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**DESARROLLO DE NUEVAS METODOLOGÍAS PARA LA
DETERMINACIÓN DE PLASTIFICANTES Y RETARDANTES DE
LLAMA ORGANOFOSFORADOS Y SUS DERIVADOS EN
MUESTRAS MEDIOAMBIENTALES**

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Memoria para optar al grado de Doctora en Química

Santiago de Compostela, enero de 2010

Dña. Pilar Bermejo Barrera, Catedrática de Universidad y Directora en funciones del Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago de Compostela,

Informa:

Que **Dña. Mónica García López** ha realizado en este departamento el trabajo recogido en la memoria titulada **“DESARROLLO DE NUEVAS METODOLOGÍAS PARA LA DETERMINACIÓN DE PLASTIFICANTES Y RETARDANTES DE LLAMA ORGANOFOSFORADOS Y SUS DERIVADOS EN MUESTRAS MEDIOAMBIENTALES”** bajo la dirección de D. Rafael Cela Torrijos, Catedrático de Universidad del Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago de Compostela, y D. Isaac Rodríguez Pereiro, Profesor Titular del mismo departamento, que presenta para optar al grado de Doctora en Química.

Y para que así conste, firmo el presente informe en Santiago de Compostela, enero 2010.

Dña. Pilar Bermejo Barrera

D. Rafael Cela Torrijos, Catedrático de Universidad del Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago de Compostela, y **D. Isaac Rodríguez Pereiro**, Profesor Titular del mismo departamento,

Autorizan:

A la licenciada **Dña. Mónica García López** a la presentación del trabajo recogido en la memoria titulada **“DESARROLLO DE NUEVAS METODOLOGÍAS PARA LA DETERMINACIÓN DE PLASTIFICANTES Y RETARDANTES DE LLAMA ORGANOFOSFORADOS Y SUS DERIVADOS EN MUESTRAS MEDIOAMBIENTALES”** que ha realizado bajo su dirección en el Departamento de Química Analítica, Nutrición y Bromatología de la Facultad de Química de la Universidad de Santiago de Compostela para optar al grado de Doctora en Química

Y para que así conste, firmamos el presente informe en Santiago de Compostela, enero 2010.

D. Rafael Cela Torrijos

D. Isaac Rodríguez Pereiro

Motivaciones, decisiones y

la duda de si todo tiene que ser siempre difícil antes de ser fácil

LA ENERGÍA SE CUANTIFICA EN “PAQUETES”

Y BERNARDO ENGORDÓ DE REPENTE

La historia

En vista de que llegaba el verano, Bernardo se puso a régimen. Nada de pasta ni de filetes: por hoy, sólo cerezas. Pero como comer y rascar todo es empezar, acabó por comerse un montón de ellas. A pesar del atracón, aún se siente muy ligero. Entonces, mira la manzana que ha quedado en el plato y piensa: “No creo que por una manzana vaya ahora a engordar”. Y dicho esto, se la toma. Acaba de tragársela cuando, de repente, ¡se pone como un muñeco Michelin! Alarmado, llama por teléfono al doctor Pesapoco, su endocrino. “Amigo Bernardo, es que no ha tenido en cuenta la cuantificación de la energía”, le explica el médico. “La energía no varía en una cantidad fija sino a saltos”. Así, mientras come alimentos con un contenido calórico inferior a un cierto valor –que depende de su estructura física –, éstos no surten ningún efecto sobre usted. Por esto puede comer todas las cerezas que quiera. Pero, como las manzanas tienen un contenido calórico superior, suficiente para hacerle dar un salto cuántico, éste se traduce en el organismo en un aumento de peso instantáneo.

La explicación

En 1900 el físico alemán Max Planck llegó a la conclusión de que la materia y la radiación no se intercambian energía en cantidades pequeñas y a su gusto, como se había creído siempre, sino en “paquetes” de cantidades bien determinadas, bautizados después por Einstein como “cuantos de luz”. Un sistema cuántico, por lo tanto, no puede alterarse de forma suave: o se altera totalmente, o nada. Por ejemplo, si a un átomo lo golpea un fotón –es decir, un cuanto de luz– de la energía adecuada, un electrón suyo “salta” a una órbita más externa. Si la energía es apenas un poco inferior, entonces no sucede nada.

Muy Interesante, Nº 198 (Noviembre, 1997)

*(Con alguna modificación en lo que concierne al sujeto que engorda
y al adjetivo que se le aplica)*

AGRADECIMIENTOS

A kite cannot fly without strings

Durante estos últimos años he aprendido muchas cosas y también he olvidado bastantes, pero el balance es muy positivo ya que he podido dedicarme a algo que me gusta y ampliar mis horizontes profesionales y personales. Todo esto no hubiese tenido valor si no hubiese podido compartir mis experiencias y tantos momentos especiales con las personas que quiero, las que formaban ya parte de mi vida y las que se han ido incorporando en esta trayectoria.

“A candle loses none of his light by lighting another candle” and fortunately there are not few candles in the world ;). Gracias a todos los que hacéis que valga la pena sonreír y cantar cada día, a los que me dais un abrazo sincero cuando me voy, cuando vuelvo, cuando las cosas no van bien, cuando desbordo alegría o simplemente porque sí. A los que me escucháis sin que haga falta gritar. A todos los que echo de menos y que dais lugar a momentos inolvidables. *And we may never forget it...*

Esta Tesis Doctoral no habría sido posible sin Rafael Cela e Isaac Rodríguez, directores del presente trabajo, que facilitaron mi incorporación a este grupo de investigación y me han proporcionado en todo momento los medios necesarios para el desarrollo de la misma. Gracias por confiar en mí. Gracias Isaac por tus ideas, por estar siempre disponible, por saber sacar siempre partido a todos los resultados.

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Gracias a mis -(c)it@s por todos los momentos estupendos que hemos pasado juntos, por ser compañeros y amigos, por hacerme echar de menos el laboratorio, por hacer más fácil cada día. Gracias por las risas, por el apoyo, por los detalles, por la complicidad, por la música, por corromperme ;), por la culturización no académica, por la ayuda desinteresada en todo momento, por confiar en mí, por hacer de septiembre un mes especial.

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Oh soli miei! *Se potessi far tornare indietro il mondo farei tornare poi senz'altro voi...* grazie per avermi fatto sentire a casa in Italia e per aver messo più musica nella mia vita, non vi dimenticherò mai: sweet home Italia ;). Quando vi penso mi vengono in mente solo risate e momenti indimenticabili. *Sognami se nevicata, sognami sono nuvola... déjame atravesar el viento sin documentos...* la mia cara sorella francese, una panchina rossa sotto il sole, il napoletano, il lungomare salernitano, il Nettuno, gli amici di afluencia e ocra, l'eruzione del Vesuvio, la piazza famosa per un nome diverso dall'originale ;)...

I would like to thank Professor Caruso and his group for their warm welcome. Thanks Doc for giving me the chance to work in your group. Thank you for your support and encouraging words and e-mails. I am especially grateful to Karolin: thank you for being so nice, for your selfless help. You made my life and stay in the States really easy. Thanks for the great moments we spent together. Thanks YaoFangcita for being so kind and good. Thanks to the shuttle family! Thanks Sam and Linda for having such a big heart, thanks for being some of my candles overseas!

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Minds are like parachutes, they only function when opened

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Sections with an asterisk () at the beginning are written in English*

ABSTRACT

The analytes considered in this PhD dissertation are the triphenyl phosphine oxide and some phosphoric acid triesters and diesters. First reports on organophosphate esters date back to the late 1970s. These studies were then almost abandoned during the 1990s as most of the considered organophosphates were found to be degradable in the environment. However, research on these chemicals in the environment has re-emerged since many of them were found in indoor environments [1] and the chlorinated derivatives were included in European Union priority lists [2,3] for risk assessment and found to be rather persistent in the environment [4]. This information justifies the analytical interest of these compounds, that may be classified as “re-emerging” rather than emerging pollutants [5]. Emerging pollutants correspond in most cases to unregulated contaminants continually introduced into the environment due to anthropogenic activity. These pollutants may be candidates for future regulation depending on research on their potential health effects and environmental impact [6]. Currently, risk assessment of the three chlorinated derivatives included in the 2nd and 4th priority lists of aquatic pollutants is being undertaken in the European Union.

The general aim of the present work was to develop sensitive and selective methodologies for these selected analytes in environmental matrices of different complexity, both solid (dust and sediments) and aqueous (tap water, river water and wastewater). Available sample preparation methodology was, at the beginning of this PhD dissertation, rather limited and mainly based on classical extraction techniques, which are laborious and involve consumption of large amounts of organic solvents. Regarding this, our efforts have principally focused on the sample preparation step, aiming to minimize the length of the analysis, their cost, the solvent intake and, as a consequence, waste generation.

Additionally, the capabilities of different chromatographic techniques (gas and liquid chromatography) and detection systems (nitrogen-phosphorus detector and mass spectrometer with electron impact, inductively coupled plasma or electrospray ionization sources) for the determination of the considered organophosphate esters were assessed.

Finally, the suitability of the developed methodologies was tested applying them to the analysis of real samples.

[1] H. Carlsson, U. Nilsson, G. Becker, C. Östman, *Environ. Sci. Technol.* 31 (1997) 2931.

[2] *Off. J. Eur. Commun.* L231 (1995) 18.

[3] *Off. J. Eur. Commun.* L273 (2000) 5.

[4] Y. Kawagoshi, S. Nakamura, I. Fukunaga, *Chemosphere* 48 (2002) 219.

[5] T. Reemtsma, J.B. Quintana, R. Rodil, M. García-López, I. Rodríguez, *Trends Anal. Chem.* 27 (2008) 727.

[6] D. Barceló, *Trends Anal. Chem.* 22 (2003) xiv.

I. JUSTIFICACIÓN Y OBJETIVOS

Los analitos considerados en esta Tesis Doctoral son el óxido de la trifenil fosfina y algunos triésteres y diésteres derivados del ácido fosfórico. Los primeros trabajos sobre estos compuestos se remontan a finales de los años 70. Durante los 90 casi se abandonó su estudio por ser considerados degradables. Sin embargo, a finales de esa misma década se retomaron las investigaciones como consecuencia de su detección en ambientes interiores [1] y la inclusión de los derivados clorados, que son bastante persistentes [2], en listas europeas de contaminantes acuáticos prioritarios [3,4]. Esto justifica el interés analítico de los organofosforados seleccionados en el presente estudio que, en base a los comentarios anteriores, pueden considerarse contaminantes “reemergentes”, más que emergentes [5]. Los contaminantes emergentes son compuestos normalmente no regulados que se están liberando de forma continua al medioambiente como resultado de la actividad humana. Estos contaminantes son candidatos a regirse en un futuro por medidas de control, en función de los resultados que se desprendan de las investigaciones sobre sus posibles riesgos para la salud o su impacto medioambiental [6]. En la actualidad, se está llevando a cabo en la Unión Europea la evaluación de los posibles riesgos derivados del uso de los tres organofosforados clorados incluidos en la 2ª y 4ª listas de contaminantes acuáticos prioritarios.

El objetivo general de esta Tesis Doctoral ha sido el desarrollo de metodologías sensibles y selectivas para la determinación de los analitos seleccionados en muestras medioambientales de diversa complejidad, tanto sólidas (polvo y sedimentos) como acuosas (agua de grifo, río y residual). La metodología disponible en lo que a preparación de muestras se refiere era, en el momento en que se inició esta Tesis, bastante limitada y basada fundamentalmente en técnicas de extracción clásicas, laboriosas y con un gran consumo de disolventes. Por ello, nuestros esfuerzos se han centrado fundamentalmente en la etapa de preparación de muestra, intentando minimizar el tiempo de análisis, los costes, el consumo de disolventes y, por ende, la generación de residuos.

Igualmente, se ha pretendido evaluar la capacidad de distintas técnicas de separación cromatográfica (cromatografías de gases y líquidos) y diferentes sistemas de detección (detector de nitrógeno-fósforo, espectrómetro de masas con ionización mediante impacto electrónico, plasma de acoplamiento inducido o electrospray) para la determinación de los organofosforados considerados.

Finalmente, además de la puesta a punto de los métodos de determinación, tras la optimización de diferentes variables, se evaluó su validez aplicando las metodologías propuestas al análisis de muestras reales.

[1] H. Carlsson, U. Nilsson, G. Becker, C. Östman, *Environ. Sci. Technol.* 31 (1997) 2931.

[2] Y. Kawagoshi, S. Nakamura, I. Fukunaga, *Chemosphere* 48 (2002) 219.

[3] *Off. J. Eur. Commun.* L231 (1995) 18.

[4] *Off. J. Eur. Commun.* L273 (2000) 5.

[5] T. Reemtsma, J.B. Quintana, R. Rodil, M. García-López, I. Rodríguez, *Trends Anal. Chem.* 27 (2008) 727.

[6] D. Barceló, *Trends Anal. Chem.* 22 (2003) xiv.

II. INTRODUCCIÓN

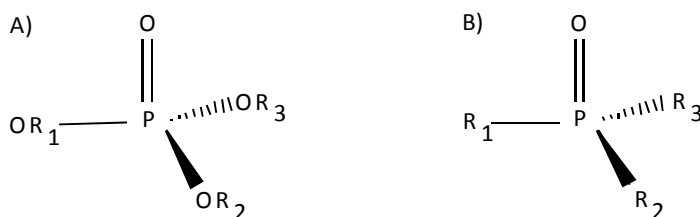
1. COMPUESTOS

1.1. Estructura y propiedades

Los compuestos considerados en la presente Tesis Doctoral son derivados tri- y disustituidos del ácido fosfórico y el óxido de la trifenil fosfina (TPPO). Los triésteres derivados del ácido fosfórico y el TPPO son compuestos de origen antropogénico, utilizados fundamentalmente como plastificantes y retardantes de llama [1]. Los diésteres se utilizan normalmente como extractantes de elementos radiactivos [2,3]. Éstos, además de producirse de manera intencionada, pueden resultar de la hidrólisis de los triésteres, como se ha observado por irradiación del tri-n-butil fosfato (TBP) en la industria nuclear [4], o de la degradación microbiana del trifenil fosfato (TPP).



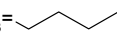


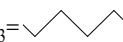
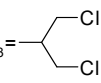

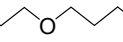
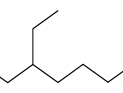


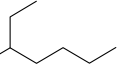

Las estructuras de los compuestos organofosforados (OPs) considerados se muestran en la **Figura II.1** y sus nombres completos, abreviaturas y algunas de sus propiedades físico-químicas se recogen en la **Tabla II.1**.

Figura II.1. Estructura de los tri- y diésteres del ácido fosfórico (A) y del óxido de la trifenil fosfina (B). R_1 , R_2 , R_3 especificados en la **Tabla II.1**.



Desde el punto de vista de sus características físico-químicas, los OPs no constituyen un grupo homogéneo ya que sus propiedades varían notablemente en función del tipo de sustituyentes que posean (**Tabla II.1**). Así, los logaritmos de sus constantes de partición octanol-agua se encuentran dentro del intervalo de 1.44 a 9.49 correspondientes al TCEP y al TEHP, respectivamente, y sus presiones de vapor varían entre 2.50×10^{-8} y 6.13×10^{-2} Torr. Los diésteres son más hidrofílicos que sus correspondientes triésteres y son, además, compuestos muy ácidos (pK_a : 1.12 (DPP), 1.47 (DEHP), 1.53 (DBP)) [5]. Desde el punto de vista analítico, esa heterogeneidad es una dificultad adicional a la hora de desarrollar metodologías válidas para el análisis simultáneo de los compuestos seleccionados.

Tabla II.1. Identidad y propiedades físico-químicas de los compuestos organofosforados considerados en esta Tesis Doctoral.

| Abrev. | Nombre | Nº CAS | PM | log K _{ow} | P _v (25°C)/Torr | Sustituyentes |
|-------------|-----------------------------------|------------|-------|---------------------|----------------------------|--|
| TPrP | Tripropil fosfato | 513-08-6 | 224.2 | 1.87 | 4.33 x 10 ⁻³ | R ₁ =R ₂ =R ₃ =  |
| TiBP | Tri(isobutil) fosfato | 126-71-6 | 266.3 | 3.60 | 1.28 x 10 ⁻² | R ₁ =R ₂ =R ₃ =  |
| TBP | Tri-n-butil fosfato | 126-73-8 | 266.3 | 4.00 | 1.13 x 10 ⁻³ | R ₁ =R ₂ =R ₃ =  |
| TCEP | Tri(2-cloroetil) fosfato | 115-96-8 | 285.5 | 1.44 | 6.13 x 10 ⁻² | R ₁ =R ₂ =R ₃ =  |
| TCPP | Tri(cloroisopropil) fosfato | 13674-84-5 | 327.6 | 2.59 | 2.02 x 10 ⁻⁵ | R ₁ =R ₂ =R ₃ =  |
| TPeP | Tripentil fosfato | 2528-38-3 | 308.4 | 5.29 | 1.67 x 10 ⁻⁵ | R ₁ =R ₂ =R ₃ =  |
| TDCP | Tri(1,3-dicloroisopropil) fosfato | 13674-87-8 | 430.9 | 3.65 | 7.36 x 10 ⁻⁸ | R ₁ =R ₂ =R ₃ =  |
| TPP | Trifenil fosfato | 115-86-6 | 326.3 | 4.59 | 6.28 x 10 ⁻⁶ | R ₁ =R ₂ =R ₃ =  |
| TBEP | Tributoxietil fosfato | 78-51-3 | 398.5 | 3.75 | 2.50 x 10 ⁻⁸ | R ₁ =R ₂ =R ₃ =  |
| TEHP | Tri(2-etilhexil) fosfato | 78-42-2 | 434.6 | 9.49 | 8.45 x 10 ⁻⁸ | R ₁ =R ₂ =R ₃ =  |
| TPPO | Óxido de la trifenil fosfina | 791-28-6 | 278.3 | 2.83 | 2.62 x 10 ⁻⁸ | R ₁ =R ₂ =R ₃ =  |
| DBP | Di-n-butil fosfato | 107-66-4 | 210.2 | 2.29 | 9.6 x 10 ⁻⁵ | R ₁ =-H R ₂ =R ₃ =  |
| DEHP | Di(2-etilhexil) fosfato | 298-07-7 | 322.4 | 6.07 | 4.65 x 10 ⁻⁸ | R ₁ =-H R ₂ =R ₃ =  |
| DPP | Difenil fosfato | 838-85-7 | 250.2 | 2.88 | 2.23 x 10 ⁻⁶ | R ₁ =-H R ₂ =R ₃ =  |

Abrev., abreviatura; PM, peso molecular; log K_{ow}, logaritmo de la constante de partición octanol-agua; P_v, presión de vapor. Propiedades físico-químicas obtenidas de [6].

1.2. Usos

Los triésteres organofosforados se utilizan habitualmente como retardantes de llama en plásticos, materiales de construcción y en la industria textil. Además, se emplean también como plastificantes, estabilizantes, antiespumantes en anticongelantes y aditivos en lubricantes y fluidos hidráulicos [1,7-11]. De forma genérica, los organofosforados clorados se emplean como retardantes de llama y los no halogenados como plastificantes [12].

Debido al gran número de aplicaciones, los triaril(aril) fosfatos se usan en las industrias electrónica, textil y de manufactura de plásticos, así como en los sectores de la construcción y de la automoción, entre otros. Estos compuestos se encuentran presentes en diversos productos [1]: espumas de poliuretano flexibles y rígidas (que se utilizan tanto para aislamientos térmico y acústico como en colchones, sofás, etc.), pinturas, pegamentos, barnices, plásticos, resinas, lacas, electrodomésticos, ordenadores, tapicerías, etc.

A principios de los años 90, alrededor del 17% del consumo global de retardantes de llama correspondía a ésteres organofosforados [13]. En los últimos años, su demanda se ha incrementado como consecuencia de la prohibición o introducción de restricciones en el uso de ciertos difenil éteres polibromados (polybrominated diphenyl ethers, PBDEs), utilizados como retardantes de llama, debido a su persistencia, bioacumulación y efectos adversos [14-16]. Así, por ejemplo, el consumo de retardantes organofosforados en Europa oriental fue de alrededor de 83000 toneladas en el año 2001, aproximadamente 91000 toneladas en 2005 y un 7.1% más en el año siguiente [17]. TCEP, TCPP, TDCP, TPP, TBEP y TBP están incluidos en la lista de productos químicos de gran producción en Europa [18].

Una de las tendencias en un futuro inmediato es la de combinar nuevos derivados bromados con compuestos organofosforados para incrementar sus propiedades como retardantes de llama y adaptarse a la nueva legislación. Comercializado por una de las principales compañías productoras de retardantes, la estadounidense Great Lakes, el Firemaster 550 ha surgido para reemplazar a los prohibidos penta y octabromodifenil éteres en sus aplicaciones. El Firemaster 550 es una mezcla de aril ésteres halogenados y aril fosfatos, con un contenido del 4.3% de fósforo y 27.1% de bromo [19], entre los que se han identificado el trifenil fosfato, el tri(propilfenil) fosfato y el octiltetrabromo benzoato [20].

Los diésteres se producen industrialmente y se utilizan para la extracción de metales, como plastificantes y en la industria textil, entre otras aplicaciones [21].

1.3. Toxicidad

La información sobre toxicidad e impacto medioambiental de estos compuestos organofosforados es escasa. En base a los datos de los que se dispone, algunos de ellos son prácticamente inocuos, otros han sido clasificados como ligeramente tóxicos y algunos son sospechosos de ser carcinogénicos [7-9,11].

El TCEP está incluido en la segunda lista prioritaria europea [22], mientras que TCPP y TDCP figuran en la cuarta lista prioritaria [23]. El borrador publicado en 2006 sobre la evaluación

de riesgos para el TCEP concluye que no hay necesidad de realizar más estudios ni tomar medidas en lo que concierne a la protección medioambiental. La evaluación de riesgos para la salud humana no se ha completado todavía, sin embargo, se reconoce la carcinogenicidad, elevada toxicidad y persistencia medioambiental de este compuesto. A pesar de esto, el TCEP no cumple el criterio de persistencia, bioacumulación y toxicidad (PBT) ya que no es bioacumulativo [24]. La evaluación de los riesgos de los otros dos clorados no se ha completado todavía pero la carcinogénesis del TDCP parece bastante clara y el TCPP es también sospechoso de ser carcinogénico. A parte, estos dos compuestos presentan constantes de partición octanol-agua mayores que las del TCEP, por lo que puede que sí cumplan el criterio PBT y haya que tomar medidas de control en un futuro.

En trabajadores expuestos a una mezcla de aril fosfatos se observó un descenso del número de monocitos [25]. En humanos, el TPP es un potente inhibidor de la carboxilesterasa [26], presenta toxicidad hemolítica [7] y causa dermatitis de contacto [27,28].

TBP y TCPP causan irritación de la piel [8,9] mientras que TDCP y TCPP originan efectos hemolíticos [29].

1.4. Distribución medioambiental

En general, los retardantes de llama pueden utilizarse como aditivos, en cuyo caso se mezclan con el material al que se adicionan pero no están químicamente unidos a él, o bien como reactivos, estableciendo enlaces químicos con el material, con lo que la emisión durante el tiempo de vida del producto es más limitada. Los ésteres derivados del ácido fosfórico son un ejemplo de retardantes que se emplean habitualmente como aditivos y, por ello, pueden liberarse al medio durante la producción, uso y destrucción de los productos que los contienen con relativa facilidad [1]. Posteriormente, su distribución y acumulación en el medioambiente o en matrices biológicas depende de sus propiedades físico-químicas, que a su vez están condicionadas por los sustituyentes del grupo éster. Así, por ejemplo, el TCEP es muy polar y bastante volátil mientras que el TEHP es altamente lipofílico y muy poco volátil, **Tabla II.1**, por lo que es esperable que los niveles del primero en aguas y aire sean superiores a los del segundo, que tenderá a adsorberse a materia particulada, sedimentos... Por otro lado, como se ha comentado ya, los diésteres se disocian a valores muy ácidos de pH por lo que en el medioambiente se presentarán como especies aniónicas y, por tanto, la adsorción a materia particulada y distribución en matrices biológicas es poco probable [30].

Como consecuencia de su uso generalizado, estos compuestos se encuentran ampliamente distribuidos y se han detectado en distintos compartimentos medioambientales [31]: aire, polvo, sedimentos, lodos, agua dulce, agua de mar, aguas residuales, etc.

1.4.1. Aire

1.4.1.1. Atmósferas interiores

Los OPs se añaden a materiales de construcción, electrodomésticos, tapicerías, barnices, etc. En consecuencia, su concentración en ambientes cerrados (oficinas, vehículos, bibliotecas,

tiendas de muebles y electrodomésticos...) es significativamente superior a la de ambientes exteriores [32].

En la **Tabla II.2** se recogen las concentraciones promedio, mínima y máxima determinadas para algunos de los organofosforados de interés en ambientes interiores. En la tabla sólo se reflejan los trabajos que proporcionan datos para un mínimo de cuatro muestras. Además, los valores por debajo de los límites de cuantificación no se han considerado para estimar las concentraciones medias.

Tabla II.2. Concentraciones promedio (ng m^{-3}) de algunos OPs en aire de atmósferas interiores. Los valores mínimos y máximos se proporcionan entre paréntesis.

| N | TBP | TCEP | TCPP | TDCP | TBEP | TPP | TEHP | Ref. |
|---------------------|------------------|-------------------|--------------------|------------------|------------------|-------------------|-------------------|------|
| 6 | 70 (<1.8-100) | 30 (<1.4-30) | n.d. | n.d. | 20 (<0.4-30) | 10 (<1.2-10) | n.d. | [33] |
| 17 | 20 (<0.2-120) | 129 (<0.4-730) | 118 (5.1-570) | 36 (<0.2-150) | 7 (<0.2-55) | 7 (<0.1-23) | <0.2-14) | [34] |
| 5 | 22 (7-35) | 94 (11-250) | 31 (14-41) | n.d. | 3 (1.4-5.9) | 0.7 (0.5-0.8) | 10 (<0.5-10) | [32] |
| 6 | 14 (9-18) | 25 (15-36) | 21 (14-28) | n.d. | 29 (20-36) | 19 (12-40) | n.d. | [35] |
| 16 | 11 (<0.1-29) | 17 (<0.2-56) | 110 (<0.1-260) | < 0.1 | < 3 | 2.8 (<1.5-5.7) | 2.4 (0.24-3.4) | [36] |
| 30 | 20 (1-172) | 78 (1-870) | 273 (1-2300) | 6 (5-7) | 16 (<1-130) | 6 (<1-17) | 3.8 (1-18) | [37] |
| 4 | 42 (4-138) | 44 (11-110) | 58 (10-112) | n.d. | 31 (0.8-46) | 12 (0.5-35) | 2.7 | [38] |
| 18 | 4 (<0.4-30.6) | 1.3 (<0.7-136) | 1.9 (<0.9-1260) | <0.6 | 1.8 (<0.6-14) | <5.4 | n.d. | [39] |
| 50 | n.d. | n.d. | 52 | n.d. | n.d. | n.d. | n.d. | [40] |
| Media global | 25 | 52 | 84 | 21 | 15 | 6 | 5 | |

N, número de muestras; n.d., no detectado.

TBP, TCEP y TCPP son los organofosforados predominantes en atmósferas interiores. A pesar de que los niveles determinados son en general bajos, se han detectado valores máximos de hasta 172, 870 y 2300 ng m^{-3} de TBP, TCEP y TCPP, respectivamente [37].

Si calculamos la suma de las concentraciones medias globales de los alquil fostatos clorados (TCEP, TCPP y TDCP), que son los que más preocupación despiertan, ésta excede los 150 ng m^{-3} . Asumiendo un volumen de aire respirado diario de 20 m^3 [34,36], su absorción diaria a través del sistema respiratorio es de alrededor de 3 μg .

La variabilidad de concentraciones de los OPs dentro de cada serie de muestras y entre series pone de manifiesto la influencia de los distintos materiales y productos presentes en el lugar de muestreo en los niveles de los compuestos encontrados. Así, por ejemplo, se sabe que los televisores y pantallas de ordenador son una importante fuente de emisión de TPP [1,41], mientras que los aislamientos de poliuretano y los barnices contribuyen a la presencia de OPs halogenados [39,42] y TBEP [1], respectivamente. Así mismo, los OPs clorados se han detectado a niveles extremadamente elevados en hospitales y cárceles debido a su uso extendido como retardantes de llama en colchones. Por otro lado, la utilización de TCPP en tapicerías y componentes plásticos en vehículos y medios de transporte públicos conlleva la presencia de niveles de 10 a 100 veces superiores en estos ambientes que en viviendas privadas [37,38].

1.4.1.2. *Atmósferas exteriores*

Aunque es de esperar que la presencia de OPs en ambientes exteriores sea inferior que en interiores, varios trabajos han revelado niveles significativos de OPs en aire de atmósferas exteriores y matrices relacionadas.

El transporte atmosférico desempeña un papel crucial en la distribución global de contaminantes en el medioambiente y es el responsable de la presencia de los mismos en regiones remotas [43]. Así, por ejemplo, Aston y col. [44] detectaron tres alquil fosfatos en hojas de pino de las Montañas de Sierra Nevada (Estados Unidos), a saber: TCEP (1950 ng g^{-1}), TCPP (763 ng g^{-1}) y TDCP (1320 ng g^{-1}). Ciccioioli y col. [45] detectaron varios compuestos de origen antropogénico, entre ellos alquil fosfatos, asociados a la materia particulada de muestras de aire recogidas durante una expedición a la Antártida. Laniewski y col. [46] analizaron agua de lluvia y nieve de varios lugares remotos del hemisferio norte y detectaron TCEP y TCPP, además de otros compuestos orgánicos clorados. Los niveles máximos de TCEP y TCPP detectados fueron 21 y 3 ng L^{-1} , respectivamente.

Hasta el momento, los OPs no han sido incluidos en estudios de monitorización en regiones Árticas. Sin embargo, recientemente, se ha hecho una evaluación teórica de contaminantes árticos potenciales, incluyendo en el estudio más de 10^5 productos químicos con similitudes físico-químicas con contaminantes árticos conocidos [47]. El TCEP, por su estructura y propiedades, es predeciblemente un contaminante ártico. Así pues, el transporte atmosférico de OPs es un factor a tener en cuenta.

1.4.2. *Matrices sólidas*

Muchos de los organofosforados considerados tienen puntos de ebullición altos, presiones de vapor bajas, presentan coeficientes de adsorción relativamente elevados y, generalmente, exhiben baja solubilidad en agua [7-9,11]. Por ello, no es de extrañar que muestren una tendencia acusada a concentrarse en sedimentos, materia particulada y lodos.

1.4.2.1. Polvo

El 99% de los organofosforados presentes en atmósferas interiores se encuentran asociados a materia particulada [32]. No obstante, la información disponible sobre niveles de OPs en polvo y otras matrices sólidas, así como la metodología analítica necesaria para llevar a cabo su determinación es tremendamente limitada. Desde un punto de vista toxicológico, el análisis de polvo es de especial utilidad para evaluar exposiciones promedio (por vías oral, dérmica y respiratoria) a compuestos semivolátiles en atmósferas interiores. Además, la composición química de la materia particulada en atmósferas interiores se está empezando a relacionar con problemas de alergias y con el síndrome de *sick building* [48].

El número de trabajos en los que se determinan los niveles de OPs en aire supera al de aquellos en los que se analiza el polvo depositado. Wilkins y col. [49] determinaron niveles de TCEP inferiores a 5 ng g^{-1} en el polvo de edificios públicos daneses. Hansen y col. [50] detectaron también ese compuesto en el análisis del polvo procedente de pisos y escuelas públicas alemanes, con concentraciones muy variables. La concentración máxima de TCEP en los pisos fue de 44 ng g^{-1} , mientras que en los colegios se detectaron hasta 2200 ng g^{-1} . El TBEP también estaba incluido en ese estudio y su concentración en las escuelas variaba entre 100 y 1300 ng g^{-1} . Quintana y col. [51] analizaron una muestra de polvo recogida en una zona con intenso tráfico y detectaron TCEP (51 ng g^{-1}), TCPP (320 ng g^{-1}), TPP (290 ng g^{-1}) y TEHP (220 ng g^{-1}). En la **Tabla II.3** se recogen otros trabajos en los que se han determinado los niveles de OPs en polvo.

Tabla II.3. Concentraciones promedio ($\mu\text{g g}^{-1}$) de algunos OPs en polvo. Las concentraciones mínima y máxima se proporcionan entre paréntesis.

| N | TBP | TCEP | TCPP | TDCP | TBEP | TPP | Ref. |
|------------------------|---------------------|-------------------|--------------------|---------------------|------------------|-------------------|------|
| 15 | 0.54 (0.07-2.2) | 11.3 (0.2-94) | 11.0 (0.5-73) | 9.5 (0.2-67) | 419 (14-5300) | 11.4 (0.9-110) | [1] |
| 436/983 (TCPP/TCEP) | ◇ | 2.7 (<0.1-121) | 1.85 (<0.1-375) | ◇ | ◇ | ◇ | [40] |
| 9 | 0.19 (0.04-0.9) | 5.6 (0.1-40) | 8.4 (1.2-40) | 6.1 (<0.05-11) | 11.4 (1.6-48) | 1.5 (0.4-4.9) | [52] |
| 8 | 0.25 (0.07-0.65) | 1.7 (0.25-9.8) | 3.9 (0.35-10.3) | 0.35 (<0.05-1.1) | 9.9 (1.2-19) | 2.6 (0.3-9.5) | [53] |
| Media | 0.33 | 5.3 | 6.3 | 5.3 | 147 | 5.2 | |

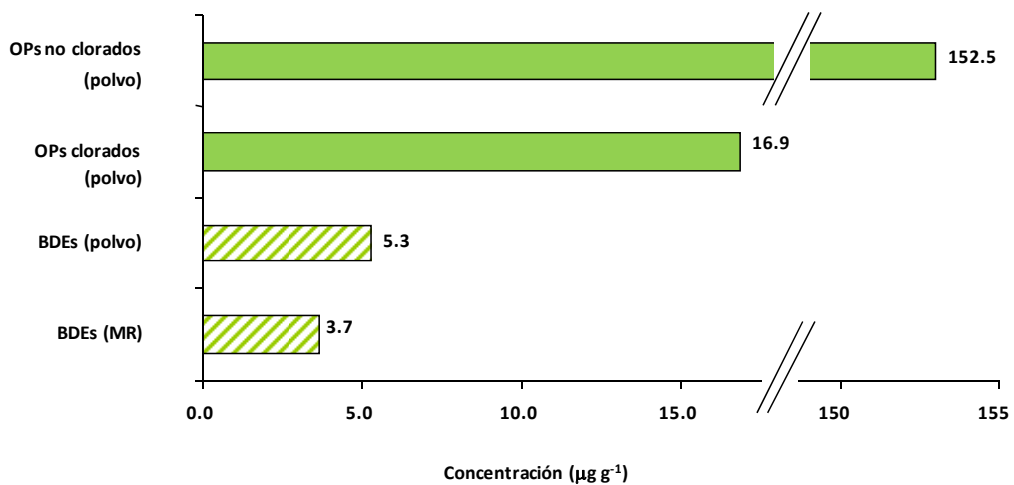
N, número de muestras analizadas; ◇, no determinado.

