UV 360	0.83	658.6	40	336.3 (25)	224.2 (35)

RESULTS

Compound	LOD[a] (ng·L⁻¹)	LOQ[b] (ng·L⁻¹)	RSD[c] (%)	Recovery[d] (%)
UV P	0.73	2.43	6.28	91.3
UV 329	0.94	3.13	7.62	89.7
UV 326	2.41	8.03	9.31	88.5
UV 328	1.17	3.90	7.27	79.0
UV 327	0.96	3.20	9.77	81.7
UV 571	4.18	13.93	8.32	77.7
UV 360	1.05	3.50	6.97	64.8

[a] S/N=3
[b] S/N=10
[c] n=6
[d] n=3

MRM chromatogram of milli-Q water spiked with 1 ng mL⁻¹ of BUVS mixture and on-line SPE stages

BUVSs concentrations (ng L-1) measured in seawater and wastewater samples of Gran Canaria Island (Spain)*

Map location	UVP	UV329	UV326	UV328	UV327	UV571	UV360
1	Nd+	Nd	Nd	Nd	Nd	Nd	<LOQ
2	Nd	Nd	Nd	Nd	Nd	Nd	Nd
3	2.91	Nd	Nd	Nd	Nd	Nd	Nd
4	2.55	Nd	Nd	Nd	Nd	Nd	4.13
5	2.96	Nd	Nd	Nd	Nd	Nd	3.78
6	4.18	Nd	Nd	Nd	Nd	Nd	4.89
A	7.31	Nd	Nd	6.61	Nd	Nd	5.46
B	4.52	Nd	Nd	8.43	Nd	Nd	5.98
C	Nd	Nd	Nd	8.01	<LOQ	Nd	Nd
D	6.27	Nd	<LOQ	5.99	Nd	Nd	Nd
E	<LOQ	Nd	9.81	11.72	4.02	Nd	Nd
F	Nd	Nd	Nd	<LOQ	Nd	Nd	Nd
G	8.48	3.71	Nd	<LOQ	3.69	Nd	Nd

* Mean of three determinations; + Nd: no detected

CONCLUSIONS

The automation provided by on-line SPE procedure minimises sample contamination or loss during handling and improves the reproducibility of the analysis. Another advantage is the reduction of the required sample volume. The results indicate that the on-line procedure provides for better sensitivity and reproducibility and is faster and easier than the off-line procedure. Thus, it allows for the pre-concentration, clean-up, separation and detection of the analytes in only 5 minutes. This procedure is a viable analytical approach for routine analysis of BUVSs.

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ACKNOWLEDGMENTS

-Spanish Ministry of Science and Innovation, Research Project CTQ2010-20554.
-Canary Agency of Investigation, Innovation and Information Society

Hyphenated techniques in sample preparation and Hyphenated techniques in chromatography

(Brujas, Bélgica, 2012).

IV. Otras publicaciones

1) **Autores:** T. Vega Morales, S. Montesdeoca Esponda, J.J. Santana Rodríguez, S. Efremova Aaron, J.J. Aaron.

   Título: "Luminescence methods for study and determination of pollutants in the environment (a review)."

   Revista: Macedonian Journal of Chemistry and Chemical Engineering

   Volumen: 29

   Páginas: 1-42

   Fecha: 2010

2) **Autores:** R. Guedes-Alonso, C. Afonso Olivares, S. Montesdeoca Esponda, Z. Sosa Ferrera, J.J. Santana Rodríguez .

   Título: "An assessment of the concentrations of pharmaceutical compounds in wastewater treatment plants on the island of Gran Canaria (Spain)."

   Revista: Springer Plus

   Volumen: 2

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3) **Autores:** Jiří Plíšek, Lenka Krčmová, Jana Aufartová, Tanausú Vega-Morales, Sarah Montesdeoca-Esponda, Roman Oros, Eva Kasalová, Luboš Sobotka, Petr Solich, Dagmar Solichová.

   Título: "New approach for clinical monitoring of 25-OH D_3 and 25-OH D_2 by UHPLC-MS/MS - how to overcome problem with non-standardized materials."

   Revista: Bioanalysis (enviado)

   Volumen:

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V. Otras comunicaciones presentadas en congresos

1) **Autores:** S. Montesdeoca Esponda, E. Estévez, M.C. Cabrera, Z. Sosa Ferrera, J.J. Santana Rodríguez.

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   Congreso: XV Reunión de la Sociedad Española de Química Analítica

   Tipo de participación: Póster

   Lugar de celebración: San Sebastián, España

   Fecha: Julio 2009

2) **Autores:** S. Montesdeoca Esponda, E. Estévez, M.C. Cabrera, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Temporal evolution of pharmaceutical residues in water samples from an area of gran canaria island using SPE-HPLC-ESI-MS/MS method."

   Congreso: 7º ANQUE International Congress. Integral Water: Present a future.

   Tipo de participación: Póster

   Lugar de celebración: Oviedo, España

   Fecha: Junio 2010

3) **Autores:** S. Montesdeoca Esponda, E. Estévez-Navarro, M.C. Cabrera-Santana, Z. Sosa Ferrera, J.J. Santana Rodríguez.

   Título: "Determination of pharmaceutical residues in waters from Gran Canaria island using a SPE-HPLC-ESI-MS/MS method."

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   Tipo de participación: Póster

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Fecha: Abril 2011

4) **Autores:** L. Krčmová, J. Plíšek, R. Oros, T. Vega Morales, S. Montesdeoca Esponda, E. Kasalová, D. Solichová, M. Bláha, L. Sobotka.

Título: "Utilization of UHPLC in connection with fast MS detector for monitoring of vitamin D in current clinical research."

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Tipo de participación: Póster

Lugar de celebración: Hradec Králové, República Checa

Fecha: Octubre 2012