Todas las muestras analizadas son de polvo recolectado con aspiradoras en áreas confinadas. Ingerowski y col. [40] analizaron el polvo de múltiples viviendas alemanas además de algunas muestras procedentes de colegios y oficinas. Marklund y col. [1] determinaron los OPs en el polvo procedente de distintos ambientes interiores (un hospital, un hotel, una biblioteca, un cine, un salón de baile, una prisión, dos casas, una oficina, etc.) y en dos de los trabajos que forman parte de esta Tesis Doctoral [52,53] se analizaron muestras de polvo de varias viviendas españolas así como del interior de vehículos.

Con la excepción del relativamente volátil TBP, las medias globales para los restantes OPs son superiores a $5 \mu\text{g g}^{-1}$, correspondiendo la máxima concentración al TBEP. En lo que concierne al número de muestras procesadas, Ingerowski *et al.* [40], son los que han llevado a cabo un trabajo más completo aunque, desafortunadamente, sólo consideraron TCEP y TCPP en su estudio. Sus concentraciones promedio se encuentran entre 1 y $3 \mu\text{g g}^{-1}$.

Los niveles proporcionados en la **Tabla II.3** son del mismo orden de magnitud, o incluso superiores, a las concentraciones publicadas para los BDEs más abundantes en 3 materiales de referencia de polvo de ambientes interiores y sus valores medios en 17 muestras de polvo recogido en viviendas privadas en Estados Unidos, **Figura II.2** [31]. Otro estudio llevado a cabo en Japón reveló concentraciones superiores de OPs que de BDEs en atmósferas interiores [39].

Figura II.2. Comparación de las concentraciones medias de OPs clorados (TCEP+TCPP+TDCP) y no clorados (TBP+TBEP+PPP) [datos de **Tabla II.3**] en polvo de ambientes interiores y BDEs (BDE-47+BDE-99+BDE-100+BDE-209) en polvo de ambientes interiores y en 3 materiales de referencia (MR) para esa misma matriz.



En base a estos resultados, la ingestión por vía oral de polvo podría representar una contribución importante a la exposición a OPs en ambientes interiores.

1.4.2.2. Sedimentos

El número de trabajos publicados en los que se determinan OPs en sedimentos es también muy limitado. En la **Tabla II.4.** se recogen los niveles (ng g^{-1}) encontrados para algunos OPs (de los OPs analizados sólo se recogen aquellos que han sido seleccionados en esta Tesis Doctoral).

Tabla II.4. Concentraciones (ng g^{-1}) de algunos OPs en sedimentos.

| Niveles OPs | País | Ref. |
|--|----------|------|
| TBP, TCPP, TPP: n.d. TCEP: 13-28 TDCP: n.d.-17 | Japón | [54] |
| TCEP: n.d.-160; TCPP: n.c.-1300; TPP: n.d.-160; TDCP: n.d.-n.c.; TBP: n.c.-50; TBEP: 2.4-130; TEHP: n.d.-140 | Austria | [55] |
| TBP: n.d.-253; TCEP: n.d.-7395; TCPP: n.d.-1181; TDCP: n.d.-709; TPP: n.d.-130; TBEP: n.d.-1969; TEHP: n.d.-7122 | Japón | [56] |
| TBP: n.d. | Alemania | [30] |
| TPP: n.d.-6000 | EEUU | [57] |

n.d., no detectado; n.c., no cuantificado.

Las concentraciones determinadas son, en general, muy bajas, salvo excepciones; tal es el caso del trabajo de Kawagoshi y col. [56], que analizaron muestras de suelo de un vertedero japonés y del área circundante y cuantificaron hasta 7395 ng g^{-1} de TCEP.

1.4.2.3. Lodos

El análisis de estos compuestos en lodos se restringe a unas cuantas publicaciones y los valores determinados son en general elevados, con concentraciones máximas del orden del $\mu\text{g g}^{-1}$. Marklund y col. [18] analizaron los lodos (liofilizados) de 11 depuradoras suecas y detectaron varios OPs, entre ellos: TCPP ($61\text{-}1900 \text{ ng g}^{-1}$), TBEP ($<5.1\text{-}1900 \text{ ng g}^{-1}$), TiBP ($27\text{-}2700 \text{ ng g}^{-1}$), TBP ($39\text{-}850 \text{ ng g}^{-1}$), TPP ($52\text{-}320 \text{ ng g}^{-1}$), TDCP ($3.0\text{-}260 \text{ ng g}^{-1}$) y TCEP ($6.6\text{-}110 \text{ ng g}^{-1}$). Bester [58] determinó niveles de TCPP entre 1700 y 2200 ng g^{-1} en lodo con un contenido acuoso del 60%, procedente de una depuradora alemana. Harrison y col. [59] hicieron una revisión de los niveles de 516 compuestos orgánicos en lodos en Estados Unidos, entre ellos varios OPs, encontrando niveles de TBP y TPP de hasta 2.4 y $1.9 \mu\text{g g}^{-1}$, respectivamente.

1.4.3. Matrices acuosas

Los organofosforados se han detectado tanto en aguas superficiales [54,60], subterráneas [61,62] y residuales [21,63] como en agua potable [64]. Las plantas de tratamiento de aguas residuales se consideran la principal fuente de contaminación de las aguas superficiales [62], mientras que la filtración de agua superficial a través del suelo puede ser la razón de la presencia de OPs en aguas subterráneas, especialmente de los compuestos más polares y menos degradables, por su baja probabilidad de adsorción y degradación al atravesar el suelo [62].

Además, los OPs se han detectado también en muestras de nieve. Así, Marklund y col. [65] determinaron niveles de TCPP y TBP en muestras de nieve recogidas en una carretera y un aeropuerto suecos de hasta 170 y 25000 ng kg^{-1} , respectivamente. La presencia de algunos de esos compuestos se relaciona con los materiales y fluidos empleados en la fabricación de vehículos y aviones.

1.4.3.1. Aguas subterráneas, superficiales y potables

En la **Tabla II.5** se recogen algunos de los trabajos en los que se han analizado OPs en aguas subterráneas, superficiales y potables. En esta tabla sólo se indican los compuestos analizados que se encuentran dentro del grupo de los OPs de interés en esta Tesis Doctoral.

Tabla II.5. Estudios sobre la incidencia de algunos OPs en aguas subterráneas, superficiales y potables.

| OPs | Matriz acuosa | País | Año | Ref. |
|--|------------------------------------|---------------|------------|------|
| TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP | Varios ríos, bahía de Osaka | Japón | 1976-1990 | [66] |
| TBP, TCEP, TCPP, TPP | Ríos y agua de mar | Japón | 1980 | [54] |
| TBP, TPP, TBEP | Ríos Elba y Weser | Alemania | 1983-1985 | [67] |
| TCEP, TCPP | Río Rin | Alemania | 1994, 1995 | [68] |
| TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP | Agua (vertedero marino) | Japón | 1996,1997 | [56] |
| TBP, TCEP, TBEP | Aguas superficiales y subterráneas | Alemania | 2000 | [61] |
| TiBP, TBP, TCEP, TCPP, TDCP, TPP, TBEP | Río Ruhr | Alemania | 2002 | [60] |
| TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP | 4 ríos | Austria | 2005 | [55] |
| TCEP | 3 ríos | Corea del Sur | 2004-2005 | [69] |
| TCEP | Aguas subterráneas, Río Rin | Alemania | 1995 | [68] |
| TBP, TCEP, TCPP, TDCP, TPP, TBEP | Lixiviado de vertedero | Suecia | 1998 | [70] |
| TBP, TCEP, TBEP | Aguas subterráneas, Río Oder | Alemania | 2000-2001 | [62] |
| TBP, TiBP, TCEP, TCPP, TDCP, TBEP | Aguas de una potabilizadora | Alemania | N.D. | [60] |
| TCEP, TBP, TDCP, TPP | Agua potable | E.E.U.U. | 2001 | [71] |
| TPrP, TiBP, TBP, TCEP, TCPP, TDCP, TPP, TBEP, TPPO | Agua potable y de río | Italia | 2006 | [72] |
| TPrP, TiBP, TBP, TCEP, TCPP, TDCP, TPP, TBEP, TPPO | 3 lagos italianos | Italia | 2006-2007 | [73] |

N.D., no disponible.

Los compuestos encontrados y los niveles detectados están condicionados por las emisiones locales y el grado de dilución. En un estudio sobre el río Ruhr se detectaron concentraciones de OPs entre 10 y 200 ng L⁻¹, encontrándose los niveles más altos en el curso del río posterior a zonas de descarga de depuradoras de agua residual [60].

A mediados de los 90, en el río Rin, la concentración de TCEP excedía la de TCPP [68]. Probablemente, como consecuencia de que el TCEP está siendo reemplazado por el TCPP, 10

años después se detectaron concentraciones comparables de ambos compuestos en aguas superficiales en Alemania [60] y Austria [55]. Aunque no se pueden proporcionar concentraciones típicas de OPs, algunos organofosforados alquilados y clorados parecen ubicuos. En la **Tabla II.6** se muestran los niveles de algunos OPs en aguas superficiales y subterráneas.

Tabla II.6. Concentraciones (ng L^{-1}) de algunos OPs en aguas subterráneas y superficiales.

| TiBP | TBP | TCEP | TCPP | TDCP | TBEP | TPP | Ref. |
|------|---------|-----------|----------|---------|---------|---------|------|
| ◇ | n.d.-36 | n.d.-347 | n.d.-176 | 23-136 | ◇ | n.d.-31 | [54] |
| ◇ | 1-1605 | n.d.-198 | ◇ | ◇ | 1-2010 | ◇ | [61] |
| ◇ | 69-1044 | n.d.-1236 | ◇ | ◇ | 121-952 | ◇ | [62] |
| ◇ | 20-110 | 13-137 | 33-170 | n.c.-19 | 24-500 | n.c.-10 | [55] |

◇, no incluido en el estudio; n.d., no detectado; n.c., no cuantificado.

1.4.3.2. Aguas residuales

En la **Tabla II.7** se recogen algunos de los trabajos en los que se han analizado OPs en aguas residuales y en la **Tabla II.8** los niveles de algunos OPs en esas depuradoras. En general, las concentraciones más altas corresponden al TBEP, TCEP y TCPP.

Tabla II.7. Estudios sobre la incidencia de algunos OPs en aguas residuales.

| OPs | Matriz acuosa | N | País | Año | Ref. |
|--|---------------|----|------------------------|-----------|------|
| TCPP | Inf. y Ef. | 1 | Alemania | 2002 | [58] |
| TiBP, TBP, TCEP, TCPP, TDCP, TPP, TBEP | Inf. y Ef. | 2 | Alemania | 2003 | [12] |
| TCEP, TCPP | Inf. y Ef. | 8 | Varios países europeos | 2003-2004 | [63] |
| TPrP, TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP | Inf. y Ef. | 11 | Suecia | 2003-2004 | [18] |
| TiBP, TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP, TPPO | Inf. y Ef. | 1 | Alemania | 2004 | [74] |
| DPP, DBP, DEHP | Inf. y Ef. | 1 | Alemania | 2004 | [21] |
| TBP, TCEP, TCPP, TDCP, TPP, TBEP, TEHP | Inf. y Ef. | 16 | Austria | 2005 | [55] |
| TCEP | Ef. | 7 | Corea del Sur | 2004-2005 | [69] |
| TiBP, TBP, TCEP, TCPP, TDCP, TBEP, TPP, TEHP, TPPO | Inf. y Ef. | 1 | España | N.D. | [75] |

N, número de plantas de tratamiento de agua residual consideradas en el estudio; N.D., no disponible; Inf., influente; Ef., efluente.

Tabla II.8. Concentraciones promedio (ng L^{-1}) de algunos OPs en aguas residuales. Las concentraciones mínima y máxima se proporcionan entre paréntesis.

| | TiBP | TBP | TCEP | TCPP | TDCP | TBEP | TPP | Ref. |
|--------|-------------------|-------------------|-----------------|-------------------|-----------------|-------------------|-----------------|------|
| Inf./2 | 1300 (* -2200) | 1200 (* -5500) | 290 (* -640) | 2000 (* -5800) | 100 (* -180) | 3700 (* -6100) | 130 (* -690) | [12] |
| Ef./2 | 160 (* -290) | 520 (* -2300) | 350 (* -410) | 3000 (* -6600) | 130 (* -180) | 440 (* -790) | 70 (* -250) | |
| Inf./7 | ◇ | * (6600-52000) | * (90-1000) | * (1100-18000) | * (210-450) | * (5200-35000) | * (76-290) | [18] |
| Ef./7 | ◇ | * (360-2100) | * (350-890) | * (1500-24000) | * (130-340) | * (3100-30000) | * (41-130) | |
| Ef./7 | ◇ | 292 (n.d.-810) | 110 (80-150) | 730 (310-960) | 81 (27-160) | 967 (13-5400) | 26 (n.c.-87) | [55] |
| Inf./1 | * | 590 | 330 | 3100 | 210 | 12000 | n.d. | [74] |
| Ef./1 | * | 130 | 350 | 2600 | 130 | 67 | n.d. | |
| Inf./1 | ◇ | ◇ | ◇ | 520 (240-1000) | ◇ | ◇ | ◇ | [58] |
| Ef./1 | ◇ | ◇ | ◇ | 380 (230-610) | ◇ | ◇ | ◇ | |

*, dato no disponible; ◇, no incluido en el estudio; n.d., no detectado; n.c., no cuantificado; Inf., influente; Ef., efluente; / número de depuradoras al que corresponden los datos.

Meyer y Bester [12] determinaron siete organofosforados en los influentes y efluentes de dos depuradoras alemanas involucrando tratamientos secundario y terciario y concluyeron que TCPP, TCEP y TDCP no se eliminaban durante el tratamiento de las aguas residuales, mientras que los niveles de TiBP, TBP, TBEP y TPP en el efluente eran inferiores a los del influente, **Tabla II.8.** Marklund y col. [18] analizaron algunos OPs en varias depuradoras suecas, utilizando muestras de agua compuestas representando el promedio de una semana. Las concentraciones de TBEP detectadas (hasta $35 \mu\text{g L}^{-1}$) fueron aproximadamente un orden de magnitud superior a las publicadas en el estudio alemán anterior. Al igual que en el trabajo de Meyer y Bester, los OPs clorados apenas se eliminaban en el tratamiento del agua residual. Los niveles más altos correspondieron al TCPP y al TBEP (hasta varios $\mu\text{g L}^{-1}$). Análogamente, Rodil y col. [74] concluyeron que la concentración de los organofosforados clorados resulta prácticamente inalterada a su paso por la EDAR mientras que los derivados alquilados se eliminan más fácilmente. Fries y Püttmann [62] proporcionaron concentraciones de TBP, TCEP y TBEP entre 50 y 7178 ng L^{-1} en los efluentes de cuatro depuradoras a pesar de las altas tasas de degradación (64-100%) en el proceso de depuración.

Debido a su persistencia, los triésteres clorados se han incluido en estudios recientes de seguimiento de contaminantes en depuradoras de varios países europeos [63,69]. Los resultados obtenidos confirman la presencia rutinaria de TCEP y TCPP en los efluentes a nivel de varios

cientos de ng L^{-1} . En la actualidad, la concentración de TCPP en efluentes supera a la de TCEP [63], reflejando, como ya se ha comentado, la tendencia de los últimos años de reducción del uso de este último a favor del primero, en parte por la reconocida carcinogenicidad del TCEP. El TCEP se elimina ligeramente (alrededor de un 20%), no así el TCPP [63].

En algunos de los estudios anteriores también se incluyó el TEHP en el grupo de OPs a analizar, sin embargo, no se detectó en ninguna de las muestras de agua residual [55,74]. Su gran tendencia a la adsorción, debido a su carácter lipofílico ($\log K_{ow} = 9.49$), explica este comportamiento.

La información sobre la presencia de diésteres en depuradoras de agua residual es prácticamente nula. La ausencia, hasta hace unos años, de un método adecuado para su determinación [21] y el hecho de que solo unos pocos están disponibles comercialmente justifican esta falta de información. En esta Tesis Doctoral se presenta un método para el análisis de algunos diésteres en aguas y se proporcionan datos para varias muestras de agua residual. Quintana y col. detectaron varios diésteres y un monoéster, siendo el DEHP el que se presentaba a niveles más altos [21]. El DBP y el DPP se incluyeron también en un estudio multiresiduo y se detectaron niveles de 109 (52) ng L^{-1} y 464 (554) ng L^{-1} en influente (efluente), respectivamente [76].

1.5. Matrices biológicas

La información sobre niveles de OPs en matrices alimentarias es inexistente y en lo que concierne a otras matrices biológicas escasa [77-82].

LeBel y Williams [78] analizaron 16 muestras de tejido adiposo humano de las que cinco contenían TDCP (0.5-110 ng g^{-1}), cuatro TBEP (4-26.8 ng g^{-1}) y sólo una presentaba TBP (9 ng g^{-1}). En un estudio posterior [79] detectaron TBEP y TDCP en 41 y 31 de las 115 muestras de tejido adiposo analizadas, respectivamente, con concentraciones entre 0.5 y 257 ng g^{-1} .

Jonsson y col. [80] detectaron concentraciones de TPP entre 0.13 y 0.15 $\mu\text{g g}^{-1}$ en muestras de sangre procedentes de tres individuos. Shah y col. [81] únicamente analizaron una muestra de sangre y detectaron niveles en torno a 0.17 ng mL^{-1} de TBP. El TPP estaba también presente en esa muestra, pero la bolsa utilizada para almacenar la sangre fue identificada como el origen del compuesto.

Hudec y col. [77] detectaron TDCP a niveles de entre 5 y 50 ng mL^{-1} en plasma seminal.

Schindler y col. [82] analizaron 30 muestras de orina de las que un 50% contenía di(2-cloropropil) fosfato (DCEP) y un 30% DPP. Los niveles máximos detectados fueron 27.5 y 4.1 $\mu\text{g L}^{-1}$ de DCEP y DPP, respectivamente.

1.6. Biodegradación

Por varias razones el comportamiento de los organofosforados parece ser ventajoso desde el punto de vista ecológico [31] ya que:

- 1) El grupo fosfato-éster es muy común en organismos vivos, por ejemplo, está presente en el ATP por lo que las enzimas que actúan sobre estas moléculas son igualmente válidas para catalizar la hidrólisis de los OPs.
- 2) Los alcoholes liberados tras la hidrólisis de los ésteres del ácido fosfórico son, en general, fácilmente degradables (por ejemplo, el grupo fenol del TPP, los grupos metilo y etilo del trimetil y trietil fosfato, respectivamente, etc.). Además, algunos OPs exhiben otros grupos éter en la cadena alifática que también pueden ser hidrolizados.

De hecho, las investigaciones en influentes y efluentes de depuradoras de agua residual sugieren que algunos triésteres son biodegradables mientras que otros, especialmente los halogenados, son bastante recalcitrantes y no se eliminan mediante tratamientos tales como la ozonización o el uso de filtros multicapa. Sin embargo, pueden eliminarse mediante filtración a través de carbono activo y también de suelo [83]. La eficacia de la eliminación depende de parámetros como el tiempo de residencia y las características del suelo.

Se han llevado a cabo estudios para evaluar si los OPs son o no biodegradables y si sufren sólo hidrólisis o pueden ser completamente mineralizados. TPP, TBP y TiBP se degradan [84,85] pero la mineralización no siempre es completa [85]. El TEHP no es fácilmente biodegradado, la biodegradación de los triésteres aromáticos es más rápida que la de los alifáticos y los triésteres halogenados son especialmente estables y presentan muy baja degradabilidad [84,85].

En un estudio reciente se evaluó si los diésteres y monoésteres se formaban durante la biodegradación de los triésteres [21], como habían sugerido algunos trabajos previos [30,85]. En el estudio se incluyeron tres OPs (TiBP, TPP y TBEP) y se observó la formación de sus respectivos diésteres como intermedios de su degradación. DPP y dibutoxietyl fosfato (DBEP) desaparecieron tras 25 días mientras que, tras ese mismo período de tiempo, sólo se había degradado un 50% de di(isobutil) fosfato (DiBP). Los monoésteres no se detectaron durante el curso de la degradación de los triésteres [31].

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2. Preparación de muestras sólidas

La etapa de preparación de muestra es la principal fuente de errores dentro del proceso analítico [1] y, sin embargo, es uno de los eslabones que menos ha evolucionado, como demuestra el hecho de que en la actualidad la extracción Soxhlet, introducida en 1879, siga siendo una de las técnicas de extracción más empleada para muestras sólidas [2]. Además, esta etapa condiciona en gran medida la validez de los resultados y es la que limita la duración total del análisis.

En la presente Tesis Doctoral se ha abordado la determinación de OPs en distintas matrices, tanto sólidas como acuosas. En este apartado se describen los fundamentos y aspectos generales de algunas de las metodologías más relevantes para la preparación de muestras sólidas, ofreciendo una explicación más pormenorizada de aquellas que se han utilizado en esta Tesis. El apartado II.3 se centra en la preparación de muestras acuosas.

En la mayoría de las técnicas analíticas disponibles para la determinación de analitos orgánicos es necesario que éstos se encuentren en disolución. Por tanto, en el caso de muestras sólidas es necesario separar el analito de la matriz empleando un extractante. Por otro lado, las interacciones analito-matriz en este tipo de muestras son más fuertes que las que se establecen en el caso de matrices líquidas. Por ello, se suelen emplear condiciones de extracción más enérgicas, lo cual disminuye la selectividad de la extracción y, normalmente, hace necesaria la inclusión de etapas posteriores de purificación. Consumo reducido de disolventes, rapidez, sencillez, posibilidad de procesar simultáneamente varias muestras y bajo coste son atributos deseables a la hora de seleccionar una técnica de extracción. En base a esta información y otros aspectos prácticos de interés, en la **Tabla II.9** se establece una comparativa entre algunas de las técnicas disponibles para la extracción de analitos orgánicos de matrices sólidas: extracción Soxhlet, extracción asistida por ultrasonidos o sonicación, extracción asistida por microondas (microwave-assisted extraction, MAE), extracción con líquidos presurizados (pressurized liquid extraction, PLE), extracción con fluidos supercríticos (supercritical fluid extraction, SFE) y dispersión de matriz en fase sólida (matrix solid-phase dispersión, MSPD).

Los datos proporcionados en la **Tabla II.9** son orientativos y reflejan condiciones y valores típicos.

Tabla II.9. Técnicas de extracción comunes para matrices sólidas y semisólidas [3-5].

| Técnica | Tiempo | Vol. Disolvente | Coste | Ventajas | Inconvenientes |
|-------------------|-----------|--|----------|--|---|
| Soxhlet | 4-48 h | 50-500 mL | Bajo | Muestra en contacto repetido con porciones frescas de disolvente; fácil manejo; no se requiere filtración; recuperaciones excelentes. | Tiempos de extracción largos; grandes volúmenes de disolvente; necesidad de concentración del extracto; temperatura máxima es la T_{ab} del disolvente. |
| Sonicación | 5-60 min | 30-200 mL | Bajo | Fácil manejo; posibilidad de extracciones simultáneas si se utiliza un baño ultrasónico. | Grandes volúmenes de disolvente; pueden ser necesarias extracciones sucesivas; requiere filtración y concentración del extracto; no automatizable; el tamaño de partícula es crítico. |
| SFE | 10-60 min | 2-5 mL (Trampa sólida), 5-60 mL (Trampa líquida) | Alto | Extracciones múltiples y rápidas; el CO_2 no es tóxico; bajo consumo de disolvente; no requiere filtración; automatizada; selectividad controlable con condiciones de atrapado. | Optimización compleja; las matrices con alto contenido en agua son problemáticas. |
| MAE | 5-30 min | 10-40 mL | Moderado | Rapidez; bajo consumo de disolvente; se pueden utilizar temperaturas elevadas; extracción simultánea de varias muestras; automatizable; fácil manejo. | Extracción simultánea de muestras similares; habitualmente el disolvente ha de absorber microondas; tiempo de espera hasta enfriamiento; necesidad de filtración y limpieza del extracto. |
| PLE | 5-30 min | 10-50 mL | Alto | Extracciones múltiples y rápidas; bajo consumo de disolvente; no requiere filtración; fácil manejo; automatizada; se alcanzan altas temperaturas y presiones que favorecen la extracción; posibilidad de purificación simultáneamente. | Selectividad limitada por lo que se necesita etapa de purificación; consumo de disolvente adicional para el lavado de las celdas. |
| MSPD | 5-20 min | 2-20 mL | Bajo | Posibilidad de integrar extracción y purificación; consumo reducido de disolventes; rapidez; sencillez. | Requiere la presencia del analista si no está automatizada. |

Vol., volumen; T_{eb} , temperatura de ebullición

2.1. Extracción Soxhlet

La extracción Soxhlet ha sido, y en muchos casos continúa siendo, la técnica estándar de extracción de muestras sólidas, además de ser el principal método de referencia con el que se comparan otros procedimientos de extracción [5,6]. Con esta última finalidad es con la que se ha empleado esta técnica en la presente Tesis Doctoral.

En las extracciones Soxhlet la muestra sólida finamente pulverizada se coloca en un cartucho poroso de celulosa o en un dedal de vidrio que se sitúa en la cámara del extractor de Soxhlet (**Figura II.3**). El disolvente extractante, situado en el matraz, se calienta, sus vapores se condensan y caen, gota a gota, sobre el dedal que contiene la muestra, extrayendo así los analitos solubles. Cuando el nivel del disolvente condensado en la cámara alcanza la parte superior del sifón lateral, el disolvente, con los analitos disueltos, desciende por el sifón y retorna al matraz de ebullición. Este proceso se repite hasta que se completa la extracción de los analitos de la muestra y se concentran en el disolvente [5].

Figura II.3. Extractor Soxhlet.



Con este método de extracción se obtienen excelentes recuperaciones para compuestos térmicamente estables. La razón es que la muestra está en contacto en cada ciclo con porciones frescas de disolvente caliente, lo cual favorece la solubilización de los analitos. Los largos tiempos de extracción, el gran volumen de disolvente empleado y, como consecuencia, la necesidad de evaporación del mismo para la concentración de los analitos son las principales desventajas de la extracción Soxhlet. Además, genera extractos complejos que requieren una purificación exhaustiva. Aunque existen equipos comerciales para llevar a cabo la extracción Soxhlet de forma automatizada, reduciendo notablemente los tiempos de extracción y el consumo de disolventes, su presencia no es habitual en la mayoría de los laboratorios analíticos.

2.2. Extracción asistida por ultrasonidos

Los ultrasonidos son ondas sonoras con una frecuencia de vibración por encima del intervalo audible para humanos (1-16 kHz): desde 20 kHz hasta varios GHz. A diferencia de la radiación electromagnética, los ultrasonidos son vibraciones mecánicas que se transmiten a través de la materia [7]. Cuando se irradia una disolución con ultrasonidos se produce el fenómeno conocido como cavitación acústica, que consiste en la formación de burbujas y su posterior implosión. El colapso de esas burbujas tiene como resultado la generación de microgradientes elevados de presión y temperatura durante intervalos de tiempo extremadamente cortos [6,8].

La extracción asistida por ultrasonidos o sonicación se basa en la exposición a la energía de ultrasonidos de la muestra, inmersa en un disolvente orgánico o mezcla extractante. La

irradiación se lleva a cabo habitualmente introduciendo una sonda en el recipiente que contiene la muestra o sumergiendo éste en un baño de ultrasonidos. Tras un tiempo de extracción controlado, el extractante se separa por centrifugación y/o filtración. El proceso se repite varias veces, añadiendo en cada extracción una nueva fracción de disolvente, combinando finalmente todos los extractos obtenidos [5].

Entre las variables que más influyen en la eficacia de los ultrasonidos están las que siguen: frecuencia e intensidad de la radiación, tiempo de irradiación, tipo de disolvente, temperatura y presión externas y tamaño de partícula de la muestra [9].

Como resultado de la cavitación acústica, la irradiación con ultrasonidos incrementa la temperatura, que conlleva un aumento de la solubilidad de los analitos y favorece la difusión de los mismos, y la presión, lo cual favorece la penetración del disolvente en los poros y el transporte de los analitos. Todo ello contribuye a una reducción del tiempo de extracción. Además, en comparación con la extracción Soxhlet, es adecuada para la extracción de especies termolábiles; sin embargo, exhibe menor reproducibilidad, requiere la filtración y/o centrifugación de los extractos y el consumo de disolvente es bastante elevado.

2.3. Extracción con fluidos supercríticos

La extracción con fluidos supercríticos emplea un disolvente en condiciones supercríticas, esto es, en condiciones de presión y temperatura por encima del punto crítico. Este punto, característico de cada sustancia, está definido por una presión y temperatura críticas que son aquéllas a partir de las cuales un gas no puede condensarse por compresión isotérmica. Los fluidos supercríticos presentan propiedades intermedias entre un gas y un líquido: la densidad es relativamente elevada, semejante a la de los líquidos, la viscosidad es similar a la de los gases y el coeficiente de difusión, intermedio entre el de los líquidos y los gases. Esta combinación de propiedades resulta en un fluido más penetrante, con un poder solvatante mayor, capaz de extraer analitos más rápida y eficazmente que los líquidos [4-6,10].

Existen varias sustancias que pueden utilizarse como fluidos supercríticos pero el dióxido de carbono es el más utilizado en la práctica, por presentar unas condiciones críticas fácilmente accesibles (31.3 °C y 73 bares) y una toxicidad e inflamabilidad bajas; además, no es corrosivo y su coste es asequible. El CO₂ es un disolvente excelente para la extracción de analitos lipofílicos y moderadamente polares. Sin embargo, para la extracción de analitos polares y/o fuertemente retenidos por la matriz, se requiere la adición de un modificador o codisolvente para incrementar la solubilidad de los analitos en el medio extractante. Los modificadores más habituales son: metanol, acetona, ácido fórmico... Éstos pueden adicionarse al fluido (adición dinámica) o a la matriz (adición estática). El procedimiento más adecuado para la introducción del modificador depende de las características de la matriz y del analito. El modo dinámico es aconsejable cuando el analito es poco soluble en CO₂ puro, mientras que el estático se utiliza para romper interacciones analito-matriz fuertes. Con el objetivo de aumentar el rendimiento de la extracción de especies polares e iónicas también es habitual el uso de reacciones de derivatización química, normalmente *in situ*.

La recuperación de los analitos se realiza habitualmente utilizando un adsorbente sólido o un disolvente orgánico, aunque también es posible el acoplamiento en línea con diferentes métodos cromatográficos.

La eficacia de la extracción está afectada por un gran número de parámetros tales como la naturaleza del fluido supercrítico, la temperatura, la presión, el flujo, el tiempo de extracción, el tamaño de partícula, la humedad de la muestra y el sistema de recolección. Por ello, SFE permite un alto grado de selectividad y la obtención de extractos bastante limpios, sin embargo, la necesidad de controlar tantos parámetros hace que su optimización sea tediosa y complicada. La técnica requiere tiempos de extracción cortos, permite la extracción de compuestos termolábiles y la preconcentración de los analitos. Como limitaciones fundamentales cabe señalar la dificultad para extraer compuestos polares e iónicos y la ya comentada complejidad de la optimización en comparación con otros métodos.

2.4. Extracción asistida por microondas

La primera aplicación de la energía de microondas en química analítica se remonta al año 1975 en el que se publicó un trabajo donde se hacía uso de un microondas casero para asistir la digestión de muestras biológicas. Desde entonces, las microondas se han utilizado extensamente con funciones diversas [2,11]: digestión de muestras para análisis elemental, la extracción con disolventes orgánicos, secado de muestras, generación de plasmas, etc.

2.4.1. Principios

Las microondas son ondas electromagnéticas cuyo intervalo de frecuencias se extiende desde 300 a 3×10^6 MHz. Su radiación origina el movimiento de moléculas debido a la conducción iónica y a tránsitos rotacionales, sin producir cambios en la estructura molecular [4-6,12,13]. La conducción iónica se produce por la migración de iones al aplicar un campo electromagnético y depende de su concentración, de su movilidad y de la temperatura. La resistencia de la disolución a este flujo de corriente origina pérdidas de energía, debidas a la fricción, que conllevan el calentamiento de la disolución. Las moléculas con momentos dipolares (permanentes o instantáneos) pueden interactuar con el campo eléctrico aplicado dando lugar a la alineación de los mismos. Cuando deja de aplicarse el campo eléctrico se restaura el desorden molecular, debido a la agitación térmica, y la energía absorbida se disipa en forma de calor. A causa de la inercia de las moléculas, el retorno al desorden no es inmediato sino que requiere un cierto tiempo, denominado tiempo de relajación dieléctrica, que se define como el lapso necesario para que el 63% de las moléculas vuelvan al desorden. A 2450 MHz, frecuencia utilizada en los aparatos comerciales, el proceso orden-desorden tiene lugar aproximadamente 5×10^9 veces por segundo, dando lugar a un calentamiento muy rápido.

El calentamiento de una muestra mediante la acción de microondas depende del factor de disipación, $\tan \delta$, que se define como la razón entre la pérdida dieléctrica de la muestra (ϵ'' , que es una medida de la eficacia para convertir energía electromagnética en calor) y su constante dieléctrica (ϵ' , que es una medida de la polarizabilidad de una molécula en un campo eléctrico, esto es, expresa la capacidad de la molécula de absorber radiación):

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'}$$

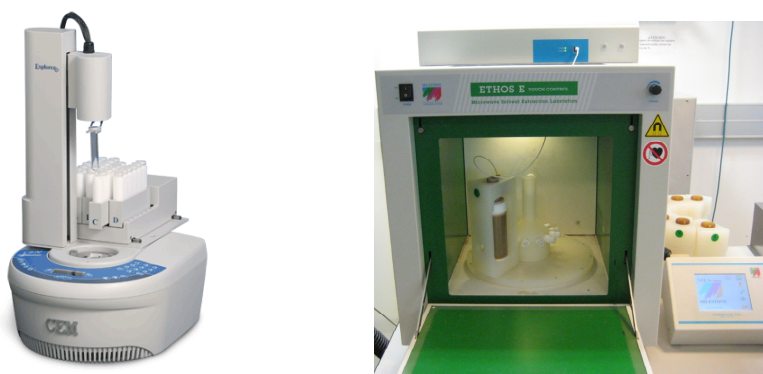
La energía absorbida por una muestra aumenta con el factor de disipación. Otros factores a tener en cuenta en el calentamiento con energía de microondas son: la profundidad de penetración de la radiación (que depende inversamente del producto de la frecuencia de la radiación y el factor de disipación), la temperatura (la solubilidad y estabilidad térmica son factores dependientes de esta variable), la viscosidad (la viscosidad de una muestra determina su capacidad para absorber energía de microondas porque afecta a la rotación molecular) y el tamaño de la muestra (cuanto menor sea la cantidad de muestra, tanto menos energía de microondas puede absorber).

El calentamiento convencional se produce por conducción y, por ello, es un proceso lento y la temperatura del fluido heterogénea, ya que se establece un gradiente de temperatura debido a corrientes de convección. Sin embargo, el calentamiento mediante energía de microondas es selectivo, pues no todos los materiales absorben esta radiación (en consecuencia, se calientan las muestras pero no el recipiente que las contiene), homogéneo y rápido (la microondas inciden sobre la totalidad de la muestra).

2.4.2. Aspectos prácticos

La instrumentación utilizada para la extracción con microondas puede clasificarse en dos grandes grupos (**Figura II. 4**) en función de cómo se suministra esa energía a la muestra [14]: (1) sistemas multimodo, en los que la radiación de microondas se dispersa en una cavidad en la que se encuentra la muestra y (2) sistemas de enfoque, en los que la energía incide sólo sobre la zona en la que se sitúa la muestra, sometida a un campo electromagnético más intenso que en el caso previo. En esta Tesis se han empleado ambos sistemas [15, 16].

Figura II.4. Sistema de enfoque (izquierda) y uno multimodo (derecha).



La extracción asistida por microondas se lleva cabo en recipientes transparentes a esa radiación y puede efectuarse en sistemas abiertos o cerrados. Cuando la extracción se realiza en vasos a presión atmosférica la temperatura está limitada por el punto de ebullición del disolvente o mezcla de disolventes a esa presión. En los sistemas cerrados no existe esa limitación, sin

embargo, tras la extracción hay que esperar a que descienda la temperatura antes de abrir el vaso, lo cual alarga el tiempo total de esta etapa.

El calentamiento mediante microondas puede ocurrir a través de tres mecanismos distintos dependiendo de la naturaleza de la muestra y del disolvente o mezcla de disolventes empleados [5]. Así, la extracción puede efectuarse utilizando:

- Un disolvente o mezcla de disolventes con coeficientes de pérdida dieléctrica altos.
- Una mezcla de disolventes con alta y baja pérdida dieléctrica.
- En el caso de muestras con alta pérdida dieléctrica, la extracción puede efectuarse con un disolvente o mezcla de disolventes transparentes a la radiación.

2.4.3. Variables que afectan a la eficacia de la extracción

La naturaleza y volumen de disolvente, la temperatura y tiempo de extracción, así como las características de la muestra son las variables más habitualmente estudiadas.

La elección del disolvente es clave para conseguir una extracción eficaz. A la hora de seleccionarlo han de tenerse en cuenta su comportamiento ante la radiación de microondas, su interacción con la matriz y la solubilidad de los analitos en él. En la **Tabla II.10** se recogen las pérdidas y constantes dieléctricas y los factores de disipación de algunos disolventes.

Tabla II.10. Pérdida y constante dieléctricas y factor de disipación de algunos disolventes a 3 GHz y 25 °C [17].

| | ϵ' | ϵ'' | $\tan \delta \times 10^4$ |
|------------------|-------------|----------------------|---------------------------|
| Agua | 76.7 | 12.04 | 1570 |
| NaCl (ac.) 0.1 M | 75.5 | 18.12 | 2400 |
| Metanol | 23.9 | 15.296 | 6400 |
| Heptano | 1.9 | 1.9×10^{-4} | 1 |

El volumen de disolvente utilizado habitualmente en MAE varía entre 10 y 40 mL y debe ser tal que asegure que toda la muestra se encuentra inmersa en él.

Uno de los parámetros más estudiado en MAE es la temperatura ya que, al igual que en otras técnicas de extracción, este factor contribuye, en la mayoría de los casos, a mejorar las recuperaciones. Temperaturas elevadas normalmente mejoran la eficacia de extracción, como consecuencia del aumento de la difusividad del disolvente y la disminución de su viscosidad y tensión superficial, que facilitan la desorción de los compuestos de la matriz y, en definitiva, la solubilización de éstos.

La naturaleza de la matriz y su contenido en humedad ejercen también un marcado efecto sobre las recuperaciones de los compuestos.

2.4.4. Ventajas e inconvenientes

Como principales ventajas cabe destacar que es una técnica rápida, con un consumo moderado de disolventes y la optimización del proceso no es demasiado complicada. Existen disponibles comercialmente equipos para la extracción asistida por microondas que permiten procesar simultáneamente varias muestras. Como limitaciones más destacadas señalar que, a pesar de los tiempos normalmente cortos de extracción, la etapa de enfriamiento de los recipientes de extracción puede durar tanto o más que la propia extracción. Los extractos obtenidos han de ser filtrados y/o centrifugados y, aunque la extracción es medianamente selectiva, usualmente es necesaria la introducción de alguna etapa adicional de limpieza para la eliminación de otros compuestos de la matriz coextraídos a las temperaturas habitualmente elevadas. Por otro lado, los equipos son relativamente costosos.

2.5. Extracción con líquidos presurizados

Inicialmente denominada extracción acelerada con disolventes (accelerated solvent extraction, ASE), con el tiempo han surgido nomenclaturas alternativas a esa denominación comercial, tales como extracción con fluidos presurizados (pressurized fluid extraction, PFE) o extracción con líquidos presurizados, siendo esta última la más generalizada [18]. PLE combina la utilización de temperaturas y presiones elevadas con disolventes en estado líquido, para proporcionar una extracción rápida y eficaz [3,4,19-22]. La presión elevada es necesaria para mantener el disolvente en estado líquido a las temperaturas de trabajo, normalmente superiores a su punto de ebullición a presión atmosférica. El uso simultáneo de presiones y temperaturas elevadas acelera la cinética de extracción en comparación con técnicas tradicionales como la extracción Soxhlet, con la ventaja adicional de utilizar volúmenes inferiores de disolventes. Se ha demostrado que, en términos de recuperación y precisión, esta técnica es equivalente o superior a otras metodologías, siendo incluso utilizada como método de referencia por la Agencia de Protección Medioambiental estadounidense (EPA) en uno de sus métodos (método 3545A) [23] para la extracción de compuestos volátiles y semivolátiles en algunas matrices medioambientales sólidas.

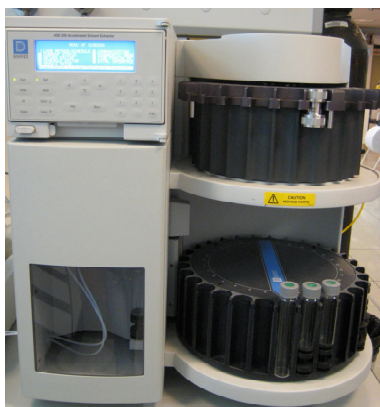
2.5.1. Principios

La utilización de disolventes líquidos a temperaturas y presiones elevadas mejora el rendimiento de las extracciones por varias razones: (1) el incremento de temperatura aumenta la velocidad de difusión y la solubilidad de los analitos; (2) a la vez, disminuye la viscosidad y tensión superficial de los disolventes. (3) Además, el incremento de la temperatura aumenta la energía cinética de las moléculas y favorece la ruptura de las interacciones entre analitos y componentes de la matriz. Todo esto favorece la transferencia de masa entre muestra y disolvente. (4) Por otro lado, las altas presiones favorecen la penetración del disolvente en los poros de la matriz y mantienen el disolvente en estado líquido [3,5,19,20].

2.5.2. Aspectos prácticos

Existen disponibles en el mercado varios extractores [19]. La mayoría de las aplicaciones publicadas han utilizado el extractor ASE 200, **Figura II.5**, de la casa comercial Dionex (Sunnyvale, CA, USA), hasta hace poco el único disponible comercialmente. En la actualidad existen otros equipos de la misma casa comercial [24] además de la alternativa de Applied Separations (Allentown, PA, USA) [25] y otros dispositivos no comerciales [20].

Figura II.5. Extractor ASE 200.



La extracción puede llevarse a cabo en modo estático o dinámico [19]. En la extracción estática se bombea el disolvente hasta rellenar la celda de extracción, que después se mantiene durante un tiempo controlado a la temperatura y presión deseadas. A continuación, el disolvente de extracción se transfiere a un vial y las conexiones y la celda se lavan con un pequeño volumen de disolvente. Al final, se purga el sistema con nitrógeno para garantizar la recuperación completa del disolvente que ha estado en contacto con la muestra. En el modo dinámico, que normalmente se lleva a cabo utilizando extractores de fluidos supercríticos o dispositivos no comerciales, el disolvente presurizado se bombea de forma continua a través de la celda a un flujo constante durante un tiempo determinado. De acuerdo con la primera ley de difusión de Fick, el contacto continuo entre la muestra y porciones frescas de disolvente acelera la transferencia de masa. Consecuentemente, la eficacia de extracción debería mejorar y el tiempo de extracción reducirse. A pesar de la mayor flexibilidad de esta última modalidad, su utilización es más limitada, fundamentalmente debido a la ausencia de un equipo disponible comercialmente [18]. Por otro lado, el aspecto más desfavorable de la modalidad dinámica respecto a la estática es la mayor dilución de los analitos. Sin embargo, la naturaleza dinámica del proceso facilita el acoplamiento con otros sistemas dinámicos y así la automatización del proceso analítico, integrando también, por ejemplo, la concentración.

La utilización de altas presiones y temperaturas acelera la extracción y permite obtener recuperaciones elevadas pero como contrapartida disminuye la selectividad de la extracción. Por este motivo, es habitual la necesidad de introducir etapas posteriores de limpieza, que pueden integrarse o no en el proceso de extracción. La posibilidad de llevar a cabo simultáneamente extracción y purificación supone una reducción de la manipulación de muestra y del tiempo total de análisis, además de una disminución del consumo de disolventes.

Procedimiento de extracción estática

El proceso de extracción estática, que es el que se ha utilizado en uno de los trabajos incluidos en esta Tesis [26], consta de varias etapas que se describen más pormenorizadamente en esta sección [24,27].

Preparación de muestra. La preparación de la muestra es una parte esencial de cualquier proceso de extracción. La eficacia de éste será tanto mayor cuanto mayor sea la superficie de contacto entre el disolvente y los analitos, por lo que es recomendable la reducción del tamaño de partícula. Por la misma razón, ha de evitarse la agregación de partículas de muestra. Para ello, se utilizan agentes dispersantes como la arena o la tierra de diatomeas.

El secado de muestras con un alto contenido en humedad es aconsejable, en especial, cuando se trata de recuperar analitos poco polares con disolventes apolares, ya que la presencia de agua dificulta la penetración del disolvente. Para esto, se recurre a la utilización de agentes desecantes: celulosa, tierra de diatomeas, sulfato sódico anhidro, etc. El sulfato sódico anhidro no debe usarse nunca con disolventes polares ni con muestras con alto contenido en humedad ya que puede ocluir las líneas del equipo de extracción; la tierra de diatomeas no presenta estos problemas. La liofilización, el secado en horno o al aire libre son otras alternativas, aunque los dos primeros pueden comprometer la extracción de analitos volátiles.

Preparación de celda. Se utilizan filtros de celulosa y/o fibra de vidrio para colocar en los extremos de la celda con el fin de evitar la obturación del sistema. Los filtros de fibra de vidrio se usan normalmente en extracciones acuosas para las que los de celulosa proporcionan una filtración inadecuada o cuando éstos son una fuente de interferencias. La celda se rellena con la muestra y con una matriz inerte (tierra de diatomeas, arena...) para ocupar el volumen muerto. Se pueden introducir además adsorbentes para conseguir una purificación en línea del extracto.

Extracción estática. La celda se introduce en el horno a la temperatura de extracción, se rellena con disolvente y se presuriza. Es posible también la introducción de una etapa de precalentamiento de la celda previamente a su contacto con el disolvente. Tras un cierto intervalo de tiempo (tiempo de equilibración), alcanza la temperatura deseada y se mantiene a esa temperatura durante un tiempo controlado (tiempo de extracción estática). Se pueden efectuar uno o varios ciclos de extracción estática. A continuación, se abre la válvula estática y se recoge el disolvente en el vial de extracción. Seguidamente, se bombea disolvente fresco a través de la celda y los tubos conectores. Este volumen de disolvente (*flush volume*), que viene expresado como tanto por ciento del volumen de celda, se utiliza para lavar la muestra y los conectores y arrastrar los analitos que hayan podido quedar en el sistema. Finalmente, el disolvente remanente en la celda se purga con nitrógeno y se recoge en el vial. Además, el equipo tiene la opción de introducir lavados entre muestras. En la **Tabla II.11** se recogen los valores que permite fijar el ASE 200 para algunos parámetros importantes en el proceso de PLE.

Si la extracción se realiza utilizando varios ciclos estáticos, el volumen de lavado (*flush volume*) se divide entre ciclos, de tal forma que si se hacen, por ejemplo, 2 ciclos y se establece un *flush volume* del 60%, se hace pasar un 30% de disolvente al final de cada ciclo. El tiempo de equilibración térmica, que no aparece en la tabla, viene preestablecido en función de la temperatura de extracción, hasta un máximo de 9 min para 200 °C. Para temperaturas de extracción entre 40 y 100 °C la duración de esta etapa es de 5 minutos.

Tabla II.11. Intervalo de valores para algunos parámetros controlables en el ASE 200.

| Parámetro | Intervalo de valores |
|----------------|-----------------------------|
| Ciclo estático | 0-99 min |
| Flush volume | 0-150% |
| Purga | 0-300 s |
| Ciclos (nº) | 1-5 |
| Temperatura | 40-200 °C |
| Presión | 500-3000 psi/3.45-20.68 MPa |

Tratamientos *in situ*

Las condiciones energéticas de la extracción hacen que la selectividad de la misma sea baja y que sea necesaria la introducción de alguna etapa de limpieza adicional en el caso de matrices complejas, lo cual alarga el tiempo total de análisis y, normalmente, aumenta el consumo de disolventes. Una opción para mejorar la selectividad del proceso es llevar a cabo la purificación de los extractos en línea, simplificando así el esquema de preparación de muestra y reduciendo la manipulación de la misma y el consumo de disolventes. La introducción del adsorbente apropiado en la celda de extracción o la utilización de un ciclo de lavado previo a la extracción son dos opciones posibles. En la mayoría de las aplicaciones de PLE para la extracción de analitos lipofílicos de matrices grasas, los lípidos se coextraen con los compuestos de interés. La eliminación de los lípidos coextraídos se ha conseguido introduciendo sílica ácida [28], Florisil [29] o alúmina activada [30] en la celda de extracción. Draisci y col. [31] resolvieron el problema con la introducción de varios ciclos de lavado con hexano, previamente a la extracción de los analitos de interés, eliminando la necesidad de limpieza posterior.

La derivatización *in situ* se ha propuesto también como forma de simplificar los protocolos de preparación de muestra. David *et al.* [32] desarrollaron un protocolo para la derivatización de herbicidas polares añadiendo el agente derivatizante directamente en la celda de extracción y obtuvieron mejores resultados que con la derivatización post-extracción. Pörschmann y col. [33] evaluaron el impacto de varios procedimientos de derivatización (acetilación, sililación y metilación) sobre la recuperación de distintos analitos polares (fenoles, esteroides y ácidos carboxílicos) añadiendo los agentes derivatizantes al disolvente de extracción. La recuperación del proceso no se vio afectada por la reacción de derivatización y la eficacia de ésta fue análoga a la obtenida llevando a cabo el tratamiento posteriormente a la extracción.

2.5.3. Variables que afectan a la eficacia de extracción

El tipo de disolvente, la temperatura de trabajo y el tiempo de extracción son los factores que más afectan a la eficacia de la extracción.

La elección correcta del disolvente o mezcla de disolventes es crucial para obtener una extracción eficaz. En PLE se puede utilizar una gran variedad de disolventes excepto aquellos con

temperatura de autoignición dentro del intervalo de temperaturas de trabajo (40-200 °C), tal es el caso del disulfuro de carbono o el éter dietílico. No se pueden utilizar ácidos y bases fuertes porque son corrosivos, pero se pueden emplear ácidos y bases débiles en porcentajes bajos, no superiores al 5%. En tal caso, hay que tener siempre la precaución de lavar las líneas con disolvente orgánico puro o agua al finalizar, para evitar el deterioro de las mismas. De cualquier forma, la tolerancia de los sistemas de extracción a valores de pH más extremos se está mejorando. El ASE 350, uno de los equipos de extracción más recientes de la marca comercial Dionex, está equipado con celdas que admiten la introducción de muestras que hayan sido sometidas a hidrólisis o control de pH, siempre que la concentración total de ácido o base no sea superior a 0.1 M.

La temperatura es el parámetro más importante en PLE. Cuando se desarrolla un nuevo método, se suele comenzar trabajando a 100 °C o, si los analitos tienen una temperatura de degradación térmica conocida, se suele comenzar a 20 °C por debajo de ese valor [24]. Temperaturas entre 100 °C y 200 °C son las más habituales, siendo las extracciones a 100 °C las más usuales [19,34].

La utilización de altas presiones tiene como finalidad mantener el disolvente en estado líquido y, en general, no ejerce ninguna influencia en la eficacia de extracción. En la mayoría de los trabajos se utilizan entre 1450 psi (10 MPa) y 2176 psi (15 MPa). Es más, los equipos más recientes desarrollados por Dionex, el ASE 150 y el ASE 350, trabajan a una presión fija de 1500 psi (10.34 MPa) y no permiten variaciones de este parámetro. Sin embargo, en algunos trabajos con matrices sólidas húmedas [4,20] se observó un efecto beneficioso de la presión.

Los tiempos de extracción en PLE son muy cortos en comparación con los de las técnicas tradicionales de extracción sólido-líquido. En ciertas matrices, los analitos pueden estar retenidos dentro de los poros u otras estructuras. En estos casos, el incremento del tiempo de extracción puede permitir la difusión de estos compuestos hacia el disolvente de extracción. El efecto del tiempo de extracción siempre se debe evaluar en conjunción con el número de ciclos, con el objetivo de conseguir una extracción completa de la forma más eficaz posible [23].

2.5.4. Ventajas e inconvenientes

La técnica es rápida y el consumo de disolvente, con la selección apropiada del tamaño de celda, moderado. La optimización es sencilla ya que no son muchos los parámetros que afectan a la eficacia de extracción (fundamentalmente temperatura y disolvente) y los extractos obtenidos están filtrados. Los dispositivos comerciales existentes permiten la extracción secuencial y/o simultánea de varias muestras.

Las condiciones enérgicas de la extracción hacen que la selectividad de la misma sea baja, siendo necesaria la introducción de alguna etapa de limpieza adicional en el caso de matrices complejas, lo cual alarga el tiempo total de análisis y normalmente aumenta el consumo de disolventes. La preparación de las celdas y su lavado son un poco tediosos. El/los disolvente/s de lavado de las líneas del extractor tras cada extracción y para la limpieza de los 11 elementos que componen cada celda ha de sumarse al consumo de disolventes de la extracción,

por lo que puede decirse que éste no es bajo. El elevado coste de la instrumentación es también un hándicap importante.

2.5.5. Extracción con agua subcrítica

La utilización de agua como extractante es una alternativa atractiva por su inocuidad, bajo coste y disponibilidad [19,35-37]. A temperatura ambiente y presión atmosférica la polaridad del agua es demasiado alta para extraer eficazmente la mayoría de las especies orgánicas no iónicas. Sin embargo, su constante dieléctrica se puede reducir a valores similares a los de disolventes orgánicos sin más que aumentar la temperatura bajo presiones moderadas para mantenerla en estado líquido. Por encima de su punto crítico (374.1 °C y 22.1 MPa) su constante dieléctrica alcanza valores entre 5 y 15. Sin embargo, en esas condiciones el agua es corrosiva, por lo que para reducir costes y evitar el deterioro de la instrumentación y la posible degradación de los analitos, se prefiere trabajar por debajo del punto crítico. Además de la disminución de la polaridad, a temperaturas elevadas se produce también una reducción drástica de la viscosidad y de la tensión superficial del agua, lo cual favorece la extracción. Esta modalidad de extracción se denomina extracción con agua subcrítica (subcritical water extraction, SWE) o extracción presurizada con agua caliente (pressurized hot water extraction, PHWE). SWE puede considerarse una variedad de PLE en la que el disolvente de extracción utilizado es agua, de ahí su inclusión en este subapartado.

No existe instrumentación específica para llevar a cabo SWE pero se pueden utilizar los extractores de SFE y PLE, con temperaturas máximas de trabajo limitadas a 150 y 200 °C, respectivamente [36]. Se han descrito también aplicaciones con dispositivos no comerciales [38-42], algunos de los cuales permiten alcanzar temperaturas elevadas, superiores a los 200 °C [38,41,42].

Los analitos pueden analizarse directamente del extracto acuoso, utilizando la cromatografía de líquidos (LC) como técnica de separación, o pueden extraerse de éste mediante extracción líquido-líquido [42,43], extracción líquido-líquido con membranas microporosas [44,45] o microextracción en fase sólida [46,47], por ejemplo. Otra alternativa, que requiere un menor consumo de disolvente, es la utilización de trampas de adsorbentes en línea con el sistema de extracción [48,49].

La adición de modificadores tanto orgánicos [50-52] como inorgánicos [53,54] al agua se ha utilizado para mejorar la eficacia de extracción; esta adición altera también la temperatura y presión críticas. La utilización de mezclas binarias de agua con disolventes orgánicos miscibles (metanol, isopropanol, acetonitrilo, acetona, etc.) normalmente proporciona recuperaciones y una selectividad aceptables a temperaturas por debajo de los 200 °C, lo cual reduce además el riesgo de descomposición térmica de los analitos, que aumenta con la temperatura [55,56].

En general, las recuperaciones con SWE son buenas, comparables a las obtenidas con otras técnicas de extracción [37]. Por otro lado, es importante tener en cuenta que, a veces, aunque diferentes métodos de extracción son válidos para un mismo propósito en términos de recuperaciones, la calidad de los extractos difiere. Así, por ejemplo, Hawthorne y col. [41] evaluaron cuatro técnicas para la extracción de hidrocarburos aromáticos policíclicos (polycyclic

aromatic hydrocarbons, PAHs) de suelos, a saber: Soxhlet, PLE, SFE y SWE. Las cuatro técnicas proporcionaron recuperaciones análogas sin embargo la coloración de los extractos era superior para los obtenidos con Soxhlet y PLE y los cromatogramas correspondientes más complejos.

2.6. Dispersión de matriz en fase sólida

2.6.1. Principios

La dispersión de matriz en fase sólida es una técnica relativamente reciente, introducida en 1989 por el grupo de Barker [57]. Los principios de la técnica son sencillos [38,57-60]. La muestra se dispersa con un adsorbente sólido que actúa como agente abrasivo, provocando la ruptura mecánica de la estructura de la matriz y consiguiendo su distribución sobre las partículas de adsorbente. Si el adsorbente tiene una fase enlazada su misión es doble ya que además de proporcionar un material finamente dividido, ayuda a disolver y dispersar los componentes de la muestra en su superficie. Los componentes de la matriz se distribuyen sobre la superficie basándose en su polaridad relativa: los compuestos no polares se dispersan en la fase orgánica no polar dependiendo de sus coeficientes de distribución en la fase y los cambios dinámicos que tienen lugar a lo largo del proceso; las moléculas polares y de menor tamaño, como el agua, se asocian con grupos polares como los grupos silanol sobre la superficie de las partículas de sílice y dentro de los poros del soporte sólido, así como con componentes de la matriz capaces de establecer enlaces de hidrógeno; las partículas más grandes y de naturaleza más apolar se distribuyen a través de la superficie de la estructura bifásica: fase ligada/lípidos de la fase dispersada. El hecho de que toda la muestra esté en contacto íntimo con el extractante mejora la eficacia de extracción.

La mezcla se transfiere a una columna o cartucho y los analitos se eluyen con un disolvente apropiado. Equilibrios de partición y/o adsorción, similares a los que ocurren en una columna cromatográfica, son los que rigen la distribución de los analitos entre la muestra dispersada y el disolvente de elución. La distribución homogénea y la fina capa de muestra alrededor de las partículas de dispersante conllevan una transferencia de masa eficaz, responsable de las buenas recuperaciones que se consiguen bajo condiciones suaves de extracción y con un consumo moderado de disolventes.

2.6.2. Aspectos prácticos

El procedimiento de MSPD consta de las siguientes etapas [57-60]:

(1) La muestra líquida, viscosa, semisólida o sólida se mezcla en un mortero con el adsorbente y se dispersa hasta obtener una mezcla homogénea. El mortero y la mano del mortero han de ser de vidrio o ágata porque se ha comprobado que la porcelana y otros materiales porosos pueden dar lugar a pérdidas de analito. Las proporciones típicas muestra:adsorbente están dentro del intervalo 1:1 a 1:4. En algunos procedimientos se utilizan también agentes desecantes como el sulfato sódico anhidro para obtener un material seco, lo cual es importante si la elución de los analitos se lleva a cabo con un disolvente apolar.

(2) La mezcla se transfiere a una columna vacía o se coloca sobre un adsorbente o varias capas de adsorbentes. La columna es normalmente el cuerpo de una jeringa o un cartucho de extracción en fase sólida con una frita de acero inoxidable o polipropileno, un filtro de celulosa o un tapón de lana de vidrio en su parte inferior. También se coloca una segunda frita o tapón sobre la mezcla para llevar a cabo la compresión de la misma. La introducción de la mezcla en la columna de separación sigue los principios de la cromatografía de adsorción convencional, es decir, deben evitarse la formación de canales en la columna y el material no debe estar comprimido en exceso ya que ralentizaría, o incluso impediría totalmente, el flujo de disolvente a través del sistema.

(3) En lo que respecta a la elución, puesto que la totalidad de la muestra está presente en la columna, es posible llevar a cabo eluciones múltiples de la misma. De esta forma, se pueden aislar distintos grupos de compuestos presentes en una misma muestra. La mayoría de las eluciones se lleva a cabo por gravedad, aunque en algunos casos se ha aplicado un poco de presión o vacío para controlar el flujo del disolvente de elución.

Limpieza

A pesar de la selección cuidadosa de dispersante y disolvente, a veces, los extractos obtenidos mediante MSPD no son directamente analizables [38]. En estos casos, se puede integrar una etapa de limpieza en el proceso, sin más que introducir una o varias capas de adsorbentes en el extremo inferior del cartucho de MSPD, debajo de la muestra dispersada. Materiales de fase normal [61,62], carbono [63] o sílica ácida [64,65], para analitos con gran estabilidad química, se han utilizado con esta finalidad.

Otra posibilidad para aumentar la selectividad de la extracción es la inclusión de una etapa de lavado previa a la elución de los analitos de interés, combinada o no con la purificación en línea con adsorbentes. La utilización de agua pura [66] o combinada con un pequeño porcentaje de un disolvente polar [67] para la eliminación de compuestos polares o el lavado con hexano [68] o diclorometano [69] para arrastrar las interferencias apolares tales como lípidos y ácidos grasos son un ejemplo.

2.6.3. Variables que afectan a la eficacia de extracción

Adsorbente y disolvente

La selectividad de un proceso de extracción basado en la MSPD depende de la combinación adecuada de adsorbente y disolvente. Las aplicaciones clásicas de la MSPD utilizan materiales de fase reversa, fundamentalmente C18 [70,71] y C8 [72]. Aunque también se han utilizado adsorbentes de fase normal tales como el Florisil [73], la alúmina [74] o la sílica [75]. En este caso, el mecanismo de interacción con los componentes de la muestra es la adsorción y no se produce la solubilización de los componentes de la matriz como con las fases enlazadas. Sin embargo, las propiedades de estos adsorbentes pueden ajustarse dependiendo de su contenido en agua y su carácter ácido o básico.

La elección del disolvente de elución también es importante para recuperar eficazmente los analitos, evitando en lo posible la coextracción de interferentes. Se han utilizado desde disolventes apolares como el hexano [76] o mezclas hexano-diclorometano [77] hasta disolventes con una polaridad media o alta tales como el acetato de etilo [78], el acetonitrilo [69], la acetona [68] o el agua [79].

Temperatura y presión

Para conseguir extracciones más eficaces se ha propuesto la utilización de MSPD con extractantes presurizados y/o a temperaturas elevadas [38,60]. PLE y MSPD comparten principios de funcionamiento por lo que, a veces, la frontera entre ambas técnicas es difícil de establecer. De hecho, en PLE es habitual que la muestra se disperse con arena o tierra de diatomeas e incluso, más recientemente, con materiales en fase reversa y normal.

2.6.4. Ventajas e inconvenientes

MSPD es una técnica sencilla, con tiempos de extracción cortos, que reduce notablemente la manipulación de la muestra. No requiere instrumentación costosa y específica y el consumo de adsorbentes y disolventes orgánicos es bajo, por lo que el coste de las extracciones es asequible. Las condiciones de extracción en MSPD son normalmente suaves (temperatura ambiente y presión atmosférica) lo cual, junto con una combinación adecuada de dispersante y disolvente de elución, proporciona cierta selectividad y recuperaciones aceptables. Además, en muchos casos es posible integrar la extracción y purificación, con lo que se reduce todavía más la manipulación de la muestra y, en consecuencia, la probabilidad de errores en el análisis.

Como desventaja fundamental se puede señalar que requiere la presencia del analista, si no está automatizada, si bien un cierto grado de automatización es posible, excepto en la etapa de dispersión, utilizando por ejemplo extractores de PLE.

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3. Preparación de muestras líquidas

3.1. Técnicas tradicionales

El grueso de los trabajos centrados en la determinación de OPs en muestras acuosas se basa en la utilización de las extracciones líquido-líquido (liquid-liquid extraction, LLE) y en fase sólida (solid-phase extraction, SPE). En la presente Tesis se ha utilizado esta última técnica para la extracción, concentración y limpieza de los OPs en muestras líquidas [1] y también en la etapa de purificación de los extractos obtenidos para muestras sólidas (polvo y sedimentos) mediante otras técnicas (MAE y PLE) [2-4]. A continuación, se exponen muy brevemente los principios de LLE y, con un poco más de detalle, los fundamentos de SPE que, como se ha mencionado, ha sido utilizada en varios de los trabajos incluidos en la presente memoria.

3.1.1. Extracción líquido-líquido

La extracción líquido-líquido se basa en la distribución o reparto de los analitos entre dos fases inmiscibles regido por la constante de distribución K_D , que se define según la ecuación siguiente:

$$K_D = [A]_2/[A]_1$$

donde $[A]_2$ y $[A]_1$ son las concentraciones de analito en la fase orgánica y en la muestra, respectivamente. La constante de distribución es característica de cada analito y depende de la temperatura [5].

Frecuentemente, la constante de distribución no es lo suficientemente grande como para que la extracción del analito sea cuantitativa en una única extracción por lo que se necesita repetir el proceso varias veces para conseguir resultados cuantitativos. El número de repeticiones es tanto mayor cuanto menor sea el valor de la constante de distribución.

La selectividad y la eficacia de la extracción dependen de la correcta elección del disolvente inmiscible con la muestra pero también de otros factores como el pH, la adición de un agente complejante, la utilización de un reactivo formador de pares iónicos o la adición de sales neutras a la fase acuosa para reducir la solubilidad del analito (efecto salino o *salting out effect*).

LLE es una técnica lenta y laboriosa, difícil de automatizar, con elevado consumo de disolventes de alta pureza (por lo que requiere una etapa adicional de evaporación del disolvente para la concentración de los analitos) y plagada de numerosos problemas prácticos tales como la formación de emulsiones.

3.1.2. Extracción en fase sólida

3.1.2.1. Principios

La extracción en fase sólida es una técnica de preparación de muestra de uso extendido que se basa en la retención selectiva sobre un adsorbente de los analitos presentes en un fluido [5-8]. Posteriormente, éstos se eluyen con el disolvente adecuado o bien se desorben térmicamente.

La SPE puede utilizarse con varios objetivos [5]:

- **Preconcentración de analitos:** se consigue cuando el analito presente en un volumen elevado de muestra queda retenido en la fase sólida y es eluido posteriormente con pequeños volúmenes de disolvente. Por ejemplo, en la determinación de contaminantes orgánicos en aguas es posible extraer los compuestos presentes en 1-2 L de agua con 5-10 mL del disolvente de elución, concentrando así entre 100-400 veces la muestra.
- **Limpieza de las muestras:** eliminación de interferencias utilizando los disolventes de lavado adecuados.
- **Almacenamiento y transporte de analitos:** es un procedimiento útil para el muestreo de gases y líquidos y el almacenamiento de compuestos con volatilidad elevada o inestables en disolución.
- **Cambio de fase:** de interés en aquellos casos en los que el analito se encuentra, por ejemplo, en un disolvente no compatible con la técnica a emplear en su determinación final. Así, una vez que el compuesto ha sido retenido en el soporte sólido se eluye con un disolvente apropiado.
- **Fraccionamiento de la muestra** en diferentes compuestos o grupos de compuestos, eluyendo cada fracción con un disolvente diferente.
- También se pueden llevar a cabo **reacciones de derivatización** entre grupos reactivos del analito y los grupos funcionales de la superficie del adsorbente.

Adicionalmente, entre las ventajas que supone la utilización de la SPE cabe mencionar las siguientes: reduce la manipulación de la muestra así como el consumo de disolventes orgánicos, permite la consecución de altos niveles de recuperación y concentración, evita la formación de emulsiones, es aplicable a un gran número de matrices y analitos debido a la disponibilidad de una extensa variedad de fases estacionarias, es posible un alto grado de automatización y es favorable para el muestreo de campo. Por todo ello y por las limitaciones de la LLE, la SPE ha ido reemplazando a ésta última en múltiples aplicaciones.

3.1.2.2. Aspectos prácticos

Dos conceptos básicos a tener en cuenta en el desarrollo de un método de extracción en fase sólida son:

- **Capacidad del adsorbente:** es la máxima cantidad de analito/interferentes que puede ser retenida en una determinada masa de adsorbente. Una vez que todos los centros activos del adsorbente están ocupados, los compuestos pasan a su través sin quedar retenidos.
- **Volumen de ruptura:** es el volumen máximo de muestra que puede hacerse pasar a través de un adsorbente sin que se produzcan pérdidas de analito.

Tipos de fases

La selección de la fase es crucial para la obtención de eficacias de extracción elevadas, así como la mejor selectividad posible. Las fases empleadas en SPE son similares a las utilizadas en la cromatografía de líquidos. Las más populares son los óxidos inorgánicos, las sílices enlazadas y polímeros como el estireno-divinilbenceno, pero también se utilizan fases de intercambio iónico, exclusión molecular, afinidad y, más recientemente, materiales de acceso restringido [9,10].

Los **óxidos inorgánicos** más utilizados en SPE son la sílice, la alúmina, el Florisil (silicato de magnesio sintético) y la tierra de diatomeas. Los grupos funcionales susceptibles de establecer puentes de hidrógeno se retienen fuertemente (carboxilos, hidroxilos, etc.), aquellos con carácter dipolar se retienen en menor medida (grupos nitro, éster, cetona...) y los grupos funcionales polarizables (anillos aromáticos, alquenos...) son los menos retenidos.

Las **sílices enlazadas** se obtienen por la reacción de organosilanos con sílice activada en la que se forman enlaces silil-éter. Estas fases se clasifican, atendiendo a la polaridad del grupo funcional, en fases invertidas (octadecil (C18), octil (C8), etil (C2), fenil (Ph), etc.) o fases normales (ciano o aminopropilos, dioles, etc.). El C18 es, con diferencia, la fase enlazada más utilizada debido a su capacidad para retener tanto analitos apolares como moderadamente polares.

Las sílices enlazadas son inadecuadas para algunas aplicaciones. Los volúmenes de ruptura para moléculas pequeñas y muy polares son, a menudo, insuficientes para su determinación a niveles traza. Además, estas fases son inestables a pH extremos ($2 < \text{pH} < 8$). Los adsorbentes poliméricos y el carbón son una posible solución a los problemas anteriores ya que son estables en todo el intervalo de pH y no poseen grupos silanoles ionizables.

Las **fases poliméricas** son las más versátiles y permiten mejorar la retención de los analitos, incrementando el área superficial y el grado de entrecruzamiento de los polímeros o introduciendo grupos funcionales afines a las moléculas de interés. Los adsorbentes poliméricos más utilizados son los copolímeros de estireno-divinilbenceno, aunque existen disponibles comercialmente otros [9]. Los cartuchos Oasis HLB, que se han utilizado en algunas de las aplicaciones de esta Tesis Doctoral, contienen una fase polimérica, concretamente, un copolímero macroporoso obtenido a partir del divinilbenceno y la N-vinilpirrolidona que combina propiedades hidrofílicas y lipofílicas. Esto permite la retención de compuestos orgánicos polares y no polares, además de hacer prescindible la etapa de acondicionamiento.

El **carbón grafitizado** se obtiene por calentamiento del carbón a 2700-3000 °C en una atmósfera inerte. En ocasiones, este adsorbente es el único capaz de concentrar solutos orgánicos altamente polares. Por otro lado, una de las desventajas que presenta es la excesiva retención de algunos compuestos. Este efecto puede contrarrestarse realizando la elución de los cartuchos en sentido opuesto al utilizado para la adsorción.

Las fases de **intercambio iónico** están formadas por un soporte silíceo o polimérico funcionalizado con grupos con propiedades ácido-base. Los intercambiadores catiónicos contienen grupos ácidos fuertes (ácido sulfónico y derivados) o débiles (ácido carboxílico)

mientras que los aniónicos presentan grupos básicos fuertes (aminas cuaternarias) o débiles (aminas primarias, secundarias o terciarias).

Las **fases de exclusión molecular** permiten la separación de compuestos en función de su tamaño molecular. Las partículas de adsorbente tienen pequeños poros en los que sólo pueden penetrar las moléculas pequeñas. De esta forma, cuando la muestra pasa a través de la fase, las moléculas más grandes no se retienen mientras que se ralentiza el avance de las pequeñas, permitiendo así la separación. Existe una gran variedad de fases estacionarias disponibles comercialmente que se ha utilizado profusamente para la purificación de muestras biológicas (suero, plasma, etc.), eliminando compuestos de elevado peso molecular (proteínas e hidratos de carbono) que suelen dificultar la determinación de analitos más pequeños como anestésicos, drogas, antibióticos, etc.

Recientemente, se han desarrollado nuevas fases conocidas como **materiales de acceso restringido**. Las partículas de estos adsorbentes tienen pequeños poros de diámetro perfectamente controlado. Además, en este caso, la superficie interna del poro posee una fase ligada, mientras que la superficie externa de la partícula es hidrofílica. De esta forma, cuando la muestra pasa a través del material, las moléculas grandes no sólo no entran en los poros sino que, además, al ser altamente hidrofóbicas pasan fácilmente, ya que tampoco quedan retenidas en la superficie hidrofílica de las partículas. Por otro lado, los compuestos de menor tamaño entran dentro de los poros y sólo aquellos que tengan afinidad por la fase ligada interna quedarán retenidos.

Las fases descritas anteriormente permiten la separación y preconcentración de grupos amplios de compuestos orgánicos por lo que no siempre es fácil la determinación final de los analitos de interés. Los **adsorbentes de afinidad**, caracterizados por su gran selectividad, están constituidos por un soporte inerte sobre el cual están inmovilizados una enzima, un anticuerpo o una hormona, que son capaces de reconocer e interactuar con su correspondiente sustrato, antígeno o receptor, respectivamente. De éstos, los adsorbentes de afinidad constituidos por anticuerpos inmovilizados, conocidos como inmunoadsorbentes, han sido los más empleados. Los inmunoadsorbentes se basan en interacciones antígeno-anticuerpo selectivas y reversibles y son una alternativa atractiva a los adsorbentes clásicos. La alta selectividad de los inmunoadsorbentes origina extractos más limpios que los obtenidos con otras fases. Sin embargo, actualmente, hay muy pocos inmunoadsorbentes disponibles comercialmente, lo cual limita sus aplicaciones prácticas.

Los **polímeros de huella molecular** (molecularly imprinted polymers, MIPs) son los análogos sintéticos a los inmunoadsorbentes. Los MIPs se obtienen sintetizando polímeros altamente entrecruzados en presencia de una molécula plantilla. Una vez obtenido el polímero, se extrae el compuesto modelo, quedando así huecos libres capaces de reconocer la molécula implicada en la formación del polímero. La síntesis del polímero tiene lugar en presencia de una gran cantidad de la molécula plantilla, que puede ser difícil de extraer cuantitativamente del polímero, reduciendo su capacidad de enlace o, lo que es más serio, las moléculas modelo residuales pueden pasar al extracto de la muestra invalidando así la determinación debido a

problemas con los blancos. Una solución a este problema es utilizar un compuesto plantilla similar al analito para la síntesis del MIP.

Algunos MIPs tienen selectividades y constantes de afinidad muy altas, comparables con sistemas de reconocimiento naturales tales como los anticuerpos monoclonales, son más fáciles de preparar que los inmunoabsorbentes y el coste y tiempo requeridos para su síntesis es menor.

3.2. Alternativas a las técnicas tradicionales

LLE es una técnica popular y versátil, que ha sido y es recomendada en muchos protocolos analíticos oficiales. Sin embargo, a pesar de su uso extendido, es una técnica laboriosa, que conlleva un consumo de grandes volúmenes de disolvente y muestra y tiene normalmente problemas de formación de emulsiones, lo cual dificulta la automatización del proceso. La extracción en fase sólida supera algunas de esas limitaciones y, lo que es más importante, permite llevar a cabo en una única etapa la extracción y concentración de los analitos. A pesar de esto, el coste de los cartuchos no es despreciable, la cantidad de muestra a procesar es también considerable, si se compara con las técnicas de microextracción, y el volumen de disolvente utilizado, aunque menor que en LLE, sigue siendo del orden de varios mililitros.

Una tendencia importante en Química Analítica es la simplificación, automatización y miniaturización de la preparación de muestra ya que esto conlleva una reducción del consumo de disolventes, de los residuos generados y de los riesgos a los que está expuesto el analista, entre otras ventajas destacables. En este contexto, han surgido diferentes metodologías, resultado de la miniaturización de la LLE y la SPE, **Figura II.6**.

Figura II.6. Esquema de las técnicas de microextracción abordadas en este capítulo.



3.2.1. Técnicas basadas en la microextracción en fase líquida

En el ámbito de la microextracción líquido-líquido (micro-LLE) han surgido varias técnicas, clasificables en dos grandes grupos: aquéllas en las que las dos fases inmiscibles están en contacto directo y aquéllas en las que media una membrana entre ellas. Dentro del primer grupo se encuadran la microextracción con gota suspendida (single drop microextraction, SDME) y la microextracción líquido-líquido dispersiva (dispersive liquid-liquid microextraction, DLLME). Las extracciones mediadas por una membrana pueden involucrar dos o tres fases y, además,

pueden llevarse a cabo en modo estático o dinámico. A continuación, se proporciona una explicación más detallada de cada una de ellas, haciendo especial hincapié en la novedosa DLLME y en la extracción con membranas microporosas huecas (hollow fiber-microporous membrane liquid-liquid extraction, HF-MMLLE), por haber sido utilizadas en dos de los trabajos que forman parte de esta Tesis [11,12].

3.2.1.1. Microextracción con gota suspendida

La microextracción en fase líquida (liquid-phase microextraction, LPME) surgió en la segunda mitad de la década de los 90 [13-15] como la alternativa miniaturizada a la LLE. El término LPME se introdujo por primera vez para describir un sistema de dos fases en el que la microextracción se llevaba a cabo con una microgota de disolvente orgánico suspendida del extremo de la aguja de una microjeringa y expuesta a la muestra: la SDME [15]. En esta modalidad de microextracción el muestreo se lleva a cabo, normalmente, en espacio de cabeza. También se puede trabajar con la gota inmersa en la muestra, en modo estático o dinámico, o ésta puede exponerse a una fina capa de disolvente orgánico con densidad inferior al agua, situada sobre la muestra acuosa [16-18].

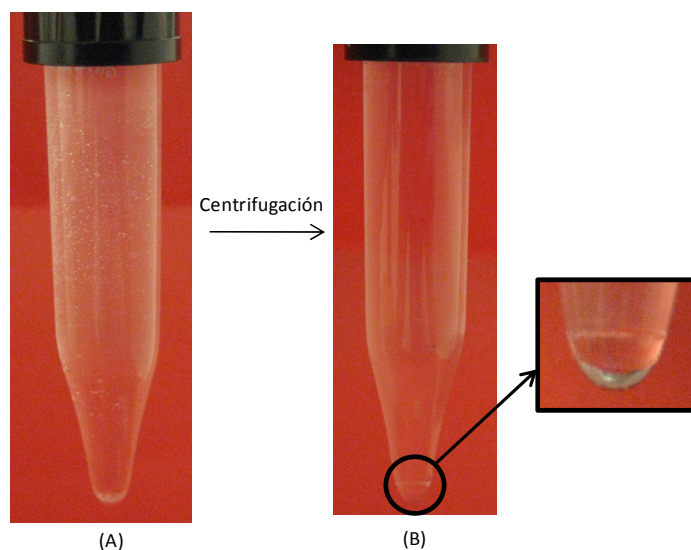
Es una técnica sencilla, de bajo coste y con una enorme capacidad de preconcentración. Su principal limitación es la inestabilidad de la gota durante la extracción.

3.2.1.2. Microextracción líquido-líquido dispersiva

3.2.1.2.1. Principios

La microextracción líquido-líquido dispersiva es una modalidad de extracción líquido-líquido miniaturizada desarrollada recientemente por Rezaee y col. [19]. Esta técnica integra en una única etapa la extracción y concentración de los analitos. La reducción notable de la razón fase aceptora-fase dadora es la responsable de su enorme capacidad de preconcentración [19-21].

DLLME se basa en la adición a una muestra líquida de una mezcla binaria compuesta por un disolvente con densidad superior a 1 g mL^{-1} (extractante) y un disolvente polar y miscible con agua (dispersante). El extractante representa alrededor de un 1-3% del volumen total de la mezcla binaria [21]. Tras la adición, normalmente mediante inyección rápida, de esta mezcla a la muestra se forma una dispersión de pequeñísimas gotas de extractante (**Figura II.7 A**) que asegura una enorme superficie de contacto entre la muestra y la fase extractante. Este hecho favorece y acelera el proceso de transferencia de masa y hace que se alcance el equilibrio muy rápidamente [19-21]. Además, conlleva tiempos de extracción muy cortos, que es una de las principales ventajas de la técnica. Tras la centrifugación de esta dispersión, las microgotas de extractante se agregan y se depositan en forma de gota en el fondo del tubo en el que se ha llevado a cabo el proceso (**Figura II.7 B**). Por razones prácticas, se utilizan tubos de vidrio de forma cónica, lo cual facilita la recuperación de la gota, que se lleva a cabo con una microjeringa.

Figura II.7. Etapas de la DLLME.

Simplicidad, rapidez, bajo coste, consumo reducido de muestra (típicamente entre 5 y 10 mL) y disolventes orgánicos, versatilidad, buenas recuperaciones y factores de concentración altos son algunas de las ventajas más destacables de la técnica. Comparada con otras técnicas de microextracción como SDME, SPME o LPME ofrece normalmente mayor precisión y tiempos de extracción más cortos [22-24]. Como desventajas cabe comentar la necesidad de una cierta destreza para la manipulación y recuperación de la gota y, sobre todo, la dificultad de automatización, aunque ya se ha propuesto algún trabajo en que se ha utilizado esta técnica en un sistema continuo para la determinación de cobre y plomo mediante espectrometría de absorción atómica en llama [25].

Cálculo de recuperaciones y factores de enriquecimiento

En la optimización de los métodos de DLLME lo más habitual es evaluar la influencia de distintas variables en términos de recuperaciones y factores de enriquecimiento, que se calculan como sigue:

$$EF = C_{sed}/C_o$$

$$R (\%) = 100 (C_{sed} V_{sed}) / (C_o V_m)$$

donde EF es el factor de enriquecimiento, C_o y C_{sed} son las concentraciones de analito en la muestra y en la fase sedimentada, respectivamente. R es la recuperación y V_{sed} y V_m son los volúmenes de la gota sedimentada y de la muestra, respectivamente.

El volumen de gota sedimentada varía en función de las condiciones de extracción. Por ello, es necesaria la utilización de ambos parámetros, recuperaciones y factores de concentración, en la optimización del método. En la selección de las condiciones óptimas se

persigue la maximización de R y EF. En la práctica, la consecución de ambos objetivos simultáneamente no siempre es posible y se deben adoptar condiciones de compromiso.

3.2.1.2.2. Variables que afectan a la eficacia de extracción

Son varios los factores que afectan a la eficacia de DLLME. Entre ellos, el tipo y volumen de disolventes extractante y dispersante así como la fuerza iónica son, en general, las variables que ejercen una mayor influencia en la eficacia de extracción. Otros parámetros habitualmente estudiados son los tiempos de extracción y centrifugación, que son normalmente cortos. Así mismo, pueden ser necesarios el control del pH y la adición de reactivos (agentes derivatizantes o quelantes, por ejemplo) para llevar a cabo la extracción de los compuestos de interés.

Disolvente extractante

La selección del disolvente extractante es un factor clave en la eficacia de DLLME. Ha de reunir las siguientes características:

- Densidad superior a la del agua.
- Baja solubilidad en agua.
- Capacidad de formar una dispersión al añadirlo, mezclado con el dispersante, a la muestra.
- Capacidad para extraer los compuestos de interés.
- Buen comportamiento cromatográfico.

El disulfuro de carbono y algunos hidrocarburos halogenados tales como el tetracloruro de carbono, clorobenceno, cloroformo, diclorometano o tricloroetano se han empleado como extractantes [20,21].

Disolvente dispersante

El dispersante actúa de puente entre el extractante y la muestra y, por ello, ha de ser miscible con ambos. Su misión es la reducción de la tensión superficial del extractante para conseguir así la formación de minúsculas gotas, que garantizan una enorme superficie de contacto entre extractante y muestra, responsable de la rapidez de las extracciones. Acetona [19], metanol [26], etanol [27], acetonitrilo [22] o tetrahidrofurano [28] pueden utilizarse como dispersantes.

Volúmenes de extractante y dispersante

Volumen de extractante. Las variaciones en el volumen de extractante no se traducen normalmente en cambios pronunciados en las recuperaciones del método. Esto es un indicativo de que las constantes de distribución de los analitos son altas y las recuperaciones ya cuantitativas para pequeños volúmenes. En el caso de compuestos más hidrofílicos sí que se produce una mejora de las recuperaciones con el aumento del volumen de extractante [11,29,30]. Por otro lado, el aumento del volumen de disolvente extractante conlleva un incremento paralelo del volumen de fase sedimentada y, por consiguiente, una disminución del

factor de preconcentración, ya que el extracto final está más diluido. Así pues, cuanto menor sea el volumen de extractante utilizado, tanto menores serán los límites de cuantificación del método. En la práctica, el volumen mínimo de extractante que se puede utilizar está limitado a aquel que tras la centrifugación de la mezcla ternaria da lugar a una gota con un volumen fácilmente manipulable, no inferior a los 5 μL . A parte de esto, la obtención de recuperaciones y factores de preconcentración altos es lo que condiciona el volumen de extractante óptimo. En general, se suelen utilizar volúmenes entre 5 y 100 μL .

Volumen de dispersante. El volumen de dispersante afecta directamente a la formación de la emulsión y, por tanto, a la eficacia de extracción. En la mayoría de los trabajos en los que se estudia la influencia de este parámetro se varía también simultáneamente el volumen de extractante, de tal forma que el volumen de la gota obtenida se mantenga constante al variar el volumen de dispersante empleado. Normalmente, se seleccionan volúmenes entre 0.5 y 1.5 mL.

Adición de sal

El aumento de la fuerza iónica de la muestra normalmente produce una disminución de la solubilidad tanto de los analitos como del agente extractante, lo cual resulta favorable en términos de recuperaciones. Ahora bien, la disminución de la solubilidad del extractante conlleva un aumento del volumen de la fase sedimentada obtenida y, en consecuencia, una dilución de los analitos o lo que es lo mismo, una disminución del factor de concentración [20].

Tiempos de extracción y centrifugación

En DLLME el tiempo de extracción se define como el intervalo que transcurre entre la adición de la mezcla binaria a la muestra y la centrifugación [19]. Esta variable no ejerce prácticamente ninguna influencia en la eficacia de extracción. La razón es la rapidez con la que se alcanza el equilibrio, de manera inmediata tras la formación de la emulsión, que garantiza una transferencia rápida de los analitos al extractante [20].

La centrifugación de la mezcla ternaria es necesaria para la separación de fases y la obtención así de la gota sedimentada. Tiempos cortos, normalmente no superiores a 5 minutos, son suficientes para tal fin.

Otras variables

DLLME es aplicable a especies en forma neutra, con un moderado o elevado carácter hidrofóbico, y poco viable para la extracción de especies hidrofílicas. La extracción de especies iónicas requiere, por tanto, la formación de derivados neutros con la suficiente hidrofobicidad para ser recuperados en la fase sedimentada. La adición de reactivos complejantes y el control del pH son la clave para la consecución de la extracción [16,20,21,31-33]. Igualmente, la extracción de especies con propiedades ácido-base puede conseguirse mediante el control del pH para desplazar el equilibrio hacia la especie neutra [28,34,35].

3.2.1.2.3. Aplicaciones

Matrices

Las características ya mencionadas de DLLME han contribuido a la publicación de un gran número de trabajos [20,21], a pesar de su reciente introducción [19]. Se ha utilizado para la extracción de analitos orgánicos e inorgánicos de diversa índole en muestras líquidas. Sin embargo, a pesar de todas las ventajas antes mencionadas, esta técnica no siempre es compatible con cualquier tipo de matriz líquida, de ahí que la inmensa mayoría de las aplicaciones desarrolladas se centren en muestras acuosas sencillas [20]. Para muestras más complejas, como es el caso de matrices biológicas (orina, plasma...) o muestras de agua residual, la interacción del extractante con componentes de la matriz puede llegar a impedir la obtención de una fase sedimentada apta para su análisis. A pesar de esto, DLLME se ha aplicado con éxito a algunas matrices complejas. Así, por ejemplo, se ha utilizado para la extracción de triclosán, metiltriclosán [36] y anilinas [37] de agua residual y fenoles volátiles de muestras de vino sin tratamiento previo [38] (más que un ajuste de pH para evitar la coextracción de ácidos orgánicos). Uno de los trabajos que forman parte de la presente Tesis Doctoral recoge también la aplicación de DLLME a muestras de agua, entre ellas agua residual [11]. Además, DLLME se ha utilizado también, en combinación con otras técnicas de extracción, como procedimiento de concentración y purificación, para la determinación de pesticidas organofosforados en vegetales [39] y organosulfurados en sedimentos [40]. En ambos casos, DLLME se aplicó tan sólo a una pequeña alícuota del extracto obtenido, diluido o no. En esta línea, Zhao y col. [39] extrajeron los pesticidas organofosforados de sandía y melón mediante extracción sólido-líquido, con 10 mL de acetonitrilo, y aplicaron DLLME a una alícuota de 1 mL del extracto orgánico resultante centrifugado. Xiong y Hu [40] sometieron muestras de sedimentos a extracción asistida por ultrasonidos con agua. A continuación, concentraron y purificaron mediante DLLME una alícuota de ese extracto acuoso centrifugado y diluido 25 veces.

Técnicas instrumentales

La DLLME se ha utilizado en conjunción con la cromatografía de gases (GC) [19,26] y la de líquidos [22,29], con distintos tipos de detección, así como con la espectrometría de absorción atómica (Atomic absorption spectrometry, AAS) [41,42]. La espectrofotometría [43] y la emisión atómica (Atomic emission spectrometry, AES) [44] también se han utilizado en combinación con DLLME. Entre ellas, las combinaciones DLLME-GC y DLLME-AAS son las más habituales, ya que en la mayoría de los casos el disolvente de extracción se puede analizar directamente sin ningún tratamiento adicional.

Para la determinación de compuestos termolábiles, no volátiles o de alto peso molecular, inadecuados para la cromatografía de gases, la cromatografía de líquidos es una buena alternativa. En la combinación DLLME-cromatografía de líquidos de alta eficacia (HPLC), el extracto de DLLME puede inyectarse directamente [22,45-47] o bien someterse a un tratamiento adicional [29,34,48]. Éste puede consistir en un cambio de disolvente [29,34], tras llevar a sequedad el extracto de DLLME, o en la combinación de DLLME con un procedimiento adicional [48]. Melwanki y Fuh [48] desarrollaron un método para el análisis de clenbuterol en aguas en el

que combinaron DLLME con la reextracción del compuesto en una fase acuosa ácida (1% ácido fórmico), contenida en una jeringa de inyección, compatible con la cromatografía de líquidos. Mediante bombeos repetitivos del émbolo de la jeringa, la fase acuosa se pone reiteradamente en contacto con la fase orgánica, hasta la reextracción total del analito.

Tendencias

DLLME se ha ido perfeccionando y se han introducido mejoras que han permitido, por ejemplo, integrar la derivatización en el proceso de extracción dispersiva para la separación mediante cromatografía de gases de compuestos polares o poco volátiles como clorofenoles [49], anilinas [37] y triclosan [36], que no podrían ser separados fácilmente sin derivatizar mediante cromatografía de gases. Llevar a cabo simultáneamente ambos procesos (extracción y derivatización) contribuye todavía más a la reducción del tiempo necesario para la preparación de la muestra.

Recientemente, se han utilizado como extractantes otros disolventes no clorados con bajos puntos de fusión, inferiores a la temperatura ambiente, tales como el undecanol, 1-dodecanol, 2-dodecanol, 1-tetradecilalcohol o el *n*-hexadecano [50,51]. En este caso, dado que la densidad de estos disolventes es inferior a la del agua, tras la centrifugación, la gota de extractante no se deposita en el fondo del tubo de extracción sino que flota sobre la superficie de la muestra. La recuperación del extractante se consigue tras la congelación del mismo, sin más que sumergir el recipiente donde se ha llevado a cabo la extracción en un baño de hielo durante un intervalo corto de tiempo (5 minutos); la gota solidificada se transfiere entonces, con la ayuda de una espátula, a un vial. En este caso, la geometría del recipiente en el que se lleva a cabo la extracción no es importante para la fácil recuperación del extractante. Este tipo de disolventes presenta una toxicidad mucho menor que los clorados, lo cual resulta atractivo no sólo desde el punto de vista medioambiental sino también desde una perspectiva toxicológica, ya que se evita la exposición del analista a reactivos nocivos. A pesar de esto, el número de publicaciones es todavía bastante limitado, probablemente por la dificultad de manipulación y recuperación de la gota solidificada.

En la misma línea de búsqueda de extractantes alternativos, la sustitución de los disolventes clorados por líquidos iónicos se presenta como una opción interesante dentro del desarrollo de una química sostenible y respetuosa con el medioambiente. Los líquidos iónicos son sales con puntos de fusión bajos, por debajo de los 100 °C, aunque desde el punto de vista analítico los más interesantes son aquellos con temperaturas de fusión próximas o por debajo de la ambiente. Estructuralmente son diferentes a los disolventes convencionales porque están compuestos por iones en vez de por moléculas. Típicamente están formados por cationes cuaternarios de nitrógeno, asimétricos y voluminosos, del tipo alquilamonio, dialquilimidazolio o alquilpiridinio. Los contraiones aniónicos pueden ser especies orgánicas o inorgánicas tales como Cl^- , AlCl_4^- , CF_3CO_2^- , SCN^- , BF_4^- , etc. Los líquidos iónicos presentan propiedades muy favorables entre las que cabría destacar que exhiben presiones de vapor muy bajas, no son inflamables, son térmica y químicamente estables y poseen la capacidad de extraer compuestos tanto orgánicos como inorgánicos [52-54]. La flexibilidad sintética de estas sales permite la combinación de

distintos aniones y cationes para ajustar ciertas propiedades físico-químicas, tales como viscosidad y miscibilidad con agua o disolventes orgánicos, a las necesidades del usuario.

Hasta el momento, el número de publicaciones centradas en el uso de la microextracción líquido-líquido dispersiva con líquidos iónicos (ionic liquids (IL)-DLLME) es aún reducido. Se han descrito aplicaciones a muestras acuosas [55] y sólidas [56,57], tras una etapa previa de extracción, para diferentes compuestos: pesticidas [58,59], fenoles [60], aminas aromáticas [61], etc. En general, no existe un procedimiento estandarizado para la realización de la extracción: en algunos trabajos se utiliza el líquido iónico en combinación con un disolvente dispersante y se procede como en la DLLME original [56,57,62]; en otros, se emplea exclusivamente el líquido iónico, sin mediación de disolvente puente [58-60]. En este último caso, se han publicado artículos en los que se sigue el protocolo habitual de DLLME, a saber, agitación de la mezcla y centrifugación para la obtención de la fase sedimentada [60]; en otros, se procede de igual forma pero con control de temperatura [58,59]: el líquido iónico se añade sobre la muestra y esta mezcla se mantiene a alta temperatura para favorecer la miscibilidad de las fases, después se enfría en baño de hielo, para disminuir la solubilidad del extractante, y por último, se centrifuga. La técnica de separación utilizada en estas aplicaciones fue la cromatografía de líquidos, lo cual no es de extrañar teniendo en cuenta la baja volatilidad y elevada viscosidad de los líquidos iónicos, que ha limitado su aplicación en cromatografía de gases al uso como fases estacionarias. No obstante, recientemente, Aguilera-Herrador y col. han desarrollado una interfase desmontable y acoplable al cromatógrafo de gases, sin necesidad de introducir ninguna modificación en el sistema, que ha permitido el acoplamiento directo de la microextracción con gota colgante utilizando líquidos iónicos con la cromatografía de gases [63-65].

Regueiro *et al.* [66] propusieron una técnica híbrida entre DLLME y la extracción líquido-líquido asistida por ultrasonidos (ultrasound-assisted liquid-liquid extraction, USALLE) que combina las ventajas de ambas: la emulsificación-microextracción asistida por ultrasonidos (ultrasound-assisted emulsification-microextraction, USAEME). El sistema ternario (muestra acuosa + mezcla de disolventes extractante y dispersante) de DLLME da paso a uno binario en USAEME que, aprovechando el poder emulsionante de los ultrasonidos, prescinde de la utilización del disolvente dispersante. El protocolo de extracción consiste en la adición del disolvente extractante a la muestra y la irradiación de la mezcla con ultrasonidos, que produce la dispersión del disolvente. Tras un tiempo controlado de irradiación, se centrifuga y se obtiene así una fase sedimentada. Esta metodología ya se ha aplicado para la determinación en aguas de pesticidas [67], bifenilos policlorados (polychlorinated biphenyls, PCBs) [68], PAHs [69], PBDEs [70] y conservantes fenólicos [71].

DLLME también se ha utilizado como etapa de purificación y concentración, combinada con otras técnicas de extracción. Esta combinación permite ampliar el ámbito de aplicación de DLLME a matrices más complejas y la consecución de factores de concentración extraordinariamente altos. Así, los factores de concentración utilizando DLLME están habitualmente dentro del intervalo de 50 a 1000 veces mientras que con la combinación SPE-DLLME pueden llegar a alcanzarse valores de hasta 17000. Normalmente, en estas aplicaciones el extracto orgánico proporcionado por otras técnicas (SPE, extracción sólido-líquido) y conteniendo los analitos de interés, se utiliza como dispersante. Si el disolvente es incompatible con DLLME se

lleva a sequedad y se reconstituye [72]. Éste, combinado con el extractante adecuado, se adiciona sobre agua para obtener así una emulsión que, tras centrifugar, da lugar a una gota sedimentada. La DLLME se ha utilizado como técnica de purificación y concentración no sólo para matrices sólidas [39,40] sino también líquidas. Fattahi y col. [73] propusieron un método de SPE-DLLME para la extracción de clorofenoles en agua con factores de concentración superiores a 17000 y límites de detección entre 4 y 20 veces menores que los proporcionados por otras técnicas. Liu y col. [72] desarrollaron un método para la determinación de PBDEs en plantas y aguas utilizando la combinación SPE-DLLME en la preparación de muestra, alcanzando así factores de concentración entre 6838 y 9405. Montes y col. [30] llevaron a cabo el análisis de fungicidas en vino mediante la utilización combinada de SPE y DLLME, que no sería posible utilizando únicamente DLLME por la precipitación de componentes del vino, sobre todo los tintos, que impiden el análisis de la gota sedimentada.

3.2.1.3. Microextracción líquido-líquido mediada por membranas

Existen numerosas variantes dentro de la extracción con membranas. De hecho, se encuentra en la bibliografía una gran riqueza de términos para referirse a procesos que realmente comparten un fundamento similar. A continuación, se presentan algunas de las modalidades más relevantes para este trabajo por haber sido utilizadas para la extracción de OPs. En base a esto, se exponen dos grupos de técnicas diferenciables por el tipo de membrana utilizada: porosa o no porosa.

3.2.1.3.1. Microextracción en fase líquida con fibra hueca

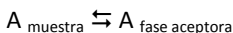
Como solución a la falta de estabilidad y robustez de la gota en SDME, Pedersen-Bjergaard y Rasmussen [74] introdujeron en 1999 una metodología alternativa en la que la fase aceptora se encuentra protegida en el interior de una membrana porosa en forma de fibra hueca. Esta modalidad se denomina microextracción en fase líquida con fibra hueca (hollow-fiber liquid phase microextraction, HF-LPME). En HF-LPME, la fase extractante se encuentra en el interior de la membrana y no está en contacto directo con la muestra. La transferencia de masa se produce a través de los poros de la membrana y las muestras pueden agitarse vigorosamente sin problemas de pérdidas de disolución aceptora.

3.2.1.3.1.1. Principios y modalidades

Para llevar a cabo la extracción, la membrana se sumerge durante un intervalo de tiempo corto en un disolvente orgánico, que así penetra en los poros y se mantiene unido a la membrana por fuerzas capilares. Alternativamente, el disolvente orgánico se puede inmovilizar desde el interior de la membrana inyectando un pequeño volumen del mismo en la fibra [75].

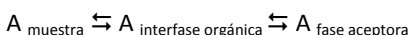
Cuando la fibra se expone a la muestra, los analitos se extraen primero a la fase orgánica intermedia y de ahí se transfieren a la fase aceptora contenida en el interior de la membrana. Esta disolución aceptora puede ser el mismo disolvente inmovilizado en la pared de la membrana o bien una disolución acuosa ácida o alcalina. Estas dos posibilidades dan lugar a dos modos de muestreo diferentes: en dos o en tres fases [17,75], **Figura II.8.**

En **LPME** involucrando **dos fases**, los analitos contenidos en la muestra acuosa (fase dadora) se extraen a través del disolvente orgánico inmovilizado en los poros de la fibra hueca en el mismo disolvente orgánico contenido en el interior de la fibra (fase aceptora). El equilibrio que se establece entre las dos fases para un analito A es el siguiente:



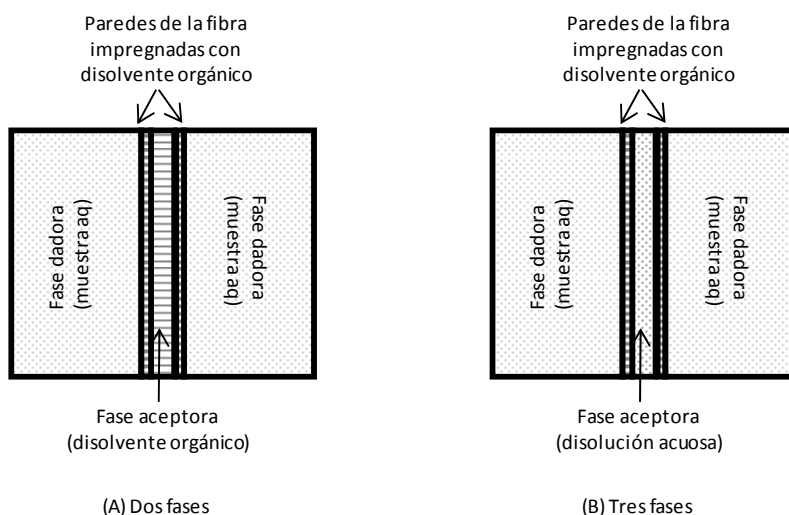
Este modo de muestreo está especialmente indicado para analitos apolares, con baja solubilidad en agua. En esta modalidad, el extracto final es compatible con la cromatografía de gases. Su inyección en un sistema de cromatografía líquida o electroforesis capilar requiere un paso previo de evaporación y reconstitución con un disolvente adecuado [76].

En **LPME** implicando **tres fases**, los analitos contenidos en la muestra acuosa se extraen a través de la interfase orgánica a otra disolución acuosa. El equilibrio que se establece en este caso es el que sigue:



Este modo de extracción está limitado a analitos hidrofílicos con propiedades ácido-base y está controlado por los valores de pH de las disoluciones dadora y aceptora. Así, por ejemplo, para analitos con propiedades ácidas el pH de la muestra debe ajustarse dentro de la región ácida, por debajo de su pK_a , para disminuir su solubilidad y facilitar su difusión a través de la interfase, mientras que la disolución aceptora ha de tener un pH alto, por encima de su pK_a . Para analitos básicos las condiciones son inversas. En esta modalidad, la disolución aceptora es directamente compatible con la cromatografía líquida o la electroforesis capilar.

Figura II.8. Sección longitudinal de una fibra hueca sumergida en una muestra acuosa en un sistema de dos fases (A) y uno de tres (B).

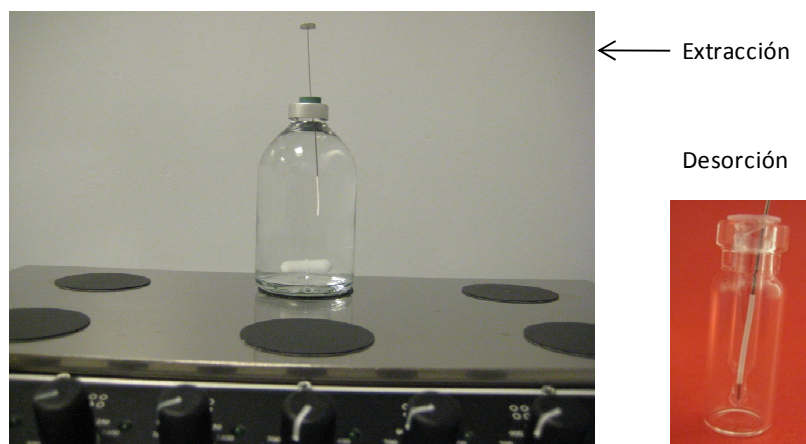


La extracción puede llevarse a cabo en modo estático o dinámico. En la modalidad estática, la disolución aceptora se encuentra en el interior de la membrana y la muestra se agita

mediante vibración o agitación magnética. En la modalidad dinámica, la fibra acondicionada se conecta a una jeringa que contiene unos pocos microlitros de disolvente orgánico inmiscible con agua y se sumerge en la disolución acuosa. Durante la extracción, pequeños volúmenes de la muestra acuosa se introducen y expulsan repetidamente de la fibra con el movimiento del émbolo de la jeringa. El modo dinámico acelera la extracción en comparación con la modalidad estática pero es más complejo, reduce la capacidad de procesamiento simultáneo de muestras, requiere el control de más parámetros e instrumentación para llevar a cabo el movimiento del émbolo de la jeringa [76].

Tradicionalmente, en HF-LPME el interior de la fibra contiene la disolución aceptora. La **extracción líquido-líquido con membranas microporosas** en forma de fibra hueca es una variante de HF-LPME con dos fases en la que la disolución aceptora está únicamente inmovilizada en los poros de la membrana y el interior no contiene disolvente orgánico, **Figura II.9** [77-79]. Esto se consigue usando un soporte inerte que ocupa el volumen interno de la fibra. Con esta modalidad se simplifica el proceso de extracción, ya que la introducción del disolvente orgánico en el interior de la membrana es un proceso bastante tedioso, además de una fuente de variabilidad. Los analitos se recuperan sumergiendo la fibra en un pequeño volumen de disolvente orgánico, que posteriormente a la desorción puede reducirse para alcanzar factores de concentración todavía superiores. Uno de los trabajos incluidos en esta Tesis Doctoral constituye la primera aplicación de HF-MMLLE para la extracción de OPs de muestras acuosas [12].

Figura II.9. Dispositivo experimental para HF-MMLLE.



3.2.1.3.1.2. Variables que afectan a la eficacia de extracción

Fibra. La fibra ha de ser hidrofóbica y compatible con el disolvente que se utilice. En la mayoría de los trabajos se utiliza el polipropileno poroso, compatible con un gran número de disolventes orgánicos y, además, capaz de proporcionar una inmovilización excelente de los disolventes utilizados en LPME. Esto es de especial relevancia cuando las muestras se agitan vigorosamente o en el caso de muestras que forman fácilmente emulsiones en presencia de disolvente orgánico [76].

El diámetro interno de las fibras utilizadas se encuentra típicamente dentro del intervalo 600-1200 μm y el espesor de la membrana suele ser de 200 μm , lo cual proporciona buena estabilidad mecánica [76]. El tamaño de poro nominal y máximo de estas membranas suele ser 0.2 μm y 0.64 μm , respectivamente, lo cual asegura una microfiltración eficaz, permitiendo sólo el paso de pequeñas moléculas a través de la membrana.

Existen varias configuraciones posibles para llevar a cabo HF-LPME: puede utilizarse un fragmento de fibra colocada perpendicularmente a la superficie de la muestra o bien puede plegarse parcialmente para dar lugar a una configuración en forma de U [75].

Disolvente orgánico. La elección del disolvente orgánico utilizado para ocupar los poros de la membrana es crucial. El disolvente debe ser inmiscible con agua, presentar baja volatilidad (pero compatibilidad con la cromatografía de gases, en el caso de LPME en dos fases) y ser fuertemente retenido en los poros de la membrana para evitar pérdidas durante la extracción. Obviamente, ha de presentar también afinidad por los analitos de interés. Los disolventes más utilizados son: octanol, éter dihélico y tolueno [75,80].

Agitación. La agitación de la muestra acelera la cinética de extracción, ya que favorece la difusión de los analitos y suele mejorar la repetibilidad del proceso. Dado que la disolución aceptora está confinada en el interior de la membrana y protegida por ésta, la técnica tolera agitación enérgica de la muestra.

La agitación puede ser magnética, introduciendo una barra agitadora recubierta de Teflón, o puede efectuarse mediante vibración. Esta última evita posibles problemas de contaminación cruzada a través de las barras magnéticas que, además, en el caso de agitación vigorosa pueden dar lugar a la formación de burbujas. Éstas, tienden a adherirse a la superficie de la fibra, acelerando así la evaporación del disolvente, introduciendo imprecisión en las medidas y reduciendo la superficie de contacto entre muestra y fibra [81].

Adición de sal y control de pH. Para algunos compuestos, el aumento de la fuerza iónica disminuye su solubilidad en la muestra y favorece así su extracción, por el efecto salino.

El control del pH es crucial en LPME en tres fases pero también puede favorecer la extracción en los sistemas de dos fases.

Tiempo de extracción. La transferencia de masa es un proceso dependiente del tiempo y su velocidad disminuye a medida que el sistema se aproxima a condiciones de equilibrio. Aunque habitualmente tiempos de extracción largos conllevan eficacias de extracción superiores, en la práctica, se suelen seleccionar tiempos de extracción que permitan procesar el mayor número de muestras posible y se opta por trabajar en condiciones de no equilibrio, por lo que el control del tiempo es crucial. Cuando son necesarios tiempos de muestreo excesivamente largos, el procesado simultáneo de varias muestras permite compensar ese hándicap.

3.2.1.3.1.3. Ventajas e inconvenientes

LPME es una técnica flexible, sencilla, con un consumo reducido de disolvente y de bajo coste. Proporciona un alto grado de preconcentración y extractos limpios, directamente compatibles con técnicas de separación cromatográfica o electroseparación. Además, las fibras son desechables, con lo que se evitan problemas de contaminación cruzada, y su pequeño tamaño de poro impide la extracción de moléculas de elevado peso molecular y partículas. Esta técnica permite procesar simultáneamente varias muestras, no requiere instrumentación específica y es válida tanto para especies neutras como iónicas. Recientemente, la modalidad de dos fases se ha automatizado completamente y combinado con la cromatografía de gases [82].

Su principal desventaja es que los tiempos de extracción pueden llegar a ser bastante largos. Una posible solución para la aceleración de la extracción puede ser la aplicación de un potencial eléctrico a través de la membrana [83].

3.2.1.3.2. Extracción con disolventes asistida por membranas

La primera aplicación de la extracción con disolventes asistida por membranas (membrane-assisted solvent extraction, MASE) fue publicada por Hauser y Popp en el año 2001 [84]. La técnica es análoga a HF-LPME pero utiliza una membrana hidrofóbica no porosa como el polietileno de baja densidad [84] o el polipropileno de alta densidad [85,86] y puede, además, alojar volúmenes de disolvente mayores (hasta 1 mL) [87]. El sistema involucra tres fases: muestra acuosa-membrana-disolvente orgánico. La membrana actúa como barrera a través de la cual deben difundir los analitos, por lo que se trata de procesos altamente selectivos, especialmente indicados para matrices complejas con un alto contenido de materia orgánica. Existe un dispositivo comercial patentado por Gerstel (Mühlheim, Alemania) que permite la automatización del proceso [86,88,89], lo que supone una ventaja adicional frente a la LPME. La membrana se une a una pieza metálica en forma de embudo a través de un anillo de teflón y se sumerge en la muestra contenida en un vial [88].

La principal limitación de la técnica es que no es adecuada para la extracción de analitos altamente hidrofílicos, muy solubles en la muestra acuosa y con baja capacidad de difusión a través de la membrana. Además, el proceso puede presentar cinéticas aún más lentas que las correspondientes a HF-LPME, por lo que, a veces, es recomendable el uso de temperaturas superiores a la ambiente para reducir el tiempo de extracción [87]. El coste de las membranas no es despreciable y, en consecuencia, suelen reutilizarse. Esto conlleva posibles riesgos de contaminación cruzada y un consumo de disolventes adicional para el lavado, al que hay que sumar al necesario para acondicionar las membranas.

Esta técnica se ha aplicado para la extracción de algunos organofosforados trisustituidos de muestras de agua residual [87]. Bajo condiciones óptimas, 100 mL de muestra con un 30% de sal se extrajeron durante 3 h a una temperatura de 60 °C. La combinación de esta técnica con LC-espectrometría de masas en tándem con ionización por electronebulización (ESI-MS/MS) proporcionó unos límites de cuantificación análogos a los obtenidos con GC-detector de nitrógeno fósforo (nitrogen-phosphorus detector, NPD), excepto para los analitos más polares. La eficacia de extracción del triéster más polar considerado en esta Tesis, TCEP, no superó el 5%. Por

otro lado, gracias a la selectividad de MASE, no se observaron problemas de supresión de señal ESI.

3.2.2. Técnicas basadas en la microextracción en fase sólida

3.2.2.1. Microextracción en fase sólida

La microextracción en fase sólida (solid-phase microextraction, SPME) es una técnica de preparación de muestra introducida en 1989 por Belardi y Pawliszyn [90]. Se basa en la utilización de una fibra de sílice fundida recubierta de una fase estacionaria de naturaleza polimérica que se expone durante un tiempo controlado a la muestra, usualmente hasta que se establece el equilibrio. Los analitos se concentran en el recubrimiento de la fibra y, posteriormente, se desorben térmicamente en el inyector de un cromatógrafo de gases o por disolución en la fase móvil, en el caso de acoplamiento con la cromatografía de líquidos.

Existe un dispositivo comercial, distribuido por Supelco (Bellefonte, PA, USA), para llevar a cabo la extracción. Consta de una fibra de sílice fundida, químicamente inerte y estable frente a altas temperaturas, recubierta de un material polimérico que puede ser un líquido de alto peso molecular, un adsorbente sólido o una combinación de ambos. La fibra recubierta se sitúa en el interior de una aguja de acero inoxidable que protege el recubrimiento de la fibra mientras no se utiliza y cuando atraviesa el séptum del inyector del cromatógrafo de gases o del vial que contenga la muestra. La aguja encaja en un sistema tipo jeringa, que sirve para llevar a cabo el muestreo y la introducción de la fibra en el cromatógrafo.

En la etapa de extracción la fibra se expone a la muestra contenida en un vial cerrado durante un intervalo de tiempo controlado tras el cual la fibra se retrae. Existen tres modalidades de extracción posibles: extracción directa o inmersión, extracción en espacio de cabeza (headspace SPME, HS-SPME) o extracción a través de membrana [5].

La eficacia de extracción se ve afectada por varios parámetros, entre los que cabe destacar: tipo de fibra y espesor del recubrimiento, tiempo de extracción, temperatura, fuerza iónica, pH de la muestra, volumen de muestra, agitación de la muestra y volumen del espacio de cabeza (en HS-SPME).

La SPME es una técnica sencilla que elimina la necesidad de utilizar disolventes orgánicos, con todas las ventajas que ello conlleva, tiene un alto poder de concentración, no requiere grandes volúmenes de muestra y reduce la manipulación de la misma notablemente. La fibra es reutilizable y existen disponibles comercialmente interfases que su acoplamiento con la cromatografía de líquidos. También se dispone de sistemas automuestreadores para GC, capaces de manipular un soporte de SPME y de realizar las distintas etapas implicadas en el proceso de extracción. Como desventajas principales se pueden comentar la fragilidad de la fibra, la posibilidad de efecto memoria y la necesidad de tiempos de extracción largos en algunas aplicaciones.

3.2.2.2. Extracción con barras agitadoras

La extracción en fase sólida con barras agitadoras (stir-bar sorptive extraction, SBSE) tiene el mismo fundamento que la SPME. La principal diferencia entre ambas técnicas es el diseño del sistema de extracción y la cantidad de adsorbente utilizado. Los materiales adsorbentes utilizados son similares pero su disponibilidad comercial es más limitada [91].

La barra agitadora está recubierta normalmente con PDMS y, dado que su superficie es superior a la de la fibra de SPME, la cantidad de recubrimiento es superior y, en consecuencia, la eficacia de extracción es también mayor que la de la SPME. La muestra líquida se agita con la barra durante un cierto tiempo, tras el cual ésta se retira y se desorbe térmicamente o con un disolvente. A diferencia de SPME, SBSE requiere una interfase especial para la desorción térmica, que además es un proceso relativamente lento debido a la mayor cantidad de recubrimiento. Los tiempos de desorción típicos son de 10 minutos, por lo que los analitos desorbidos deben reenfocarse antes de su separación mediante cromatografía de gases. Esto se lleva a cabo habitualmente con un sistema de temperatura programable con enfriamiento criogénico. Por otro lado, a diferencia de SPME, con SBSE normalmente se alcanzan recuperaciones cuantitativas.

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4. Técnicas de extracción aplicadas a compuestos organofosforados

En este apartado se presentan de forma resumida diferentes metodologías de preparación de muestra para la determinación de OPs en muestras sólidas y acuosas, que son las que han sido abordadas en la presente Tesis Doctoral.

4.1. Muestras sólidas

En la **Tabla II.12** se recogen las condiciones empleadas en algunos trabajos publicados para la determinación de OPs en diferentes matrices sólidas, a saber: polvo, sedimentos y lodos.

Tabla II.12. Métodos para la determinación de OPs en muestras sólidas.

| Técnica extracción | Disolvente | Purificación | Detección | R % | Ref. |
|--------------------|-------------------------------------|-------------------------------|-----------------------|-----------|------|
| Polvo | | | | | |
| US | DCM (2 x 25 mL) | - | GC-NPD | 97 (TPeP) | [1] |
| Soxhlet | Hexano/acetona, 8/2 (N.D.) | - | GC-EI-MS | N.D. | [2] |
| PLE | AcOEt (ca. 8 mL) | Alúmina (en línea) | GC-NPD y GC-PCI-MS/MS | 67-110 | [3] |
| Sedimentos | | | | | |
| US | AcOEt/ACN, 30/70 (2 x 30 mL) | - | LC-ESI-MS/MS | 74-104 | [4] |
| US | Acetona (100 mL)+ MeOH (100mL) | LLE (DCM) | GC-FPD | N.D. | [5] |
| Agitación | Acetona (2 x 100 mL) | LLE (DCM)+ SPE (Florisil) | GC-FPD | 78-95 | [6] |
| Soxhlet | MeOH/H ₂ O, 2/1 (150 mL) | SPE (C18) | GC-NPD | 110 (TBP) | [7] |
| Soxhlet | AcOEt (175 mL) | SPE (Florisil) | GC-FPD y GC-EI-MS | N.D. | [8] |
| Lodos | | | | | |
| Soxhlet | AcOEt (N.D.) | SPE (sílica)+GPC | GC-EI-MS | 110 | [9] |
| PLE | AcOEt (50 mL) | GPC+SPE (sílica) | GC-EI-MS | 93-117 | [10] |
| PLE | AcOEt (N.D.) | SPE (sílica)+GPC+SPE (sílica) | GC-EI-MS | 52-96 | [11] |

N.D., no disponible; EI (electron impact), impacto electrónico; PCI (positive chemical ionization), ionización química positiva; FPD (flame photometric detector), detector fotométrico de llama; DCM, diclorometano; AcOEt, acetato de etilo; ACN, acetonitrilo; MeOH, metanol; GPC (gel permeation chromatography), cromatografía de permeación en gel.

Normalmente, los OPs se extraen de matrices sólidas utilizando disolventes de polaridad intermedia (tales como el diclorometano, la acetona o el acetato de etilo). Por su parte, las técnicas más utilizadas son Soxhlet, PLE y la extracción asistida por ultrasonidos. La eficacia del

proceso se mejora utilizando temperaturas y presiones elevadas o varios ciclos de extracción. Estas condiciones favorecen además de la recuperación de los OPs, la coextracción de especies interferentes, de ahí la conveniencia de realizar una purificación de los extractos previamente a su análisis [12].

Como puede verse en la **Tabla II.12**, aunque hay trabajos en los que la utilización de técnicas de detección selectiva (GC-NPD, GC-MS en modo SIM (single ion monitoring, monitorización de un solo ión) o LC-MS/MS) ha permitido el análisis de extractos de complejidad media sin purificación adicional [1,2,4], la inclusión de esta etapa es recomendable para prolongar la vida útil de la columna analítica. En el caso de lodo liofilizado, que puede contener en torno a un 40% de carbono orgánico [13], la limpieza de los extractos es esencial. De hecho, los trabajos publicados para el análisis de OPs en lodo incluyen un protocolo de purificación bastante elaborado, combinando la utilización de SPE en fase normal y GPC [9-11].

El polvo es una matriz compleja, caracterizada por un alto contenido orgánico [14] debido a la presencia de tejidos, pelo, ácaros, etc. [15]. Los sedimentos, dependiendo de su origen, también pueden llegar a tener un contenido en carbono elevado. En consecuencia, la complejidad de los extractos obtenidos para ambas matrices hace necesaria, igualmente, la introducción de una etapa de limpieza. La dilución con agua de los extractos orgánicos resultantes de la extracción de sedimento para someterlos a una extracción líquido-líquido con diclorometano [5,6,16] o a una SPE [7] se ha utilizado como protocolo de limpieza para disminuir la complejidad de los extractos. En algunos de estos casos, el extracto resultante de la LLE se sometió a una purificación adicional mediante SPE [6,16]. Esta estrategia de purificación es bastante compleja, lenta y conlleva un elevado consumo de disolventes orgánicos, que debe sumarse al empleado en la etapa de extracción.

Como parte de la presente Tesis Doctoral se han planteado varias metodologías para la determinación de OPs en dos matrices sólidas, concretamente: polvo y sedimentos. En la **Tabla II.13** se resumen algunos aspectos relevantes de los métodos desarrollados. Para el análisis de polvo se propusieron dos metodologías: una basada en la extracción asistida por microondas con un sistema multimodo [17] y la otra en la dispersión de matriz en fase sólida [18]. La extracción presurizada con disoluciones hidroorgánicas [19] y la extracción asistida por microondas en un sistema de enfoque [20] fueron las técnicas propuestas para la extracción de los analitos de interés en muestras de sedimento.

Tabla II.13. Métodos desarrollados en esta Tesis Doctoral para el análisis de OPs en polvo y sedimentos.

| Técnica extracción | Disolvente | Limpieza | Detección | R % | Ref. |
|--------------------|---|--------------------------------------|-----------|--------|------|
| Polvo | | | | | |
| MAE | Acetona (10 mL) | SPE (Oasis HLB+sílica) | GC-NPD | 85-104 | [17] |
| MSPD | Acetona (3 mL) | Hexano (2 mL), alúmina (en línea) | GC-NPD | 80-116 | [18] |
| Sedimentos | | | | | |
| MAE | Acetona (4 mL)+ ACN (4 mL) | SPE (sílica) | GC-ICP-MS | 78-105 | [20] |
| PLE | H ₂ O/ACN, 75/25 (ca. 16 mL) | SPE (Oasis HLB) | GC-EI-MS | 77-111 | [19] |

ICP (inductively coupled plasma), plasma de acoplamiento inducido; ACN, acetonitrilo.

Una forma de reducir el número de interferentes en los extractos es optar por condiciones de extracción suaves, que minimicen la coextracción de interferencias [3,18]. En esta línea, en uno de los trabajos incluidos en esta Tesis Doctoral para la determinación de los organofosforados en polvo se propuso la MSPD como técnica de extracción, que permitió además, llevar a cabo la purificación de los extractos simultáneamente a la extracción, en una única etapa [18]. En el procedimiento desarrollado, las especies más lipofílicas se eliminan en un lavado con hexano y las más polares se retienen en una capa de alúmina situada bajo la muestra dispersada con Florisil y sulfato sódico anhidro. Análogamente, Quintana y col. [3] propusieron un método para la extracción de OPs en esa misma matriz, basado en la extracción presurizada con acetato de etilo, en el que la temperatura de extracción utilizada fue de 50 °C y la purificación se llevó a cabo en línea introduciendo en la celda de extracción 0.5 g de alúmina.

En base a la información proporcionada en este apartado, en lo que respecta a la determinación de OPs en muestras sólidas, los esfuerzos han de centrarse en la reducción del consumo de disolventes y en la simplificación de los protocolos. En especial, se necesita trabajar más en el desarrollo de métodos para la determinación de OPs en lodos ya que los disponibles son bastante largos y utilizan volúmenes elevados de disolventes; además, GPC es una técnica costosa y poco habitual en la mayoría de los laboratorios. Sin embargo, esta no es una tarea fácil ya que la complejidad de la matriz dificulta enormemente el trabajo.

4.2. Muestras acuosas

LLE y SPE son las técnicas que más se han empleado para la extracción de organofosforados de muestras líquidas, **Tabla II.14**. En LLE se utilizan típicamente volúmenes de muestra entre 0.5 y 2 L y diclorometano [4,5,10,21] o tolueno [22,23] como extractantes, aunque también hay algún trabajo en el que se ha utilizado hexano y éter dietílico [24].

LLE permite procesar muestras filtradas y sin filtrar, sin embargo la recuperación de OPs polares como el TCEP es baja (alrededor de un 39%) [22,23]. Además, LLE tiene numerosas desventajas por lo que la SPE se prefiere como técnica de extracción.

La SPE requiere la utilización de muestras filtradas para evitar la obturación de los cartuchos. Sin embargo, la filtración puede conllevar la pérdida de los OPs más lipofílicos, asociados a la materia particulada que queda adherida a los filtros. Marklund y col. [10,21] resolvieron este problema pasando la primera fracción de disolvente a través del filtro para recuperar los compuestos más hidrofóbicos (TEHP, por ejemplo), que después se utilizó para la LLE. En SPE se obtienen, en general, recuperaciones bajas para el más lipofílico de los OPs, el TEHP [29,32,34], debido a problemas de adsorción sobre el material de vidrio utilizado y por asociación a la materia orgánica disuelta. Ni la adición de un 1% de metanol a la muestra ni el lavado del material de vidrio y las conexiones de SPE con 25 mL de una mezcla metanol:agua (25:75) mejoraron significativamente las recuperaciones del TEHP [32]. Sin embargo, Rodil y col. minimizaron el problema lavando los recipientes que contenían la muestra y las conexiones de SPE con metanol puro, que después se utilizó para la elución de los cartuchos [29], o bien se combinó con el eluato de la SPE [35], alcanzando recuperaciones entre 50 y 84% en la determinación de TEHP en muestras de agua residual.

Para la extracción de OPs de muestras acuosas mediante SPE se han utilizado diferentes adsorbentes {C18 [28], polímeros del divinilbenceno (Bakerbond Speedisk Hydrophilic DVB polymer [25,30,31], Oasis HLB [29,32,36,37], Bond Elut PPL [26,27,38])} en distintas presentaciones (discos, cartuchos). La mayoría de ellos proporcionan recuperaciones satisfactorias para los OPs seleccionados en esta Tesis, tanto para muestras de agua superficial como residual (0.1 y 5 L).

Tabla II.14. Métodos para la determinación de OPs en muestras acuosas utilizando LLE o SPE como técnicas de extracción.

| Técnica | Matriz | Condiciones | R% | Detección | LOQ, ng L ⁻¹ | Ref. |
|-----------------------|--------------------------------------|--|-------------------|------------------------------------|---------------------------------|----------|
| LLE | Agua residual, superficial y potable | V _m : 1 L, Dis.: tolueno (10 mL) | 89-107 (TCEP: 31) | PTV-GC-EI-MS | 5-20 | [22, 23] |
| LLE | Agua residual y nieve | V _m : 2 L, Dis.: DCM (100 + 40 + 25 mL) | 81-100 | GC-NPD | 3-10 | [10,21] |
| LLE | Agua residual y de río | V _m : 0.5-0.8 L, Dis.: DCM (25 mL + 2 x 5 mL) | 63-94 | LC-ESI-MS/MS | 3-8 (río), 4-13 (agua residual) | [4] |
| SPE | Agua residual | Ads.: DVB-hydrophobic Speedisks 45 mm, El.:MTBE y tolueno | 75-90 | PTV-CG-MS | 1-7 | [25] |
| SPE | Agua superficiales y subterráneas | Ads.: Bond Elut PPL 100 mg, V _m : 5 L, El.: ACN/MeOH (1/1) | 83-89 | GC-MS | 1 | [26,27] |
| SPE | Agua potable | Ads.: C18, V _m : 1L, El.: acetona | N.D. | GC-MS (cloroalquil), LC-DAD (TPPO) | N.D. | [28] |
| SPE | Agua residual | Ads.: Oasis HLB 60 mg, V _m : 100 mL, El.: MeOH | 28-90 | LC-ESI-MS/MS | 3-81 | [29] |
| SPE | Agua potable y superficial | Ads.: Bakerbond Speedisk Hydrophilic DVB 200 mg, V _m : 500 mL, El.: MeOH | 20-103 | LC-ESI-MS/MS | 0.3-4 | [30,31] |
| SPE | Agua de río y residual | Ads.: Oasis HLB 60 mg, V _m : 1 L, El.: AcOEt | 24-109 | GC-NPD | 5-10 | [32] |
| SPE (mono-/diésteres) | Agua residual | Ads.: Lichrolut RP-18 500 mg, V _m : 100 mL + 11 mL tampón TrBA, El.: MeOH | 71-112 | LC-ESI-MS/MS | 7-14 | [33] |

V_m, volumen de muestra; Dis., disolvente; Ads., adsorbente; El., disolvente de elución; MTBE, metil tert-butil éter; DCM, diclorometano; ACN, acetonitrilo; MeOH, metanol; AcOEt, acetato de etilo; N.D., no disponible; TrBA, tributilamina; DAD (diode array detector), detector de diodos en serie; PTV (programmed temperature vaporizer), vaporizador con temperatura programada.

Además de LLE y SPE se han propuesto metodologías basadas en técnicas de microextracción. En la **Tabla II.15** se recogen las condiciones de extracción, recuperaciones, LOQs y técnica de detección para estos trabajos. La miniaturización, reducción del consumo de muestra y disolventes y la mejora de la selectividad de la extracción son algunas de las ventajas que ofrecen estas técnicas.

Tabla II.15 Métodos para la determinación de OPs en muestras acuosas utilizando técnicas de microextracción.

| Técnica | Condiciones de extracción | R% | Detección | LOQs, ng L ⁻¹ | Ref. |
|----------|--|--------|--------------|--------------------------|------|
| SPME | PDMS-DVB, inmersión directa, V _m : 22 mL, 300 mg mL ⁻¹ NaCl, tiempo: 40 min | N.D. | GC-NPD | 10-25 | [32] |
| MASE | Dis. (1 mL): ciclohexano, V _m : 100 mL, 300 mg mL ⁻¹ NaCl, tiempo: 3 h | 5-98 | LC-ESI-MS/MS | 1-25 | [39] |
| DLLME | Dis. (1 mL): acetona+TCE (98+2), V _m : 10 mL, 200 mg mL ⁻¹ NaCl | 23-109 | GC-NPD | 10-80 | [40] |
| HF-MMLLE | HF-PP (2 cm), inmersión directa, V _m : 115 mL, 300 mg mL ⁻¹ NaCl, tiempo: 12 h | 2-61 | GC-NPD | 8-120 | [41] |

PDMS-DVB (polydimethylsiloxane-divinylbenzene), polidimetilsiloxano-divinilbenceno; Dis., disolvente; V_m, volumen de muestra; N.D., no disponible; TCE, tricloroetano; HF-PP, fibra hueca de polipropileno

Los trabajos basados en DLLME [40] y MMLLE [41] forman parte de esta Tesis Doctoral y su optimización se aborda pormenorizadamente en los artículos recogidos en el capítulo III.

Rodríguez y col. [32] desarrollaron un método de SPME para la determinación de triésteres en muestras acuosas. Evaluaron varias fibras con polaridades diferentes y las que proporcionaron mejores recuperaciones fueron: PDMS-DVB y divinilbenceno-carboxen-polidimetilsiloxano (DVB-CAR-PDMS). Sin embargo, la fibra triple presentaba problemas de memoria para el TPP, por lo que seleccionaron la de PDMS-DVB. La extracción se lleva a cabo en modo directo durante 40 minutos, a temperatura ambiente, con agitación y con adición de sal (300 mg mL⁻¹) para favorecer la extracción de los OPs más polares. La inmersión de la fibra en la muestra es necesaria para la extracción del TCEP y el TPPO, que no se detectan en espacio de cabeza, ni aumentando la temperatura hasta 100 °C. Con el modo de muestreo directo se obtienen también mejores resultados para TCPP y TDCP; sin embargo, para TiBP, TBP, TPP y TEHP la eficacia de la extracción es superior en la modalidad de espacio de cabeza. En lo que respecta a la fuerza iónica, la adición de sal hasta 300 mg mL⁻¹ produce un aumento de la señal de hasta seis veces para TCEP, TCPP y TPPO; para los demás compuestos, la señal se mantiene prácticamente inalterada (TiBP, TBP y TPPO) o sufre una reducción de hasta un 25% (TDCP, TBEP, TPP). La técnica desarrollada no es adecuada para la determinación de TEHP ya que presenta problemas de repetibilidad y linealidad, achacables a los ya mencionados problemas de adsorción de este compuesto. Los límites de cuantificación obtenidos para los demás OPs se encuentran en el intervalo de 10 a 25 ng L⁻¹, utilizando un volumen de muestra de 22 mL, que son del orden o ligeramente superiores a los obtenidos para muestras de 1 L con SPE: 5-10 ng L⁻¹ [32].

MASE también se ha propuesto como técnica para la extracción de OPs de muestras acuosas [39]. Esta técnica mejora la selectividad de la LLE, previene la formación de emulsiones y consume un volumen de disolvente mucho menor (entre 0.5 y 1 mL). El proceso puede llevarse a cabo en un dispositivo comercial que consiste en una bolsa de polipropileno encajada en un vial a través de un embudo y un anillo de teflón. Quintana y Reemtsma [39] evaluaron la influencia de diferentes variables en el rendimiento de la extracción. Como disolución aceptora se estudiaron varios disolventes: metanol, acetonitrilo, acetona, acetato de etilo, diclorometano y ciclohexano. Los más polares no extrajeron ninguno de los analitos, el acetato de etilo proporcionó recuperaciones bajas y los menos polares fueron los que más eficazmente extrajeron los analitos de interés. Se seleccionó el ciclohexano (1 mL) por su menor toxicidad y por poseer una temperatura de ebullición superior a la del diclorometano, por lo que posibilita el calentamiento de la muestra para mejorar las recuperaciones. La influencia de la temperatura y la fuerza iónica se evaluó conjuntamente mediante un diseño experimental y, al igual que Rodríguez y col. [32], observaron que el aumento de la fuerza iónica ejerce una marcada influencia positiva en la recuperación de los OPs más polares (TCEP, TCP y TPPO). Las condiciones más favorables fueron 60 °C y un 30% (m/v) de NaCl. En las condiciones óptimas de extracción utilizaron 100 mL de muestra, con agitación. Para ese volumen de muestra el equilibrio se alcanza tras 3 horas, lo cual no compromete necesariamente el número de análisis diarios ya que pueden llevarse a cabo varias extracciones simultáneamente. En combinación con LC-MS/MS, los límites de cuantificación del método son análogos a los obtenidos con SPE y GC-NPD [32], excepto para los OPs más polares. Además, debido a la mayor selectividad de MASE, no se observaron problemas de supresión de señal en ESI para las muestras de agua residual. Sin embargo, la eficacia de extracción para el TCEP fue muy baja: 5%. Por otro lado, la manipulación de la muestra ha de hacerse de forma cuidadosa y es una fuente de errores, por lo que se obtienen desviaciones estándar relativamente altas (13-27%) para muestras con un nivel de adición bajo (20 ng L⁻¹).

Ellis y col. [42] aplicaron la SPME a la extracción de tres OPs (TBP, TBEP, TEHP) en muestras de influente con lodo en suspensión (2 mL), previamente digeridas mediante MAE durante 2 min a 100 W. La extracción se llevó a cabo en inmersión a 65 °C durante 40 min, utilizando una fibra de PDMS de 100 µm expuesta a la muestra digerida, a la que añadieron un 10% de NaCl. Los LOQs del método calculados utilizando una muestra de agua bidestilada con adición estaban entre 29 y 45 ng L⁻¹.

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5. Determinación

La separación de los organofosforados trisustituídos puede llevarse a cabo satisfactoriamente tanto mediante cromatografía de gases como de líquidos [1]. Esta última, es la técnica idónea para la separación de los diésteres, cuya derivatización es imprescindible en caso de utilizar la cromatografía de gases [2]. En esta Tesis Doctoral se han utilizado ambas modalidades. El detector de nitrógeno-fósforo, la espectrometría de masas con plasma de acoplamiento inducido y con fuente de impacto electrónico han sido los detectores que se han utilizado en combinación con GC. Así mismo, se ha empleado la cromatografía de líquidos con detección por espectrometría de masas en tándem. A continuación, se explican someramente los principios básicos de funcionamiento de los sistemas de detección utilizados.

5.1. Sistemas de detección. Principios teóricos

5.1.1. Detector de nitrógeno-fósforo

El detector de nitrógeno-fósforo también denominado detector termoiónico, termoiónico alcalino o de ionización en llama alcalina ha evolucionado de los detectores de ionización en llama (flame ionization detector, FID) en los que la llama se dopaba con sales alcalinas [3]. La presencia de estas sales minimiza las ionizaciones de los grupos C-H y aumenta la ionización de los grupos que contienen heteroátomos, en particular nitrógeno y fósforo (aunque también azufre, boro o halógenos). El resultado es una considerable selectividad para los compuestos que contienen esos átomos, lo cual permite su análisis en muestras complejas y ha encontrado grandes aplicaciones en el campo medioambiental.

Los sistemas actuales utilizan como soporte de las sales alcalinas (sales de potasio y rubidio, normalmente) un material cerámico o vidrio montado sobre un cable eléctrico, que se encarga de la calefacción, situado unos milímetros por encima de la boca del *jet* de llama y por debajo del colector. La respuesta del detector está condicionada por el estado del elemento activo, por la composición química del gas que rodea su superficie y por la temperatura de ésta. El aumento de la temperatura del detector, el suministro de potencias elevadas al elemento activo o la utilización de flujos de hidrógeno elevados conllevan un aumento de sensibilidad, a costa de disminuir la vida útil de la perla. Contrariamente, el aumento del flujo de aire o de gas portador, enfrían ligeramente la zona activa y, en consecuencia, disminuye la respuesta del detector.

5.1.2. Espectrometría de masas con plasma de acoplamiento inducido

Un plasma es una mezcla gaseosa neutra conductora de la electricidad, que contiene una fracción significativa de partículas cargadas. Existen varias modalidades para la generación del plasma pero aquí nos centraremos en el plasma de acoplamiento inductivo, que es el que se ha utilizado en este trabajo. Una fuente de ICP típica está constituida por una antorcha de cuarzo, integrada por tres tubos concéntricos a través de los cuales fluye el argón, que se encuentra rodeada por una bobina de inducción, alimentada por un generador de radiofrecuencia. La ionización del argón que fluye se inicia por medio de una chispa que proviene de una bobina Tesla [4].

Las prestaciones analíticas de ICP-MS son función de varias variables tales como la composición del plasma, los flujos de los gases involucrados en su generación, la radiofrecuencia suministrada, el potencial de las lentes de enfoque de los iones o la utilización de una celda de colisión presurizada [5,6].

La detección de los organofosforados incluidos en este estudio se ha llevado a cabo a través de la detección del fósforo. Este elemento es monoisotópico y a su ión característico le corresponde una relación m/z 31.

La adición de pequeños porcentajes de algunos gases (N_2 , He, O_2) al plasma de Ar puede contribuir a mejorar la ionización de elementos con potenciales de ionización altos, como es el caso del fósforo [5-7]. En este tipo de plasmas mixtos la cantidad de gas adicional suele ser del orden de unos cuantos $mL\ min^{-1}$, frente a los $L\ min^{-1}$ de gas plasmógeno. Se ha demostrado que la adición de N_2 mejora la respuesta del fósforo, aunque también conlleva un aumento de la señal de fondo. La formación de iones poliatómicos tales como $^{14}N^{16}O^+H^+$, $^{15}N^{16}O^+$ o $^{12}C^{18}O^+H^+$ es responsable de este incremento. Cuanto mayor sea la señal de fondo, tanto más imprecisas serán las áreas de pico por la superposición con el ruido de fondo. A priori, la utilización de instrumentos equipados con celda de colisión es una forma de reducir esas interferencias. Esto se consigue mediante la presurización de la celda con un gas cuya colisión con los iones de la muestra permite la reducción de interferencias por alguno de los mecanismos siguientes: disociación, reacción (si el gas es reactivo) o discriminación energética. La utilización de plasmas fríos es otra forma de reducir algunas interferencias [8].

5.1.3. Espectrometría de masas con fuente de impacto electrónico

La fuente de impacto electrónico es una fuente dura que produce una elevada fragmentación de la muestra gaseosa, lo cual es útil para la elucidación estructural e identificación de los analitos [4].

Los electrones son emitidos por un filamento caliente de wolframio o renio y se aceleran, mediante un potencial de aproximadamente 70 V que se aplica entre el filamento y el ánodo. Su colisión con las moléculas da lugar a la ionización de las mismas. Los iones positivos producidos, tras su aceleración y enfoque, se transfieren al analizador de masas. En esta Tesis doctoral se ha utilizado un instrumento equipado con una trampa de iones interna, donde los fragmentos iónicos son sometidos a una radiofrecuencia que los estabiliza en órbitas circulares. A medida que se incrementan los potenciales de los electrodos que constituyen la trampa, las trayectorias de los iones se hacen inestables en orden creciente de m/z . Los iones expulsados se detectan con un multiplicador, en el que el impacto de cada fragmento genera una señal eléctrica.

5.1.4. Espectrometría de masas con ionización por electronebulización

La ionización por electronebulización se realiza en condiciones atmosféricas de presión y temperatura [4]. La disolución (fase móvil conteniendo o no la muestra) se bombea a través de un capilar metálico al final del cual se aplica un campo eléctrico (positivo o negativo) elevado,

responsable de la nebulización e ionización de la misma. ESI es una fuente de ionización blanda que origina poca fragmentación de los iones y permite detectar el ión molecular.

El espectrómetro de masas utilizado está equipado con un triple cuadrupolo que permite llevar a cabo la espectrometría de masas en tándem. En el primer y tercer cuadrupolos se aíslan iones y el segundo se utiliza como celda de colisión. Las colisiones entre los iones precursores seleccionados en el primer cuadrupolo con el argón contenido en el segundo producen fragmentaciones que dan lugar a los iones hijos.

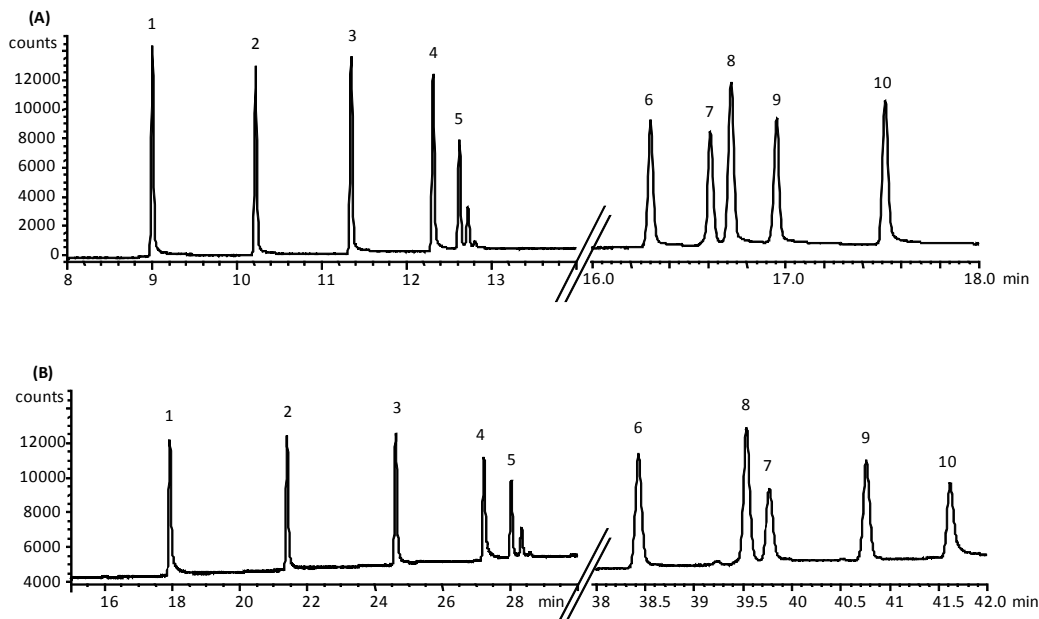
5.2. Determinación de organofosforados

5.2.1. Cromatografía de gases

La técnica cromatográfica más habitualmente utilizada para la separación de los triésteres es la cromatografía de gases, ya que los compuestos son termoestables y suficientemente volátiles. Además, el NPD, por ejemplo, proporciona una selectividad y sensibilidad aceptables y los límites de detección se pueden mejorar con la inyección de grandes volúmenes, puesto que este detector y/o la utilización de un esquema de preparación de muestra adecuado proporcionan la selectividad necesaria, al menos para matrices acuosas. De todos modos, hay que prestar especial atención a la limpieza del *liner* y de la cabeza de columna para minimizar posibles problemas de colas en los picos, especialmente en el caso del TCEP, TBEP y el TPPO [9].

La separación de los compuestos a línea base es posible utilizando una columna capilar del tipo DB-5 (5% fenil-metilpolisiloxano), con la excepción del TBEP y el TPP para los que se obtiene una resolución de aproximadamente 1.4 [10], muy afectada por el estado de la columna, su longitud y la presencia de suciedad en cabeza de la misma. Un aspecto interesante de esta columna es la variación que se produce en el orden de elución del anterior par de compuestos dependiendo de la rampa de temperatura utilizada. Con una rampa de 5 °C min⁻¹ eluye primero el TPP y el TBEP después, sin embargo, con una rampa de 15 °C min⁻¹ se invierte el orden de elución, **Fig. II.10**. Esta información es especialmente útil ya que en muestras en las que los niveles de TBEP no sean muy elevados (esto excluye el polvo, por ejemplo) puede utilizarse la rampa más rápida. Sin embargo, en los casos en los que la concentración de TBEP sea mucho mayor que la de TPP, éste último aparecería en la cola del primero, impidiendo su cuantificación, por lo que sería necesaria la utilización de la rampa más lenta. El uso de una columna más polar como la SPB-1701 (14% cianofenil-dimetilpolisiloxano), de Supleco, proporciona una buena separación de estos dos compuestos pero el TCEP coeluye con el segundo isómero del TCPP, independientemente del programa de temperatura seleccionado [10].

Figura II.10. Cromatograma obtenido mediante GC-NPD para un patrón de triésteres de 100 ng mL^{-1} , en acetato de etilo, utilizando una columna DB-5 ($30\text{m} \times 0.25\text{mm d.i.}$, d.f.: $0.25 \mu\text{m}$) y diferentes rampas de temperatura: (A) $15 \text{ }^\circ\text{C min}^{-1}$, (B) $5 \text{ }^\circ\text{C min}^{-1}$. Picos: 1. TPrP, 2. TiBP, 3. TBP, 4. TCEP, 5. TCPP, 6. TDCP, 7. TBEP, 8. TPP, 9. TEHP, 10. TPPO [10].



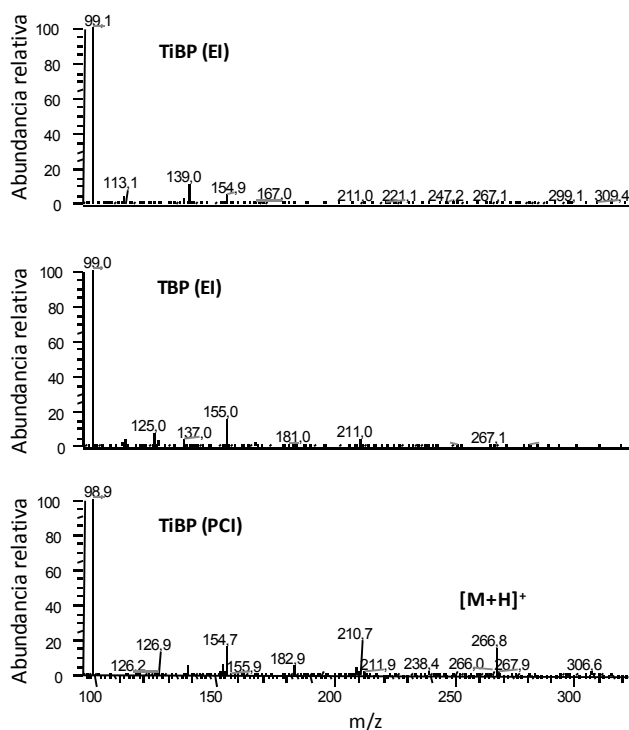
En la mayoría de los casos la detección se lleva a cabo utilizando un detector de nitrógeno-fósforo o la espectrometría de masas con ionización por impacto electrónico [11-14]. GC-EI-MS es una técnica disponible en la mayoría de los laboratorios pero conlleva una elevada fragmentación de los alquil fosfatos, dando lugar a espectros de masas bastante pobres, con un fragmento característico a una relación m/z de 99, correspondiente al ácido fosfórico protonado. Este hecho dificulta su determinación selectiva, sobre todo a bajos niveles, ya que los componentes de matriz normalmente interfieren con iones con relaciones masa-carga bajas. Por esta razón, GC-MS se utiliza normalmente como técnica de confirmación y GC-NPD se emplea de forma rutinaria para la cuantificación de muestras [15-18]. En la **Fig II.11** se muestran los espectros de masas característicos para el TiBP y el TBP, obtenidos mediante impacto electrónico e ionización química positiva.

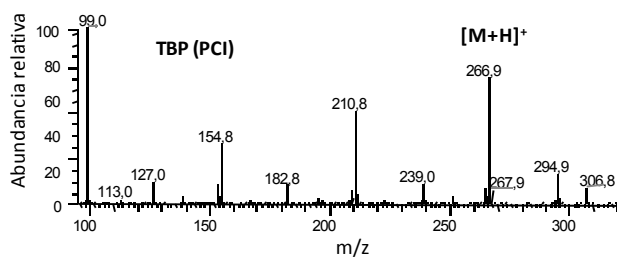
El acoplamiento GC-NPD es más sensible que el GC-EI-MS, presenta una selectividad análoga y es menos costoso, por lo que es bastante común en laboratorios analíticos. La principal desventaja del NPD es su poca estabilidad, ya que el elemento activo de Rb se degrada rápidamente con el uso y debe reemplazarse bastante frecuentemente, lo que encarece la aplicación de esta técnica. Como alternativa al NPD algunos grupos de investigación han utilizado el FPD, que proporciona una selectividad análoga pero es menos sensible.

En algunos casos la complejidad de las muestras medioambientales requiere mayor selectividad que la proporcionada por los detectores antes mencionados. Una forma habitual de aumentar la selectividad es la utilización de la espectrometría de masas en tándem, donde se

selecciona un ión precursor (preferiblemente con m/z alta) y se fragmenta por disociación inducida por colisión (collision induced dissociation, CID). Sin embargo, un problema usual en EI-CID-MS es que la mayoría de los fragmentos producidos tienen masas bajas, como consecuencia de las elevadas energías involucradas en el proceso de ionización. Utilizando una técnica de ionización más suave, como la ionización química positiva, es más probable la supervivencia de los iones más pesados para posibilitar su posterior disociación [19]. La ionización química positiva, utilizando metanol, acetonitrilo [19] o metano [20] como gases reactivos, en combinación con MS/MS se ha utilizado para el análisis de algunos triésteres. En comparación con GC-NPD, GC-PCI-MS proporciona mayor selectividad y la posibilidad de usar estándares marcados isotópicamente con fines cuantitativos. Sin embargo, incluso con PCI, es difícil obtener iones moleculares para alquil fosfatos como el TiBP, **Figura II. 11**, o el TEHP. La estructura ramificada de estos compuestos, al igual que la del TCPP o TDCP, favorece el mecanismo de reorganización de McLafferty por lo que se obtiene, incluso en condiciones de PCI, un elevado grado de fragmentación [20]. Quintana y col. [20] cuantificaron el TEHP en SIM-MS porque incluso en condiciones de PCI, el único ión que se obtuvo a niveles suficientes para su refragmentación en MS/MS fue el 99. La elevada fragmentación del TDCP y el bajo rendimiento con el que obtuvieron masas superiores a la m/z 99 para el TiBP son los responsables de los peores LODs de estos compuestos en comparación con el NPD, **Tabla II.16**.

Figura II. 11. Espectros obtenidos para el TiBP y el TBP mediante impacto electrónico e ionización química positiva.





Otras opciones para la detección selectiva de los triésteres son la utilización del detector fotométrico de llama (FPD) [21-23], la emisión atómica (AED) [24] o de la espectrometría de masas con plasma de acoplamiento inducido (ICP-MS) [25-27]. Con ambas técnicas se gana selectividad, sin embargo, la sensibilidad del AED para el fósforo es baja (alrededor de un orden de magnitud menos que con NPD).

Tabla II.16. Límites de cuantificación instrumentales (ng mL^{-1}) para los triésteres organofosforados usando distintos detectores en combinación con la cromatografía de gases.

| Detección | V_i , μL | TiBP | TBP | TCEP | TCPP | TDCP | TPP | TBEP | TEHP | TPPO | Ref. |
|------------------|-----------------------|------|-----|------|------|------|-----|------|------|------|-------|
| GC-FPD | N.D. | ◇ | 33 | 33 | ◇ | ◇ | 33 | 33 | ◇ | ◇ | [22]* |
| GC-FPD | N.D. | ◇ | 100 | 200 | 200 | 200 | 200 | 300 | 267 | ◇ | [23]* |
| GC-NPD | 1 | 5 | 5 | 10 | 10 | 10 | 5 | 10 | 5 | 5 | [17] |
| GC-ICP-MS | 2 | 5 | 5 | 5 | 10 | 5 | 5 | 5 | 5 | 5 | [27] |
| GC-EI-MS | 1 | 15 | 15 | 15 | 15 | 15 | 15 | 500 | 50 | 500 | [28] |
| LVI-GC-PCI-MS/MS | 10 | 50 | 10 | 10 | 10 | 100 | 8 | ◇ | 50 | 4 | [20] |

V_i , volumen de inyección; *, calculados a partir de LODs; ◇, no incluido en el estudio; N.D., no disponible.

El número de trabajos publicados ocupándose de la determinación de diésteres organofosforados es tremendamente limitado. Estos compuestos son muy polares, de ahí la necesidad de su derivatización previamente a su separación mediante GC: el bromuro de pentafluorobencilo (PFBBR) [2] y diazobutano [28] se han utilizado como derivatizantes. Schindler y col. [2] desarrollaron un método de GC-MS/MS para la determinación de varios diésteres en orina tras la extracción mediante SPE y derivatización con PFBBR obteniendo LOQs entre 0.3 y 3 $\mu\text{g L}^{-1}$.

5.2.2. Cromatografía de líquidos

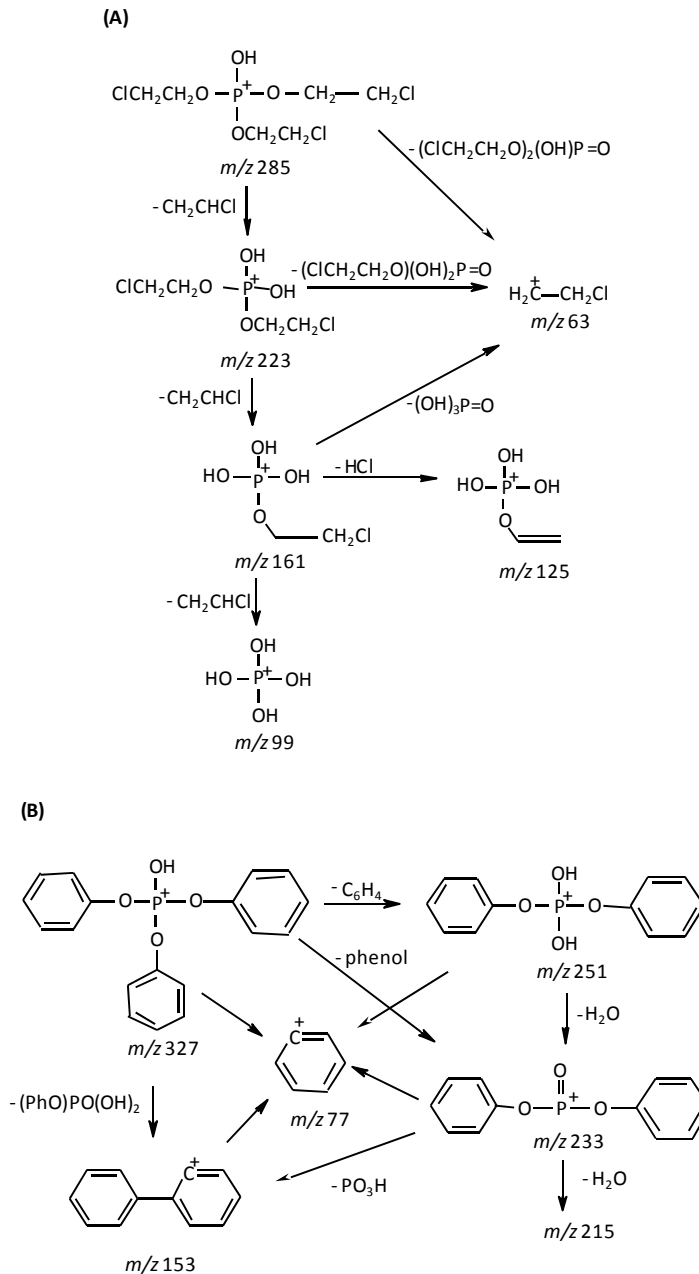
El número de trabajos que aborda la separación de los triésteres mediante LC es mucho más reducido, ya que la mayoría de ellos son susceptibles de separarse mediante GC. De cualquier forma, LC-MS ofrece algunas ventajas a tener en cuenta: (1) la posibilidad de inyección de muestras acuosas y (2) del análisis de compuestos polares y, en particular, (3) una selectividad y sensibilidad en modo MRM (multiple reaction monitoring), con triple cuadrupolo, superior a la

proporcionada por GC-SIM en instrumentos con un cuadrupolo o la de GC-MS/MS en trampas de iones, por la ya comentada dificultad para producir iones moleculares utilizando EI e incluso PCI.

El primer método de LC-MS fue desarrollado en 2003 por Amini y Crescenzi para la determinación de nueve triésteres en muestras de sangre humana [29]. En ese trabajo, la ionización química a presión atmosférica (atmospheric pressure chemical ionization, APCI) en modo positivo proporcionaba mejores resultados que ESI porque se reducían los efectos de matriz. La primera aplicación para la determinación de los triésteres y el TPPO en muestras acuosas mediante LC-MS/MS fue desarrollada por Rodil y col. y publicada en 2005 [30]. Con este procedimiento, utilizando ESI en modo positivo, fue posible el análisis directo de muestras acuosas conteniendo concentraciones en torno a $1 \mu\text{g L}^{-1}$, con lo que se pueden llevar a cabo análisis rápidos de un gran número de muestras de agua residual. Esta sensibilidad es adecuada para los triésteres más comunes en influentes. Utilizando SPE como técnica de concentración se obtienen LOQs del orden de los ng L^{-1} para muestras de 100 mL. Con el mismo enfoque analítico Bacaloni y col. [31,32] extendieron la aplicación del método a aguas superficiales y potables utilizando 500 mL de muestra. Por otro lado, en vez de SPE se puede utilizar MASE como técnica de extracción, que proporciona una mejor selectividad y menos efectos de matriz [33].

Tanto APCI como ESI en modo positivo son eficaces para la ionización de los triésteres. La fragmentación de éstos depende de los sustituyentes. En los triálquil ésteres (con cadenas alquílicas con más de dos átomos de carbono) el mecanismo de disociación está dominado por 3 reorganizaciones de McLafferty consecutivas, caracterizadas por pérdidas neutras de los sustituyentes alifáticos y la formación en última instancia del ácido fosfórico protonado (m/z 99) como fragmento predominante (**Fig. II.12**) [29,30], análogamente a EI-MS. En el caso de algunos triésteres se produce una migración de carga a un grupo alquílico y el ácido fosfórico o sus mono- o diésteres se separan como moléculas neutras y se forma un catión alquílico. Este tipo de fragmentación parece la prominente para ésteres clorados (TCEP) y para los alquil ésteres ramificados (TEHP, TiBP). Para los triaril ésteres el reordenamiento de McLafferty es menos favorable y ocurre sólo una vez. En este caso, el patrón de fragmentación es más complicado y, a parte de los esperados fragmentos resultantes de la pérdida del grupo fenilo, aparecen otras m/z como la 233 y la 153 [29,30], **Fig II.12**. La carga positiva puede migrar a los anillos aromáticos y se detectan tanto el catión fenilo (m/z 77) como el bifenilo (m/z 153) [30].

Figura II.12. Esquema de fragmentación en ESI-MS/MS para: (A) TCEP, (B) TPP [30].

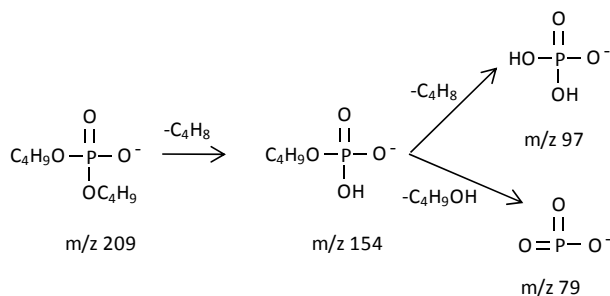


La cromatografía de líquidos es idónea para la determinación de los diésteres, que pueden detectarse como aniones utilizando ESI en modo negativo [34]. La separación cromatográfica involucra normalmente la utilización de agentes formadores de pares iónicos tales como la trietilamina o el tributilamonio [34], para ofrecer una retención adecuada de estos compuestos ácidos sobre columnas fase reversa. Alternativamente, puede utilizarse una columna de carbón activo [35]. Los resultados obtenidos en nuestro trabajo muestran que algunos

diésteres se retienen también sobre columnas tipo C18 sin necesidad de añadir agentes formadores de pares iónicos [36].

Al igual que los trialquil ésteres, los dialquil ésteres sufren también un reordenamiento de McLafferty cuyo resultado es la formación del anión del monoéster. Después se expulsa el alcohol como molécula neutra dando lugar a la formación del anión fosfito (PO_3^- , m/z 79), **Figura II. 13** [34,37]. La fragmentación del DPP está gobernada por la habilidad del anillo aromático de estabilizar la carga negativa, dando lugar al anión fenolato (m/z 93) como ión mayoritario y también se observa el ión con m/z 79 [34].

Figura II.13. Esquema de fragmentación del DBP en ESI-MS/MS [37].



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III. METODOLOGÍA DESARROLLADA

1. Muestras sólidas

1.1. Introducción

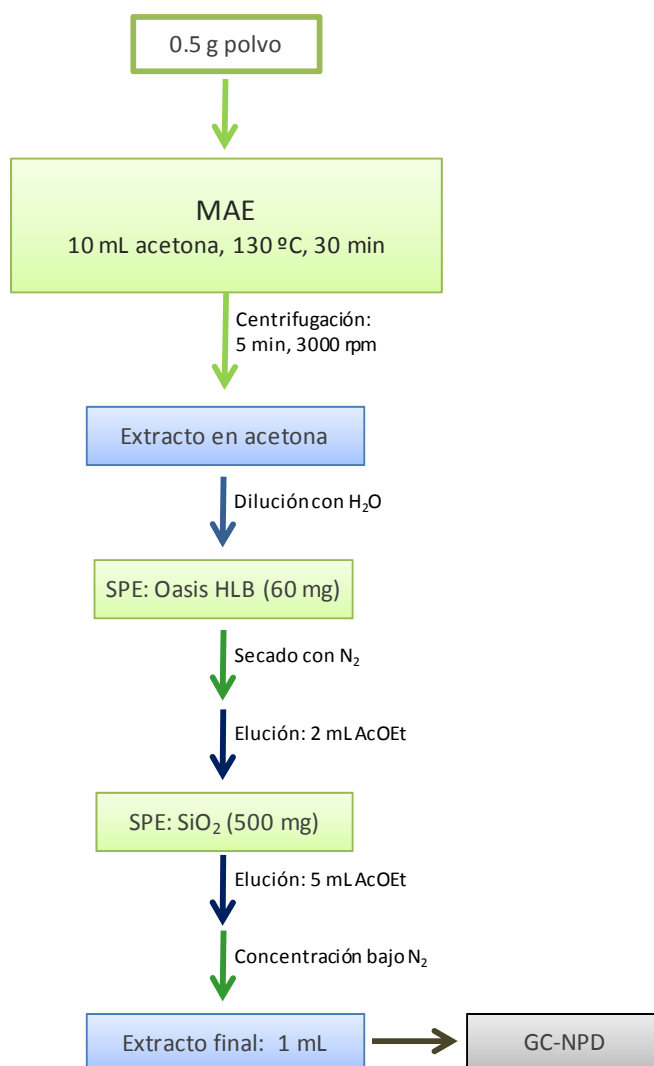
Nuestra vida transcurre mayoritariamente en ambientes interiores por lo que los riesgos para la salud que representan los contaminantes a los que estamos expuestos en ese entorno son un motivo de preocupación. Como se ha comentado en la sección II.1.4.2., las propiedades físico-químicas de algunos OPs favorecen su concentración en polvo (y otras muestras sólidas). Por ello, esta matriz es adecuada para estimar los niveles de estos compuestos en ambientes interiores y conocer el grado de exposición a los mismos.

Por otro lado, la metodología disponible para el análisis de los OPs en el momento en que se decidió abordar el análisis de polvo era tremendamente limitada y conllevaba un consumo elevado de disolventes y/o tiempos de extracción largos (ver **Tabla II.12**), de ahí el interés analítico de este campo. Inicialmente, se desarrolló un método basado en la extracción asistida por microondas y, a posteriori, con el objetivo de simplificar el protocolo de preparación de muestra, integrando extracción y purificación en una única etapa, se evaluó la MSPD como técnica alternativa. En ambos casos, se utilizó la extracción Soxhlet como método de referencia, por la ausencia de materiales de referencia certificados para estos compuestos, y GC-NPD como técnica de separación-detección.

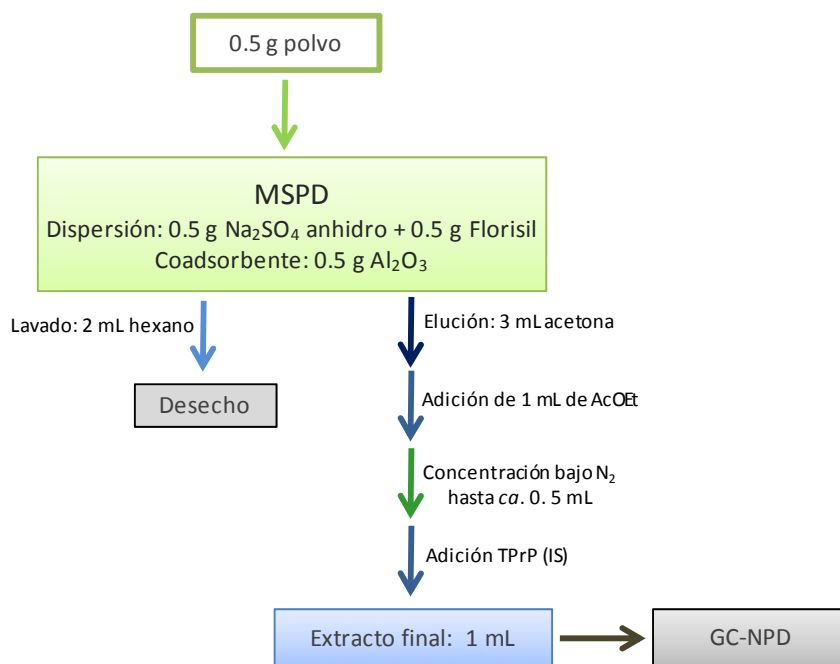
Los sedimentos son otra matriz en la que también se han detectado los OPs, a niveles generalmente bajos, y para los que los métodos de preparación de muestra disponibles suponían el uso de grandes volúmenes de disolventes orgánicos (ver **Tabla II.12**). Con el objetivo de reducir el consumo de disolventes se propusieron dos metodologías, una basada en MAE y otra en PLE con mezclas hidroorgánicas. Además, se pretendió evaluar las posibilidades de la detección mediante ICP-MS cuya selectividad y sensibilidad ya se habían explotado satisfactoriamente para el análisis de muestras complejas como la sangre o aguas residuales, pero sólo para algunos OPs y nunca mediante inyección directa de disolventes, sino siempre en combinación con SPME. En otro de los trabajos, la detección se llevó a cabo mediante GC-EI-MS.

1.2. Esquemas de los métodos desarrollados

Fig. III.1. Esquema para la determinación de OPs en polvo utilizando MAE y GC-NPD.

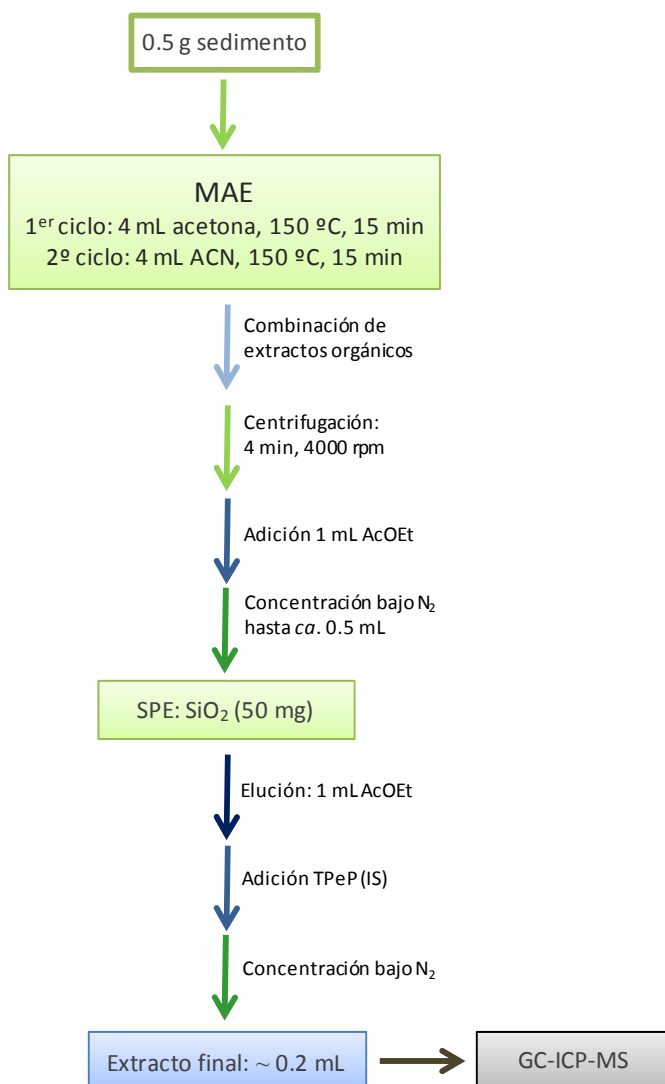


AcOEt, acetato de etilo

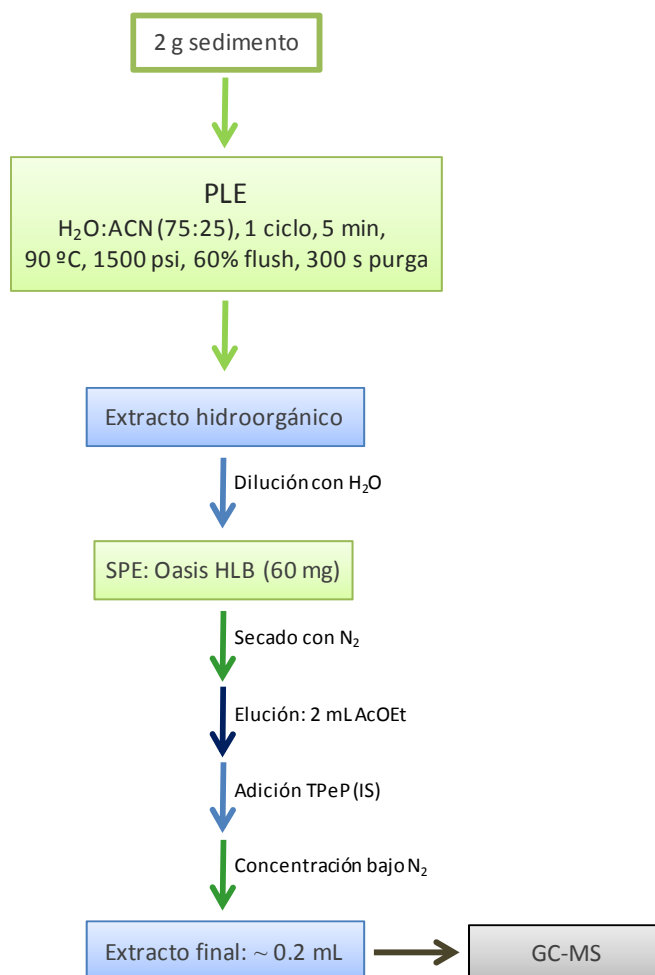
Fig. III.2. Esquema para la determinación de OPs en polvo mediante MSPD y GC-NPD.

IS (internal standard), estándar interno

Fig. III.3. Esquema para la determinación de OPs en sedimentos mediante MAE y GC-ICP-MS.



ACN, acetonitrilo

Fig. III.4. Esquema para la determinación de OPs en sedimentos mediante PLE y GC-MS.

1.3. PUBLICACIÓN:

***MICROWAVE-ASSISTED EXTRACTION OF ORGANOPHOSPHATE FLAME
RETARDANTS AND PLASTICIZERS FROM INDOOR DUST SAMPLES.**

M. García, I. Rodríguez, R. Cela

J. Chromatogr. A 1152 (2007) 280

Microwave-assisted extraction of organophosphate flame retardants and plasticizers from indoor dust samples

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Abstract

A procedure for the determination of eight organophosphate flame retardants and plasticizers in dust samples is presented. Microwave-assisted extraction and gas chromatography (GC) with nitrogen-phosphorus detection (NPD) were used for sample preparation and analytes quantification, respectively. Influence of different variables (type and volume of organic solvent, temperature, time, agitation, etc.) on the yield of the extraction step was evaluated. The most important factor was the type of solvent, with the highest efficiencies corresponding to acetone. Under final conditions 10 mL of this solvent were employed. The extraction was carried out at 130 °C and satisfactory yields, similar to those obtained with the Soxhlet technique, were achieved. Due to the high content of organic carbon in dust samples, primary acetone extracts had to be subjected to intensive clean-up. Dilution with ultrapure water followed by concentration on a reversed-phase sorbent and further purification using silica, allowed a significant reduction of co-extracted interferences. Application of the developed methodology to indoor dust from private houses showed important concentrations of several organophosphate esters. The highest levels, up to 19 µg/g, corresponded to tris(butoxyethyl) phosphate; moreover, average values of two chlorinated compounds, used as flame retardants and considered as the most concerning species in the group, exceeded the 1 µg/g level.

Keywords: Microwave extraction; Organophosphate compounds; Flame retardants; Dust analysis.

1. Introduction

Several esters of phosphoric acid are extensively employed as plasticizers and flame retardant additives in textiles, wallpapers, varnishes and polymeric materials [1,2]. In most cases, these species are not chemically bound to host materials; therefore, they can be easily emitted to the surrounding areas [3]. Other uses of organophosphate compounds (OPs), e.g. as additives in lubricants and hydraulic fluids, might contribute also to their release in the environment [4]. As a result, nowadays, OPs are ubiquitous pollutants in sewage water [5,6] and indoor atmospheres [7-9]. Wastewater contributes to their spread in the aquatic media; whereas, OPs in indoor areas represent a potential risk for human health due to oral ingestion and breathe inhalation. Although the toxicity of OPs is relatively low, at least in comparison to the brominated flame retardants [10,11], their increasing use and some negative effects reported for the chlorinated OPs [12], have increased the concern about possible long term effects associated to a chronic exposure to these species.

In general, the presence of volatile and semi-volatile chemicals in working-centres and private houses has been related to the sick building syndrome and also to some respiratory diseases, such as asthma and allergies [13]. A proper estimation of indoor exposure to OPs requires measuring their concentrations in air and settled dust. OPs in gas-phase, and those associated to airborne transported particles, can be determined after pumping a certain volume of air through a trapping device, which normally consists of a filter followed by a solid sorbent, e.g. a polyurethane foam or just a few mg of a solid-phase extraction polymer [8,9,14]. Although the sampling step is time-consuming, analytes can be easily recovered from the solid sorbent and the filter unit with a few mL of an appropriate organic solvent. Moreover, as the mass of dust and particulate matter retained in the filter is rather low, obtained extracts can be analysed directly, e.g. by gas chromatography-nitrogen-phosphorus detection (GC-NPD), without introducing a clean-up step in the sample preparation scheme [15,16]. Obtained data showed that OPs are associated mainly to airborne particles, reinforcing the interest in the analysis of dust samples [17,18].

Settled dust constitutes an important source of exposure to semi-volatile chemicals in indoor environments, especially in the case of crawling children exhibiting hand-to-mouth behaviour [19]. Conversely to air, dust samples can be easily obtained, e.g. from vacuum cleaner bags; however, the determination of organic compounds in this type of matrix requires intensive sample preparation. Dust can content up to 30% of organic matter, making difficult to achieve quantitative extractions and disturbing the further determination of OPs due to the presence of co-extracted interferences [20]. In fact, opposite to the plethora of procedures reported for air samples, only a few methods have been proposed for the analysis of OPs in dust samples. In most cases, extractions are carried out using the Soxhlet method, which requires the use of high volumes of organic solvents and, therefore, generates large amounts of organic residues [7]. In another work, dust samples were extracted using chlorinated solvents with a considerable toxicity [21].

The aim of this work is to present a sample preparation procedure for the determination of several OPs, used as flame retardants and plasticizers, in bulk dust samples from indoor environments. Microwave-assisted extraction (MAE) was chosen as the sample preparation technique in order to reduce the consumption of organic solvents and to speed up the extraction step. From the best of our knowledge, previous applications of MAE to the determination of OPs in dust samples have not been reported. Only, Ericsson and Colmsjö have described a dynamic MAE method for the elution of several organophosphate esters from glass fibre filters [22]; however, this matrix presents a much lower complexity than bulk dust, particularly considering that only 60 L of air had been previously pumped through the filter unit. A procedure for the clean-up of sample extracts using reversed followed by normal-phase commercial sorbents is also proposed. Finally, analytes were determined by GC with NPD detection.

2. Experimental

2.1. Reagents, materials and samples

HPLC-grade methanol, ethyl acetate, *n*-hexane and acetone for trace analysis were obtained from Merck (Darmstadt, Germany). Tributyl phosphate (TBP), triisobutyl phosphate (TiBP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloro-,1-chloromethylethyl) phosphate (TDCP), tris(butoxyethyl) phosphate (TBEP), tris(2-ethylhexyl) phosphate (TEHP), triphenyl phosphate (TPP) and triphenyl phosphine oxide (TPPO) were acquired from Aldrich (Milwaukee, WI, USA). Tris(chloropropyl) phosphate (TCPP) was kindly provided by Dr. J. B. Quintana (University of La Coruña, Spain). Individual solutions of each compound were prepared in acetone. Diluted standards and mixtures of the nine compounds were made in *n*-hexane and used to prepare spiked dust samples. Calibration solutions were prepared in ethyl acetate. Reversed-phase OASIS HLB (60 mg), and normal-phase silica (500 mg) cartridges were provided by Waters (Milford, MA, USA).

Dust samples were obtained from different houses (mainly flats and terraced houses) located in the northwest of Spain and used as private residences. Sample collection was carried out using conventional vacuum cleaners equipped with paper filter bags. Bags were opened in the laboratory and their content sieved. The fraction with a particle size under 60 μm was employed in this study. Sieved samples were stored at 4 °C using light protected glass vessels. Optimisation of extraction and clean-up conditions was carried out using a pool of samples containing a 23% of total organic carbon (TOC), 3.3% of nitrogen and 0.7% of sulphur. Preliminary extractions of this sample, performed using the Soxhlet method [7], showed the presence of clear and intense chromatographic peaks at retention times of TCPP, TDCP, TPP and TBEP, whereas only weak signals or no peaks were found at retention times of TiBP, TBP, TCEP, TEHP and TPPO; therefore, in order to obtain a homogeneous matrix for optimising the extraction conditions, the pooled sample was fortified with these last five compounds at the 1 $\mu\text{g/g}$ level. The spiking procedure consisted of mixing 25 g fractions of dust with 25 mL of a standard solution of the five OPs in *n*-hexane. This slurry was thoroughly stirred and left in a hood until complete evaporation of the solvent. Then, it was stored in amber glass vessels at 4 °C for at least two weeks before extraction.

2.2. Sample preparation

Samples were extracted using an Ethos Microwave Extraction System (Milestone, Leutkirch, Germany), equipped with 12 pressurized 100 mL volume vessels. Under optimised conditions, 0.5 g of accurately weighed dust were extracted with 10 mL of acetone at 130 °C for 30 min. After that, extraction vessels were cooled down to room temperature, the slurry centrifuged for 5 min at 3000 rpm and the supernatant added to 500 mL of ultrapure (Milli-Q) water in a glass beaker. This solution was passed through a 60 mg OASIS HLB SPE cartridge. Analytes were recovered from the cartridge with 2 mL of ethyl acetate [5]. This solution was loaded on the top of a 500 mg silica cartridge. OPs were eluted from the normal-phase sorbent using 5 mL of ethyl acetate [4,5,12] and the extract was finally evaporated to 1 mL.

2.3. Equipment

OPs were quantified using a HP 5890 series II GC system (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a NPD detector. A DB-5 (5%-phenylmethylpolysiloxane) type column (30 m x 0.32 I.D., d.f.: 0.25 μm), purchased from Agilent (Wilmington, DE, USA), was used for the separation of OPs. Nitrogen (99.999%) was employed as carrier gas at a constant head column pressure of 96 kPa, and also as auxiliary gas in the NPD system (25 mL/min). Synthetic air (99.995%) and hydrogen (99.999%) were used as detector gases at flow rates of 95 and 5 mL/min, respectively. Chromatographic separations were carried out using a heating rate of 7 $^{\circ}\text{C}/\text{min}$ from 50 $^{\circ}\text{C}$ (hold for 1 min) to 260 $^{\circ}\text{C}$ (hold for 8 min). The injected volume was 1 μL , the injector temperature was maintained at 270 $^{\circ}\text{C}$ and the splitless valve opened 1 min after injection. In addition to the GC-NPD, a Varian CP 3900 Gas Chromatograph (Walnut Creek, CA, USA) connected to an ion-trap mass spectrometer (Varian Saturn 2100) was employed to assess the efficiency of sample clean-up and to confirm the identity of some chromatographic peaks. This system was also equipped with a DB-5 type column, with same length and film thickness as those corresponding to the column employed in the GC-NPD instrument, but with an internal diameter of 0.25 mm. Chromatographic conditions were also the same as those used in the GC-NPD system, with the only exception of the carrier gas flow rate (helium) which was maintained at 1.2 mL/min. Electron-impact mass spectra were recorded in the range from 70 to 500 m/z units.

Concentrations of OPs compounds in dust samples were determined by external calibration, comparing the responses of the GC-NPD system for sample extracts and calibration standards prepared in ethyl acetate.

3. Results and discussion

3.1. Chromatographic analysis

Using DB-5 type capillary columns, TPP and TBEP present very close retention times; moreover, their elution order depends on the column-heating rate. Most authors employ gradients around 15-20 $^{\circ}\text{C}/\text{min}$. Under these conditions TBEP elutes before than TPP and separations can be completed in less than 20 min [5,22]. However, when sample extracts contain much higher levels of TBEP than of TPP, the second specie is difficult to quantify since it appears buried in the tail of the TBEP peak, at least using NPD detection [5]. As TBEP concentrations as high as 200 $\mu\text{g}/\text{g}$ have been reported in some dust samples [21], chromatographic conditions were adjusted to reverse the elution order of TBEP and TPP. This was achieved using a heating rate of 7 $^{\circ}\text{C}/\text{min}$, from 50 to 260 $^{\circ}\text{C}$. Using this temperature program, OPs compounds were baseline separated in approximately 35 min, figure not shown. In the case of TCPP three peaks, with identical MS spectra, were obtained. Only the most intense one was considered for quantitative purposes. Quantification limits of the GC-NPD system ranged from 0.005 $\mu\text{g}/\text{mL}$ (TiBP, TBP, TPP, TEHP and TPPO) to 0.010 $\mu\text{g}/\text{mL}$ (TCEP, TCPP, TDCP and TBEP). Repeatability of the injection for standards at different concentration levels (0.05 and 0.4 $\mu\text{g}/\text{mL}$) remained between 2 and 4%. The linearity in the response of the NPD detector was evaluated using standards, prepared in ethyl acetate, at seven different levels from 0.010 to 5.0 $\mu\text{g}/\text{mL}$.

Correlation coefficients from 0.995 to 0.998, depending on the compound, were obtained within this interval of concentrations. At higher concentrations, a significant decrease in the slope of the calibration curves was observed. On the other hand, the GC-MS system showed quantification limits around 0.015 µg/mL for TiBP, TBP, TCEP, TCPP, TDCP and TPP, 0.05 µg/mL for TEHP and values higher than 0.5 µg/mL for TBEP and TPPO. The poor sensitivity achieved for the last two species is probably related to their excessive fragmentation in the electron impact ionisation mode [2]. Therefore, GC-NPD was selected as the quantification technique and GC-MS considered only for qualitative purposes.

3.2. Extraction and clean-up conditions

3.2.1. Preliminary extraction experiments and clean-up strategy

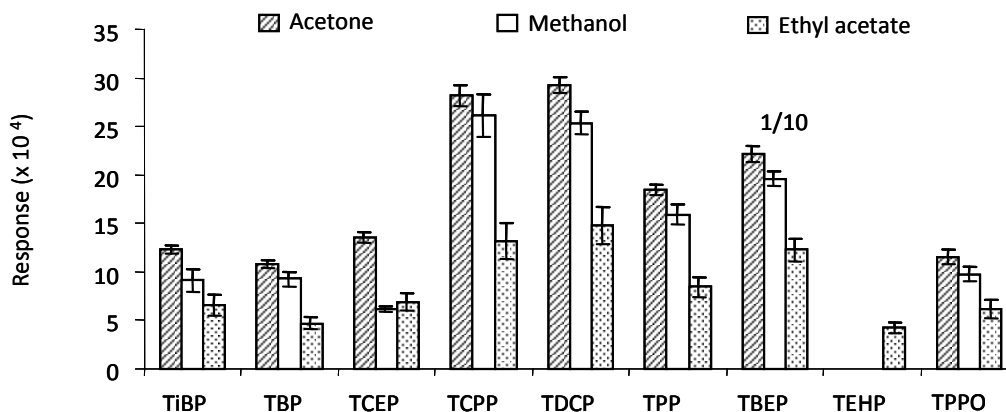
The choice of an appropriate solvent is a key factor controlling the yield and selectivity in the extraction of organic species from complex solid samples. In addition, the properties of the chosen solvent should be taken into account to design the further clean-up of primary extracts. Ethyl acetate, acetone and methanol, were initially considered for the extraction of OPs from dust. In previous articles, these solvents had been proposed for the isolation of OPs from dust [7] and other matrices with high levels of organic matter, such as sediments or sludge [4,23,24]. Dichloromethane, which is the most popular solvent for the extraction of OPs from dust [21] and airborne particles trapped on glass fibre filters [17], was not considered in this study because of its higher toxicity. As a starting point, MAE extractions were carried out at 100 °C for 15 minutes using 20 mL of each solvent and 0.5 g of the pooled dust sample. Colourful extracts unsuitable for direct GC analysis were obtained irrespective of the extraction solvent used.

In view of these results two different clean-up strategies were considered. Ethyl acetate extracts were evaporated to 2 mL and purified using a 500 mg silica cartridge. Analytes were recovered from the normal-phase sorbent with 5 mL of the same solvent [4,5,12] and concentrated to a final volume of 1 mL. The clean-up scheme applied to methanol and acetone extracts involved the sequential use of reversed and normal-phase sorbents. Primary extracts, in methanol or acetone, were diluted with ultrapure water, concentrated using an OASIS HLB cartridge and analytes recovered with ethyl acetate. These extracts were additionally purified over silica as described above. In fact, this is a rather common clean-up approach when water-soluble solvents are employed in the extraction of organic compounds from complex matrices, e.g. sludge [25]. The suitability of the OASIS HLB sorbent for the concentration of OPs from water samples had been already evaluated by our group in a previous paper [5]. The sorbent provided quantitative recoveries, except in case of TEHP, breakthrough volumes higher than 2 L and only 2 mL of ethyl acetate were necessary in the elution step. However, replacing the water sample by a mixture of methanol-water or acetone-water might affect to the retention capability of the sorbent; therefore, the breakthrough volume of the cartridge needed to be re-evaluated. With this aim, 200 mL aliquots of ultrapure water, spiked with the analytes at the 0.005 µg/mL level, and containing increased percentages of methanol or acetone, from 5 to 15%, were extracted using two OASIS cartridges sequentially connected. After the concentration step, cartridges were disconnected, dried using a stream of nitrogen and eluted separately. In the case of methanol, OPs were always retained quantitatively in the first cartridge; however, a dramatic reduction in

the breakthrough of the sorbent was observed when acetone was added to water samples. Even considering only a 5% of acetone (10 mL) in the 200 mL aqueous solution, significant amounts of the most polar OPs: TCEP (log Kow, 1.7) and TPPO (log Kow, 2.9), passed to the second cartridge. In order to improve OPs retention, without reducing the volume of acetone under 10 mL, the water volume was increased from 200 to 500 mL. In these conditions, maintaining the percentage of acetone equal or lower than 2%, all OPs were quantitatively retained in the first cartridge (more than 99%). Considering these results, methanol extracts from the pooled dust sample were diluted to 200 mL with water and concentrated using an OASIS cartridge. The 20 mL acetone extracts were first evaporated to 10 mL, diluted to 500 mL with ultrapure water and then subjected to the same process. In both cases, ethyl acetate extracts from the reversed-phase sorbent were further purified using silica.

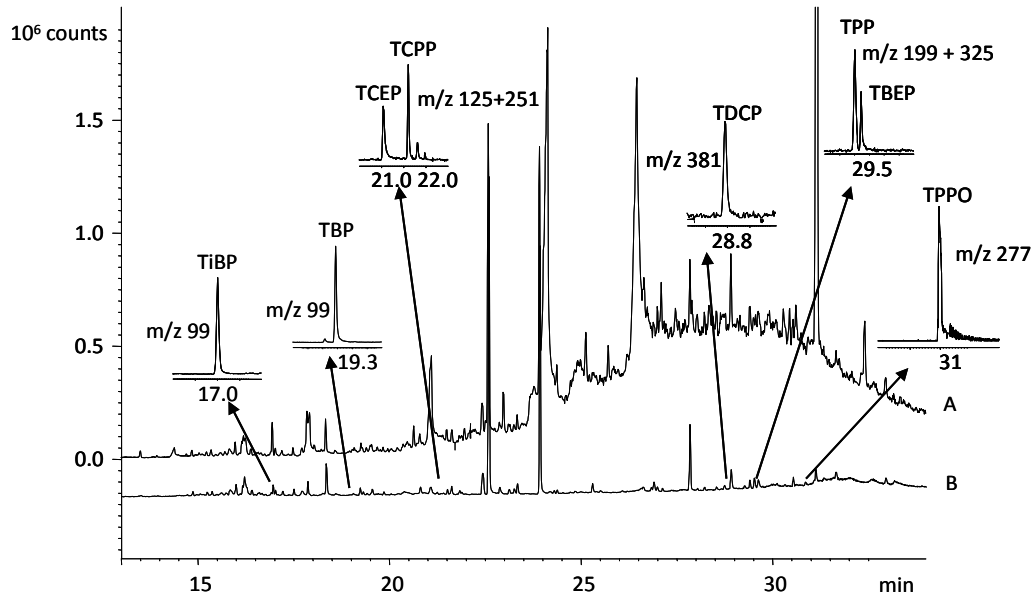
Fig. 1 compares the obtained responses for each OP compound using ethyl acetate, methanol and acetone as extraction solvents. Significantly higher signals were obtained for acetone and methanol than in the case of ethyl acetate, except for TEHP. The impossibility of recovering this compound from complex aqueous samples using reversed-phase sorbents has been explained in detail elsewhere [5].

Fig. 1. Influence of the type of solvent on the yield of OPs extracted from the pooled dust (0.5 g of sample intake). MAE at 100 °C for 15 min using 20 mL of each solvent. GC-NPD detection. Mean values with their standard deviations, $n = 3$ replicates.



Regarding the selectivity of the extraction, injection of dust extracts in the GC-MS system revealed the presence of an important hump in the chromatogram when using ethyl acetate as extraction solvent, whereas, stable baselines were obtained with methanol and acetone (**Fig. 2**). Although, apparently this hump did not disturb the NPD detection of target OPs, it might shorten the useful life of the GC chromatographic column. Thus, acetone and methanol were selected for further optimisation experiments and the sequential use of reversed and normal-phase sorbents was adopted as purification strategy. Obviously, TEHP was excluded from the group of target species since it was lost during the clean-up step.

Fig. 2. GC-MS total ion current chromatograms corresponding to the pooled dust sample using ethyl acetate (A) or acetone (B) as extraction solvents. Chromatographic peaks for OPs recorded using some of the most intense ions in their MS spectra are also plotted. Same extraction conditions as in Fig. 1.



3.2.2. Optimisation of microwave extraction conditions

In a second optimisation stage, the effects of volume, type of organic solvent (methanol or acetone), extraction time and temperature on the efficiency of the microwave process were evaluated simultaneously using a fractional 2^{4-1} experimental design with two central points [26]. This type of design allows estimating the main effect of each factor on the yield of the extraction with a minimum of experiments; however, interactions between factors are confounded. Low and high levels for the considered variables are shown in **Table 1**. After extraction, the slurry was centrifuged and the supernatant recovered with a Pasteur pipette. Extracts in methanol were diluted to 200 mL using ultrapure water and subjected directly to the clean-up procedure described previously. In the case of acetone, when required, the volume of the extract was first reduced to 10 mL and then made up to 500 mL with ultrapure water. Standardized values of main effects obtained after analysing the responses for each analyte in the experiments of the design are also presented in **Table 1**. The absolute value of the main effect for a given compound is proportional to the variation in the efficiency of the extraction for this specie, when the considered factor changes from the low to the high level established in the domain of the design. The sign means if the extraction yield increases (positive sign) or decreases (negative sign). For all compounds, the type of solvent showed a negative effect on the efficiency of the process (**Table 1**) meaning that acetone provides higher extraction yields than methanol. Moreover, this factor was statistically significant (95% confidence level) for TCEP, TPPO and TBP. On the other hand, the effect of extraction time was always positive and statistically significant for TDCP, TPP and TBEP. In general, for most of the compounds, temperature and solvent volume played

positive but non-significant effects. On the basis of these results, acetone was chosen as extraction solvent and temperature was fixed at 130 °C. The influence of the other two factors was studied with more detail considering an univariant approach.

Table 1. Experimental domain and standardized values for main effects of the factors considered in the fractional (2^{4-1}) experimental design.

| Factor | Levels | | Compounds | | | | | | | |
|-------------|---------|----------|-----------|--------------------|--------------------|-------|-------------------|-------------------|-------------------|--------------------|
| | Low | High | TiBP | TBP | TCEP | TCPP | TDCP | TPP | TBEP | TPPO |
| Solvent | Acetone | Methanol | -2.48 | -3.53 ^a | -6.91 ^a | -1.76 | -1.58 | -0.11 | -1.84 | -3.01 ^a |
| Volume (mL) | 10 | 30 | 1.73 | 1.42 | -0.40 | 1.21 | 0.99 | 0.85 | 0.87 | 0.82 |
| Time (min) | 10 | 30 | 2.01 | 1.92 | 1.51 | 1.69 | 2.76 ^a | 2.63 ^a | 3.13 ^a | 2.21 |
| Temp. (°C) | 90 | 130 | -0.05 | 0.09 | 0.20 | 0.26 | 0.64 | 1.03 | 1.90 | 1.15 |

^a Statistically significant factors at the 95% confidence level; Temp., temperature.

Extraction yields achieved using 40 and 10 mL of acetone are compared in **Table 2**. For both volumes, the process was carried out in triplicate at 130 °C for 30 min. The increase in the solvent volume did not result in any noticeable improvement on the extraction yield; therefore, it was maintained at 10 mL. This finding allowed to reduce solvent consumption and, moreover, it avoids the need of evaporating the primary sample extract in acetone, before starting the clean-up procedure.

Table 2. Comparison of extraction efficiencies at 130 °C using two different volumes of acetone.

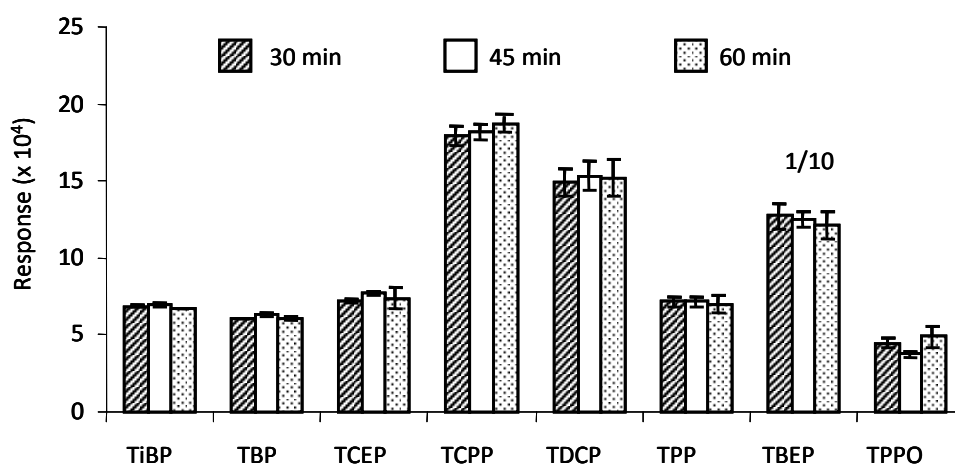
| Compound | 10 mL of acetone | | 40 mL of acetone | |
|----------|------------------|---------|------------------|---------|
| | Mean | RSD (%) | Mean | RSD (%) |
| TiBP | 100 | 4 | 87 | 12 |
| TBP | 100 | 5 | 93 | 10 |
| TCEP | 100 | 10 | 100 | 14 |
| TCPP | 100 | 8 | 97 | 9 |
| TDCP | 100 | 7 | 92 | 11 |
| TPP | 100 | 7 | 93 | 10 |
| TDCP | 100 | 6 | 92 | 10 |
| TPPO | 100 | 11 | 84 | 11 |

Normalised responses to those obtained for 10 mL of solvent, $n = 3$ replicates.

According to the information reported by the experimental design, the factor time had a positive, and in some cases statistically significant, influence on the extraction. Thus, longer periods than those explored with the experimental design were tested. Extending the exposition of the sample slurry to the microwave field from 30 to 60 min, did not increase the efficiency of the process (**Fig. 3**). Therefore, the factor time was fixed at 30 min, the upper level within the domain of the design.

The MAE system employed in this work offers the possibility of stirring the slurry contained in the closed extraction vessels, using Teflon coated stir bars, while they are exposed to the microwave field. Experimentally, it was observed that stirring did not improve the extraction efficiency, data not shown, therefore, this option was not considered. Finally, the effect of adding a few micro-litres (100-200 μL) of formic acid to the extraction solvent (10 mL of acetone) was also evaluated. Theoretically, an acidic medium might help to disrupt the structure of the sample, enhancing the yield of the extraction process. In practise, responses for target analytes remained unaffected; however, the obtained extracts presented a higher chromatographic complexity, probably because organic compounds with acidic properties from the sample were co-extracted in a higher extension in presence of formic acid.

Fig. 3. Comparison of responses obtained for OPs in the pooled dust sample using different extraction times. MAE extractions at 130 $^{\circ}\text{C}$ using 10 mL of acetone. Mean values with their standard deviations, $n = 3$ replicates.



3.3. Performance of the method

Table 3 presents the recoveries of the proposed method for two spiked dust samples. Data were calculated by dividing the difference between concentrations measured for spiked and non-spiked fractions of the same sample by the added amount. Extractions were performed in triplicate, using 0.5 g of dust, and simultaneously placing six vessels in the microwave rotor. The second column in **Table 3** summarises the recoveries obtained for the pooled dust used in the optimisation of extraction and clean-up conditions. This sample had been fortified only with TiBP, TBP, TCEP and TPPO at the 1 $\mu\text{g/g}$ level. Concentrations of these species in the non-spiked fractions of the sample ranged from non-detected, for TPPO, up to 0.4 $\mu\text{g/g}$, for TCEP. **Fig. 4** overlays the GC-NPD chromatograms for spiked and non-spiked fractions of this pooled sample. The fourth column in **Table 3** shows the recoveries for a discrete dust sample spiked with all OPs at levels similar to their original concentrations in this matrix, from less than 0.2-10 $\mu\text{g/g}$. Globally, recoveries ranged from 85% to 104% with relative standard deviations below 11% (**Table 3**).

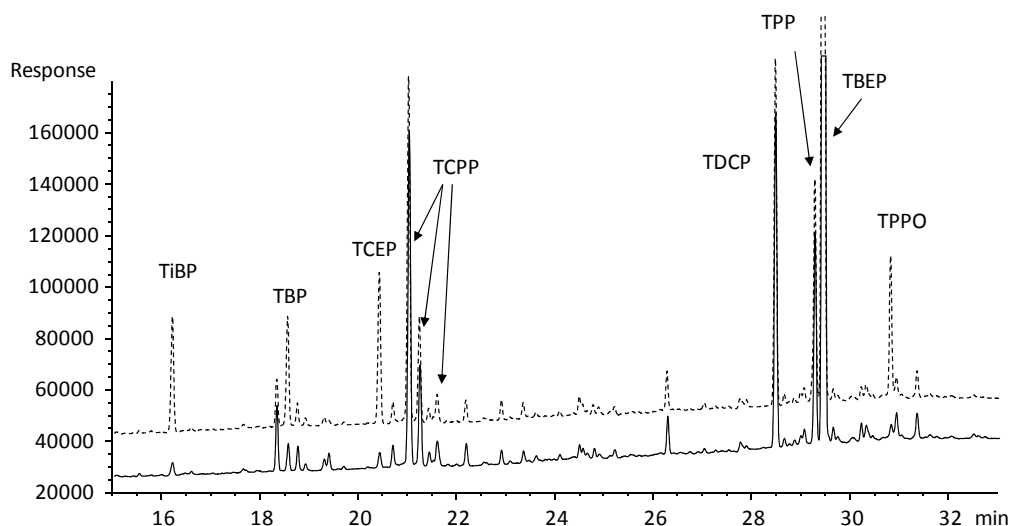
Table 3. Recoveries of the MAE method for spiked dust samples, $n = 4$ replicates.

| | Pooled dust sample ^a | | Pooled dust sample ^b | |
|------|---------------------------------|---------|---------------------------------|---------|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| TiBP | 92 | 13 | 98 | 3 |
| TBP | 98 | 10 | 100 | 5 |
| TCEP | 89 | 7 | 88 | 9 |
| TCPP | -- | -- | 93 | 4 |
| TDCP | -- | -- | 94 | 6 |
| TPP | -- | -- | 104 | 6 |
| TBEP | -- | -- | 97 | 6 |
| TPPO | 91 | 11 | 85 | 11 |

^aSpiked only with TiBP, TBP, TCEP and TPPO at the 1 $\mu\text{g/g}$ level.

^bSpiked with all OPs at different levels (from 0.2 $\mu\text{g/g}$ to 10 $\mu\text{g/g}$).

Fig. 4. GC-NPD chromatograms obtained under optimal extraction conditions for non-spiked (solid line) and aged, spiked (dotted line) fractions of the pooled dust sample. Only TiBP, TBP, TCEP and TPPO were added to the sample.



In addition to the use of spiked samples, accuracy was further assessed by comparing the results obtained for real polluted (non-spiked) dust samples using the proposed method and the Soxhlet technique. Soxhlet extractions were carried out for 15 h using 0.5 g of dust and 75 mL of acetone. The organic phase was evaporated until an approximated final volume of 10 mL and then subjected to the same clean-up procedure as supernatants from microwave extractions. **Table 4** summarizes the results obtained with both techniques for three different dust samples. Quantification of TBEP required a dilution of the final sample extract, since measured concentrations exceeded the linear response range of the NPD detector. In general, an excellent

agreement was found between both sets of average concentrations. Similar precisions were also obtained for both extraction techniques with relative standard deviations around 10%.

Table 4. Comparison of OPs levels obtained for three different non-spiked dust samples using MAE and Soxhlet extraction.

| | Sample 1 | | Sample 2 | | Sample 3 | |
|------|------------------------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|
| | MAE (mean \pm SD) | Soxhlet (mean \pm SD) | MAE (mean \pm SD) | Soxhlet (mean \pm SD) | MAE (mean \pm SD) | Soxhlet (mean \pm SD) |
| TiBP | n.q. | n.q. | n.q. | n.q. | 0.16 \pm 0.01 | 0.15 \pm 0.01 |
| TBP | 0.046 \pm 0.009 | 0.053 \pm 0.006 | 0.162 \pm 0.005 | 0.163 \pm 0.008 | 0.15 \pm 0.02 | 0.14 \pm 0.02 |
| TCEP | 1.5 \pm 0.1 | 1.79 \pm 0.08 | 3.2 \pm 0.3 | 3.4 \pm 0.3 | 0.26 \pm 0.03 | 0.30 \pm 0.03 |
| TCPP | 4.2 \pm 0.4 | 4.0 \pm 0.3 | 3.8 \pm 0.4 | 4.0 \pm 0.3 | 1.8 \pm 0.1 | 1.7 \pm 0.1 |
| TDCP | 4.1 \pm 0.3 | 3.9 \pm 0.3 | 0.107 \pm 0.006 | 0.119 \pm 0.003 | 0.86 \pm 0.05 | 0.84 \pm 0.03 |
| TPP | 1.6 \pm 0.2 | 1.62 \pm 0.09 | 0.57 \pm 0.03 | 0.57 \pm 0.06 | 0.60 \pm 0.04 | 0.55 \pm 0.04 |
| TBEP | 17.5 \pm 0.7 | 16.6 \pm 0.3 | 11.1 \pm 0.9 | 12.3 \pm 1.1 | 11.2 \pm 0.4 | 10.7 \pm 0.8 |
| TPPO | n.q. | n.q. | 0.068 \pm 0.006 | 0.075 \pm 0.004 | n.q. | n.q. |

n.q. under quantification limits: 0.040 $\mu\text{g/g}$ for TiBP, TBP, TCEP and TCPP; 0.050 $\mu\text{g/g}$ for the rest of OPs. Values in $\mu\text{g/g}$, $n = 3$ replicates.

Due to the ubiquitous presence of OPs in indoor environments, blanks of the whole analytical procedure were systematically performed. Considering MAE as the extraction technique contamination problems were never detected in blank extracts; however, important signals were detected for TiBP and TBP in blanks of the Soxhlet procedure. Cellulose thimbles were identified as the contamination source. The problem was solved replacing them by glass thimbles.

Considering a sample intake of 0.5 g, a final extract volume of 1 mL, and those quantification limits given in section 3.1 for the GC-NPD instrument, quantification limits of the overall method should range between 0.01 and 0.02 $\mu\text{g/g}$. In practise, the quantification limits were 0.04 $\mu\text{g/g}$ for TiBP, TBP, TCEP and TCPP; and 0.05 $\mu\text{g/g}$ for the rest of species. These values were determined considering the higher baseline noise of chromatograms for dust extracts when compared with those corresponding to pure standards. Increasing the sample intake and/or decreasing the volume of the final extract, did not contribute to improve the sensitivity of the method since the signal to noise (S/N) ratio of the chromatographic peaks remained constant. Anyhow, the obtained quantification limits are similar to those previously reported for dust and other complex solid samples [7,21,23].

3.4. Application to real samples

The developed method was applied to the analysis of dust samples collected in flats and terraced houses from urban areas. Only one of the samples, code 8 in **Table 5**, corresponded to a detached house in the countryside. Dust was obtained using conventional vacuum cleaners, in most cases equipped with paper filter bags. New bags, from different suppliers, were extracted using the method developed for dust samples. Traces of TiBP and significant amounts of TBP, around 0.5 $\mu\text{g/g}$, were detected in most bags; therefore, migration of these two compounds with the consequent contamination of dust samples during vacuuming cannot be excluded. The rest of the investigated OPs were never detected in the extracts from new vacuum cleaner paper bags (1 g of sample intake). **Table 5** summarises the results obtained for dust samples, fraction below 60 μm .

Table 5. Concentrations ($\mu\text{g/g}$) of OPs in dust samples.

| Sample code | TiBP | TBP | TCEP | TCPP | TDCP | TPP | TBEP | TPPO |
|-------------|------------------|------------------|----------------|----------------|------------------|----------------|----------------|------------------|
| 1 | 0.27 (0.07) | 0.23 (0.04) | 0.58 (0.07) | 10.3 (0.7) | 0.3 (0.1) | 2.6 (0.4) | 11.9 (0.9) | n.q. |
| 2 | 0.22 (0.05) | 0.226 (0.005) | 0.25 (0.03) | 5.5 (0.2) | 0.094 (0.003) | 9.5 (0.5) | 4.2 (0.2) | n.q. |
| 3 | 0.22 (0.06) | 0.15 (0.01) | 0.47 (0.05) | 2.1 (0.3) | 1.1 (0.1) | 0.8 (0.1) | 18.2 (0.8) | n.q. |
| 4 | 0.16 (0.01) | 0.34 (0.03) | 9.8 (0.8) | 5.7 (0.2) | 0.15 (0.01) | 2.2 (0.4) | 16.3 (0.9) | 0.13 (0.02) |
| 5 | 0.27 (0.08) | 0.65 (0.04) | 1.56 (0.03) | 5.6 (0.3) | 0.56 (0.03) | 1.5 (0.1) | 18.5 (1.1) | n.q. |
| 6 | 0.16 (0.02) | 0.09 (0.01) | 0.36 (0.07) | 0.35 (0.07) | n.q. | 3.6 (0.3) | 1.18 (0.09) | n.q. |
| 7 | 0.179 (0.001) | 0.23 (0.01) | 0.54 (0.01) | 0.61 (0.05) | n.q. | 0.34 (0.06) | 6.8 (0.7) | 0.085 (0.003) |
| 8 | n.q. | 0.070 (0.007) | 0.37 (0.02) | 1.10 (0.07) | 0.09 (0.01) | 0.29 (0.01) | 1.93 (0.03) | 0.064 (0.005) |
| Av. Conc. | 0.21 | 0.25 | 1.7 | 3.9 | 0.35 | 2.6 | 9.9 | 0.09 |

Av. Conc., average concentration. Values in parentheses correspond to the standard deviation of three replicates.

Five of the analytes considered in this study: TBP, TCEP, TCPP, TPP and TBEP were found in all processed samples at concentrations above the quantification limits of the proposed method. The average values in four of them were higher than 1 $\mu\text{g/g}$. Measured concentrations for two of the three chlorinated OPs, TCEP and TCPP, were in the same range that the average

levels reported for a large number of dust samples collected in private houses from Germany, using the same sampling method as in this work [7]. Both compounds have been included in the second (TCEP) and fourth (TCPP) European lists of priority pollutants [27,28] and are assumed to be the most toxic OPs flame retardants. Concentrations of TBEP close to 20 $\mu\text{g/g}$ were measured in two of the samples (**Table 5**). Although these values are relatively high, they still remain one order of magnitude lower than those found in public buildings, e.g. hospitals [21].

4. Conclusions

The applicability of MAE for the determination of several OPs used as flame retardants and plasticizers in dust samples has been demonstrated. The extraction efficiency of this technique was similar to that achieved using the Soxhlet method, with the advantage of a reduced consumption of organic solvent and the possibility of processing up to 12 samples simultaneously. Despite the excellent selectivity of the NPD detector, an exhaustive clean-up of primary extracts is recommended to avoid contamination of the chromatographic column. In fact, purification of sample extracts is the longest step of the proposed method; therefore, further developments should be focussed on shortening this stage to increase the sample throughput and also to avoid the loss of TEHP.

Data obtained for non-spiked samples revealed the ubiquitous presence of most of the selected OPs in house dust. Even assuming that values measured for TiBP and TBP might be overestimated due to dust contamination during sample collection, relatively high levels were reported for five of the other six OPs considered in this study. Particularly, the average concentrations of two chlorinated flame retardants (TCEP and TCPP) were higher than 1 $\mu\text{g/g}$, and the minimum level of TBEP exceeded this value. On the other hand, only low or not detected levels of TPPO were found in most of the samples.

Acknowledgements

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1.4. PUBLICACIÓN:

***OPTIMISATION OF A MATRIX SOLID-PHASE DISPERSION METHOD FOR THE DETERMINATION OF ORGANOPHOSPHATE COMPOUNDS IN DUST SAMPLES.**

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Optimisation of a matrix solid-phase dispersion method for the determination of organophosphate compounds in dust samples

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Abstract

A fast and inexpensive sample preparation procedure based on the matrix solid-phase dispersion (MSPD) technique is proposed for the isolation of several organophosphate esters (mainly employed as flame retardants and plasticizers) from indoor dust samples. Extraction and clean-up were carried out in a single step and target compounds were determined by gas chromatography (GC) with nitrogen-phosphorus detection (NPD). The main parameters affecting extraction yield and selectivity, such as type and amount of dispersant material, clean-up co-sorbent and extraction solvent, were evaluated and optimised. Under final conditions, 0.5 g of dust were dispersed with equal amounts of anhydrous sodium sulphate and Florisil, and loaded on the top of a polypropylene cartridge containing 0.5 g of alumina. The dispersed sample was washed with 2 mL of *n*-hexane to remove the least polar interferences, and analytes were eluted with 3 mL of acetone. Recoveries of the proposed method for spiked samples ranged from 80 to 116%, and the day-to-day variability remained between 5 and 10%. Data on levels of organophosphate species in dust from private houses and vehicles cabins are provided. In both cases, the lowest concentrations corresponded to the short chain, non-chlorinated, alkyl organophosphates, whereas, mean values above 1 $\mu\text{g g}^{-1}$ were measured for the rest of analytes.

Keywords: Organophosphate compounds; Dust; Matrix solid-phase dispersion; Gas chromatography.

1. Introduction

Organophosphate esters (OPs) comprise a broad and heterogeneous group of chemical compounds in terms of organic substituents, vapour pressures, polarities and industrial applications. Alkyl derivatives of phosphoric acid are mainly used as plasticizers in polymeric materials and as antifoaming agents in lubricants and hydraulic fluids [1,2]. On the other hand, aryl derivatives and chlorinated alkyl phosphates are usually employed as flame retardants in textiles, polyurethane foams, computer and television-set screens, electrical cable sheathing, etc [3,4]. In all the above applications, OPs are included in the structure of host materials as additive chemicals and can therefore diffuse out to surrounding areas. After that, wastewater, air and particulate matter contribute to their spread in the biosphere.

In terms of toxicity, persistence and mobility, triphenyl phosphate, tris (2-chloroethyl) phosphate, tris (chloropropyl) phosphate and tris (2-chloro,1-chloromethylethyl) phosphate are the more concerning compounds [5-7]. In fact, chlorinated OPs pass through conventional urban wastewater treatment plants without undergoing significant degradation [2,8,9]. Furthermore,

they are not removed by other treatments such as ozonization or the use of multilayer filters and therefore, they might reach surface and drinking water supply sources [10,11].

From a toxicological point of view, indoor areas (e.g. private houses, work places and other confined areas) represent the main source of human exposure to these pollutants through inhalation and oral ingestion of particulate matter and dust. In such environments, the most volatile OPs are found in the gas phase, whereas other OPs are mainly associated with airborne particulate matter and dust [3,12]. Analysis of settled dust allows estimating time-average exposures to OPs in indoor areas. Moreover, unlike air samples, which are usually obtained by active sampling methods, dust can be easily obtained, e.g. from vacuum cleaner bags.

From an analytical point of view, the determination of OPs in dust is a challenging task. The main reason is the high organic carbon content of this matrix, which might constitute up to 30% of its weight [13,14]. Previously developed procedures for the determination of OPs in dust samples are based on the combination of exhaustive extraction techniques, such as Soxhlet [15], sample soaking with large volumes of organic solvents [16] or microwave-assisted extraction [17], followed by one or several clean-up steps and determination of analytes by liquid or gas chromatography techniques involving mass spectrometry and nitrogen-phosphorus detection, respectively [5,16,18]. In most cases, clean-up of primary extracts is the longest step in the analytical procedure, limiting sample throughput and significantly affecting the overall variability and accuracy of the method [2,17].

Unlike the above mentioned exhaustive extraction techniques, matrix solid-phase dispersion (MSPD) exhibits some interesting features in terms of selectivity and simplicity. Since extraction is carried out under mild conditions (room temperature and atmospheric pressure) less interferences are co-extracted together with target analytes. Moreover, the selectivity of the process can be adjusted by appropriate selection of the dispersant sorbent and the elution solvent [19,20]. Other advantages of MSPD are: (1) limited consumption of organic solvents; (2) reduced cost, since it does not require the acquisition of dedicated instrumentation and (3) the possibility of combining extraction and clean-up in the same process, just by placing a suitable co-sorbent at the bottom of the MSPD cartridge and/or by rinsing the dispersed sample with a solvent with low affinity for the target species. Until now, MSPD has mainly been used in the analysis of food, biota and biological fluids (solid and semi-solid matrices) [21-23]; however, some applications have been also proposed for sediments, sludge and dust samples [24-26].

The aim of the present work was to determine whether MSPD can be used as an alternative sample preparation technique for the determination of OPs in settled dust. The study centered on a group of seven compounds whose presence in indoor dust samples has been demonstrated in a previous work [17]. After extraction, analytes were determined using a GC-NPD system. The effect of several parameters on the performance of the method was evaluated with both spiked and real-life polluted samples. Moreover, the extraction efficiency of the optimised method is compared with that achieved by microwave-assisted extraction. Levels of target OPs in dust from private houses and automobile cabins are provided.

2. Experimental

2.1. Reagents and standards

Acetone, dichloromethane, ethyl acetate and *n*-hexane for analyses were purchased from Merck (Darmstadt, Germany). Standards of tributyl phosphate (TBP), triisobutyl phosphate (TiBP), tris (2-chloroethyl) phosphate (TCEP), tris (2-chloro-, 1-chloromethylethyl) phosphate (TDCP), tris (butoxyethyl) phosphate (TBEP) and triphenyl phosphate (TPP) were acquired from Aldrich (Milwaukee, WI, USA). Tris (chloropropyl) phosphate (TCPP) was kindly provided by Dr. J. B. Quintana (University of La Coruña, Spain). The internal standard (IS), tripropyl phosphate (TPrP), was also purchased from Aldrich. Individual stock solutions of each analyte and the IS were prepared in acetone. Diluted standards and mixtures of target OPs were made in *n*-hexane, when used to prepare spiked samples, and in ethyl acetate, when employed as calibration standards. The stock solution of the IS was also diluted with ethyl acetate and added at a constant concentration, ca. $0.4 \mu\text{g mL}^{-1}$, to calibration standards and sample extracts.

Anhydrous sodium sulphate, Florisil (60-100 mesh), C18 (70-230 mesh) and alumina (150 mesh) were provided by Aldrich. Silica (230-400 mesh) was acquired from Merck. Normal-phase materials were activated at $130 \text{ }^{\circ}\text{C}$ for 48 hours and then allowed to cool down in a desiccator before being used as sample dispersants or co-sorbents in the MSPD process. The C18 sorbent was used as received, without any further treatment.

Empty solid-phase extraction polypropylene cartridges (15 mL volume) and polyethylene frits ($20 \mu\text{m}$) were purchased from International Sorbent Technology (Mid Glamorgan, UK). Syringe filters (Millex GV, 13 mm, $0.45 \mu\text{m}$) were obtained from Millipore (Billerica, MA, USA).

2.2. Instrumentation and determination conditions

Concentrations of OPs in dust samples were determined with a HP 5890 series II GC system (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a NPD detector. Analytes were separated in a DB-5 (5%-phenyl, 95%-methylpolysiloxane) type column ($30 \text{ m} \times 0.32 \text{ mm i.d.}$, d.f.: $0.25 \mu\text{m}$), purchased from Agilent (Wilmington, DE, USA). The oven temperature was increased from $70 \text{ }^{\circ}\text{C}$ (held for 1 min) to $260 \text{ }^{\circ}\text{C}$ (held for 8 min) at a heating rate of $7 \text{ }^{\circ}\text{C min}^{-1}$. Nitrogen (99.999%) was used as carrier gas at a constant head column pressure of 96 kPa, and also as auxiliary gas in the NPD detector (25 mL min^{-1}). Synthetic air (99.995%) and hydrogen (99.999%) were used as detector gases at flow rates of 95 and 5 mL min^{-1} , respectively. The injected volume was $1 \mu\text{L}$, the injector temperature was maintained at $270 \text{ }^{\circ}\text{C}$ and the splitless valve opened 1 min after injection. In addition to the GC-NPD system, a Varian CP 3900 Gas Chromatograph (Walnut Creek, CA, USA) connected to an ion-trap mass spectrometer (Varian Saturn 2100) was used to assess the level of co-extracted organic species. This system was also equipped with a DB-5 type capillary column of the same length and film thickness as that installed in the GC-NPD instrument, but with an internal diameter of 0.25 mm . The carrier gas flow rate (helium) was maintained at 1.2 mL min^{-1} and the column temperature programmed from $50 \text{ }^{\circ}\text{C}$ (1 min) to $260 \text{ }^{\circ}\text{C}$ (8 min), with a gradient of $15 \text{ }^{\circ}\text{C min}^{-1}$. Electron-impact mass spectra were recorded in the range from 70 to 500 *m/z* units.

2.3. Samples

Dust was obtained from two different environments: flats and houses used as private residences (all located in the northwest Spain) and vehicles cabins. In the private houses and some cars, dust was collected by use of conventional vacuum cleaners equipped with paper filter bags. Other samples from automobile cabins were obtained from industrial vacuum cleaners, furnished with stainless-steel boxes and located in car-washing areas. Bulk dust was sieved and the fraction with a particle size below 60 μm was retained for analysis. After sieving, samples were stored at 4 °C in light protected glass vessels. Their average total carbon (TC) contents were 13% and 26% for vehicle cabins and house dust, respectively. Optimisation of MSPD extraction conditions was carried out with a pool of samples containing 20.0% of total carbon, 2.3% of nitrogen and 0.3% of sulphur. A fraction of this composite sample was spiked with TiBP, TBP, TDCP and TCEP at levels between 1.2 and 2.5 $\mu\text{g g}^{-1}$. The other OPs were already present in the pooled matrix at similar or even higher concentrations. The spiking procedure consisted of mixing, with a ratio 1 g:1 mL, fractions of sieved dust with the appropriate volume of a standard solution of the four above mentioned OPs in *n*-hexane. This slurry was thoroughly stirred and left in a hood until complete evaporation of the solvent. Then, it was stored in amber glass vessels at 4 °C and aged for at least two weeks before extraction. Fractions of different discrete dust samples were also spiked with target OPs and used in assessing the method recoveries.

2.4. Sample preparation

Fractions (0.5 g) of dust samples were mixed with the same amount of anhydrous sodium sulphate and dispersed with an appropriate sorbent in a glass mortar, with a pestle, until a visually homogeneous mixture was obtained. The blend was loaded in a cartridge containing a polyethylene frit and a given amount of co-sorbent. A second frit was placed over the dispersed sample before slight compression. Cartridges were eluted by gravity flow. Under optimised conditions, Florisil (0.5 g) was used as sample dispersant and alumina (0.5 g) as co-sorbent, packed on the bottom of the MSPD cartridge, to reduce the presence of polar interferences in the final sample extract. Non-polar compounds contained in the sample were first rinsed with 2 mL of *n*-hexane, and then, analytes recovered using 3 mL of acetone.

In a further step, acetone extracts were mixed with 1 mL of ethyl acetate and evaporated at room temperature, with a gentle stream of nitrogen, to a volume of approximately 0.5 mL. After that, extracts were spiked with the IS (50 μL of a 8 $\mu\text{g mL}^{-1}$ TPrP solution in ethyl acetate) and made up to 1 mL with ethyl acetate in a volumetric flask. Finally, extracts were filtered before being injected into the chromatographic system.

Performance of the MSPD method was compared with that achieved by a previously optimised microwave-assisted extraction (MAE) procedure. In brief, the latter involves an extraction step at 130 °C, with 10 mL of acetone, followed by centrifugation, dilution of the supernatant to 500 mL with ultrapure water, concentration on a reversed-phase sorbent and final purification with silica [17].

3. Results and discussion

3.1. Performance of gas chromatography determinations

The quantification limits of the GC-NPD system remained between $0.005 \mu\text{g mL}^{-1}$ (TiBP, TBP, TPP) and $0.010 \mu\text{g mL}^{-1}$ (TCEP, TCPP, TDCP and TBEP). The relative standard deviations for six consecutive injections of standards at two different concentration levels, 0.04 and $0.4 \mu\text{g mL}^{-1}$, ranged from 1 to 3%. The linearity in the response of the NPD detector was evaluated with standards, in ethyl acetate, at seven different levels from 0.010 to $5.0 \mu\text{g mL}^{-1}$. Correlation coefficients from 0.996 to 0.999, depending on the compound, were obtained within this interval of concentrations. On the other hand, the quantification limits of the GC-MS system ranged from values similar to those achieved with NPD up to 50-fold higher levels [17]. Therefore, GC-NPD was selected as the quantification technique and GC-MS was considered only for qualitative purposes.

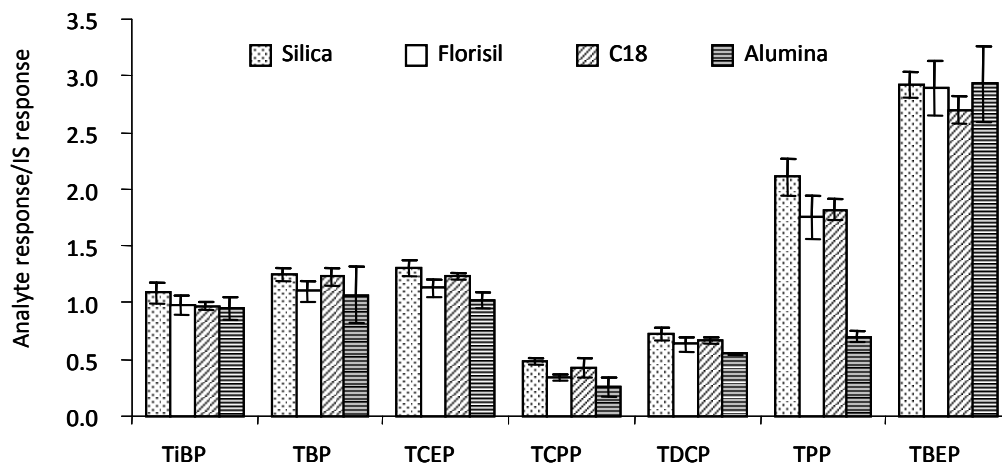
Levels of OPs in dust extracts were calculated using internal standard calibration to compensate for possible changes in the sensitivity of the NPD detector as function of the state of the active bead. Calibration curves were built by plotting the ratios between analytes and IS peak heights versus analytes concentrations.

3.2. Preliminary extraction assays

Preliminary experiments were carried out to assess the effect of several sorbents on the yield and selectivity of the MSPD process. Samples (0.5 g dust + 0.5 g of anhydrous sodium sulphate) were dispersed with 2 g of one of the following sorbents: silica, C18, Florisil or alumina, and then transferred to polypropylene cartridges containing 1 g of same material. Ethyl acetate was initially selected as the extraction solvent because it had previously been used for the extraction of OPs from complex solid matrices, e.g. lyophilised sludge [27], reversed and normal-phase materials [8,17]. Extracts (15 mL volume) were evaporated to a final volume of 1 mL and filtered. Whatever the used dispersant sorbent, a large hump was observed in the corresponding GC-MS chromatograms, which indicated a poor selectivity in the process as well as a risk of column contamination. Interfering compounds that caused the hump were able to be removed by rinsing the MSPD cartridge with 10 mL of *n*-hexane, previously to analytes extraction with ethyl acetate.

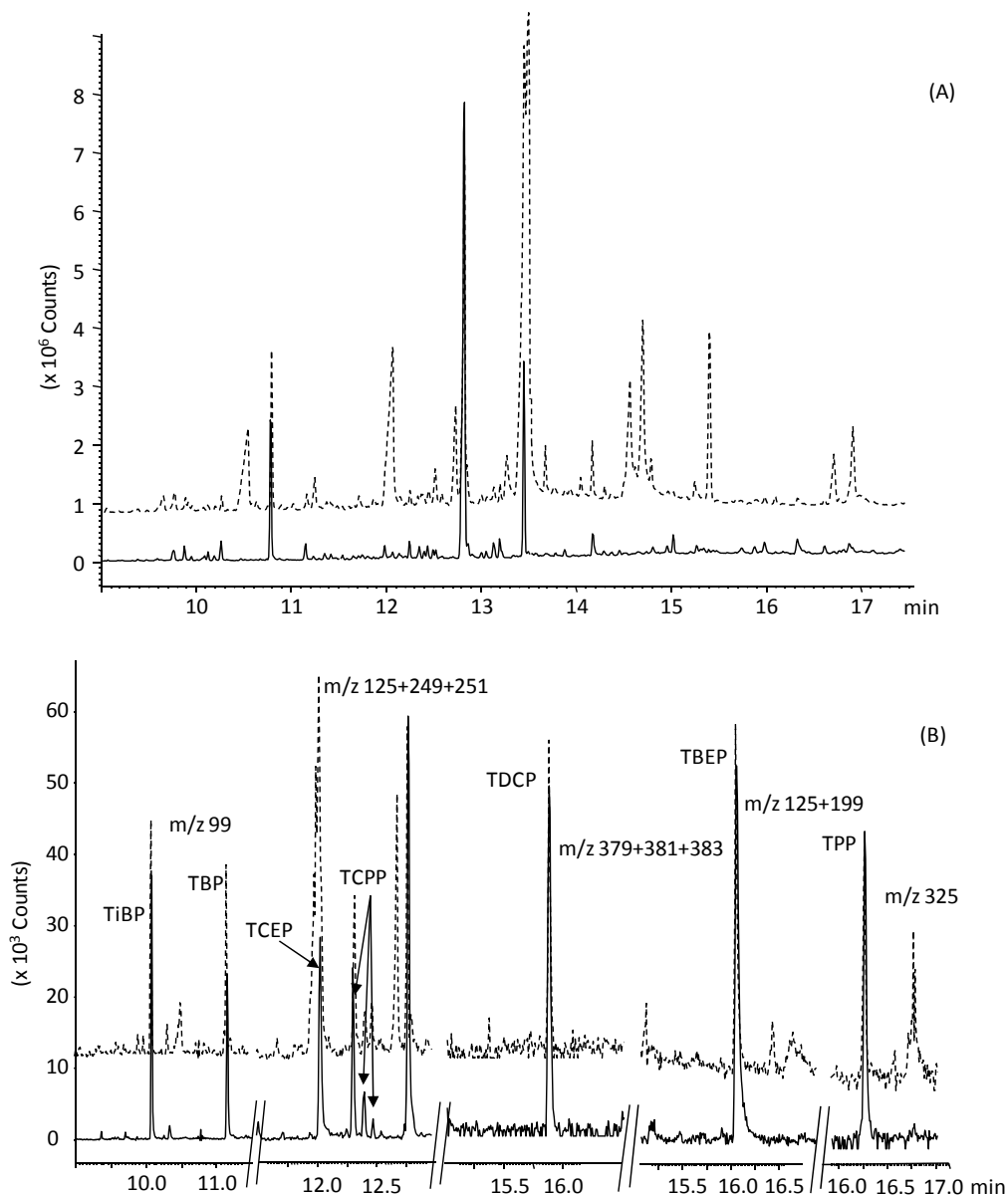
Working under above conditions and using NPD detection, it was found that the type of sorbent had little influence on the yield of the extraction for OPs compounds, except in case of TPP (**Fig. 1**). For this compound significantly lower yields were observed when alumina was used in the MSPD process. In addition to studies of extraction efficiency, the effects of dispersant and co-sorbent on the selectivity of the process were evaluated employing both GC-MS and GC-NPD detection.

Fig. 1. Effect of the type of sorbent on the efficiency of the MSPD extraction. Same material was used as sample dispersant (2 g) and clean-up co-sorbent (1 g). Data for triplicate extractions using 15 mL of ethyl acetate. GC-NPD detection.



With the first technique, it was verified that total ion current chromatograms (TIC) for C18 and silica were much more complex than those corresponding to Florisil and alumina (**Fig. 2A**). Even when the most intense ions in the MS spectra of each analyte were monitored, worse signal-to-noise ratios were obtained with silica or C18 than with Florisil or alumina, particularly at retention times of TiBP, TBP, TCEP and TCPP (**Fig. 2B**). These findings are consistent with the visual appearance of the respective extracts. Alumina and Florisil were therefore selected for further experiments. GC-NPD chromatograms corresponding to samples dispersed with either of the both sorbents revealed a lack of interferences at the retention times of most OPs. The only exception was a very intense peak close to TBP, particularly when Florisil was used as dispersant and co-sorbent. This peak was considerably reduced by the use of alumina, figure not shown. On the basis of these results, it was decided to evaluate a combination of both sorbents (Florisil and alumina) in the MSPD cartridge, to overcome possible TBP separation/quantification problems. The TPP extraction yields with Florisil as dispersant (2 g) and alumina as co-sorbent (1 g), and vice versa, were intermediate between those obtained using only Florisil or only alumina as dispersant and co-sorbent (**Fig. 1**); in addition, the magnitude of the interfering peak close to TBP was reduced with this mixed-mode column. As slightly higher TPP responses were achieved with Florisil as dispersant and alumina as clean-up co-sorbent, this combination was chosen for further experiments.

Fig. 2. GC-MS chromatograms for extracts of the spiked, pooled dust sample using Florisil (solid line) or C18 (dotted line) as dispersant and clean-up sorbents. (A) Total ionic current chromatograms; (B) signals monitored using the most intense m/z ratios for each analyte. The IS peak is not shown.



In a second optimisation stage, acetone and dichloromethane were considered as alternative elution solvents to ethyl acetate. The first has been used in the microwave-assisted extraction of OPs from dust samples [17], whereas dichloromethane is the most popular solvent for recovering OPs from dust and polyurethane sorbents, which had previously been employed

for air sampling purposes, using sonication or Soxhlet extraction [12,16]. In all cases, MSPD cartridges were first rinsed with 10 mL of *n*-hexane and then analytes extracted with 15 mL of the selected solvent. Under these conditions, dichloromethane was completely useless as extraction solvent since none of the analytes was found in the corresponding extract. Acetone and ethyl acetate provided equivalent yields for all compounds, except for TCPP, which was recovered in a greater extent with acetone, data not shown. Nevertheless, both solvents were considered in further extraction experiments.

3.3. Multi-factorial optimisation of extraction conditions

In a first approach to the systematic optimisation of the MSPD process, the influence of type and volume of extraction solvent, as well as the effects of dispersant (Florisil) and co-sorbent (alumina) masses on the yield of the extraction were simultaneously evaluated using a fractional, two-level, 2^{4-1} factorial experimental design with four central points (**Table 1**). Generation of the design matrix and data treatment were carried out with the Statgraphics software package [28]. In all experiments, MSPD cartridges were rinsed with *n*-hexane prior to extraction of analytes with acetone or ethyl acetate. To avoid losses of the less polar OPs: TiBP, TBP, TPP and TBEP (log *K*_{ow} values from 3.6 to 4.6), particularly with the lowest masses of dispersant and co-sorbent considered in the design, the volume of *n*-hexane was reduced from 10 to 2 mL.

Table 1. Domain of the two level, fractional, 2^{4-1} experimental factorial design.

| Factor | Levels | |
|---------------------|---------|---------------|
| | Low | High |
| Extraction solvent | Acetone | Ethyl acetate |
| Solvent volume (mL) | 10 | 20 |
| Florisil mass (g) | 0.5 | 2 |
| Alumina mass (g) | 0.5 | 1 |

For most OPs, higher extraction yields were obtained with acetone than with ethyl acetate as elution solvent; however, the effect was statistically significant only for TCPP (95% confidence level). In general, the other three factors (solvent volume and masses of Florisil and alumina) also showed a negative effect on the yield of the extraction (lower responses were attained for extractions accomplished at their upper values) although, the magnitude of their effects was less important than those associated with the extraction solvent. The exception was TPP, for this compound, the mass of co-sorbent (alumina) had the most relevant negative effect on the extraction process, which is consistent with the trend shown in **Fig. 1**.

In light of the above results, it was decided to fix the mass of co-sorbent (alumina) at 0.5 g and to re-evaluate the effects of dispersant (Florisil) and extraction solvent (acetone or ethyl acetate) after shifting their volumes to lower values than in the first design. In this case a full, two-level, 2^3 experimental factorial design with two central points was used. The

standardized values of main effects obtained after analysing the responses for each compound in the experiments of the design are shown in **Table 2**. The absolute value of a main effect is proportional to the variation in the yield of the extraction, for a given analyte, when the considered factor changes from the low to the high level within the domain of the design; its sign indicates whether the extraction yield increases (positive sign) or decreases (negative sign).

Table 2. Domain and standardized values for main effects of factors considered in the 2^3 experimental factorial design.

| Factor | Level | | Compound | | | | | | |
|---------------------|---------|---------------|----------|-------|------|--------------------|-------|-------|-------|
| | Low | High | TiBP | TBP | TCEP | TCPP | TDCP | TPP | TBEP |
| Extraction solvent | Acetone | Ethyl acetate | 0.97 | 0.62 | 0.49 | -5.12 ^a | 1.06 | -1.60 | 1.00 |
| Solvent volume (mL) | 3 | 10 | -1.13 | -1.08 | 0.15 | 0.92 | -0.61 | 1.12 | -1.07 |
| Florisil mass (g) | 0.5 | 2 | -1.10 | -1.35 | 1.06 | 0.52 | -1.35 | -2.73 | 0.37 |

^a Statistically significant factor at the 95% confidence level.

In general, Florisil mass and solvent volume played negative but statistically non-significant effects, as in the first design. The effect of the extraction solvent was compound-dependant and it generally remained below the statistically significant level (95%), except for TCPP, for which acetone again provided higher extraction efficiencies than ethyl acetate, exceeding the level of statistical significance. Taking these results into account, the mass of Florisil was fixed at 0.5 g, acetone was selected as the extraction solvent and its volume was temporary maintained at 3 mL.

In a further step, several dust samples (spiked and non-spiked) were processed to verify optimal volumes for washing (*n*-hexane) and extraction (acetone) solvents. In the first case, the use of more than 2 mL led to partial losses of TiBP and TBP in the washing fraction. As regards the volume of acetone, although approximately 90% of the analytes were already recovered with only 2 mL of this solvent, the working extraction volume was maintained at 3 mL.

3.4. Analytical figures of merit

The performance of the optimised method was characterized in terms of accuracy, precision and limits of quantification. **Table 3** presents the recoveries obtained for two discrete dust samples spiked with the considered OPs at levels from 1 to 3 $\mu\text{g g}^{-1}$. Depicted data were calculated by dividing the difference between concentrations measured for spiked and non-spiked fractions of the same sample, by the added amount. Extractions were performed in triplicate. The levels of OPs in the non-spiked fractions of the samples varied between 0.02 and 9 $\mu\text{g g}^{-1}$. Recoveries ranged from 80 to 116%, with relative standard deviations below 17%.

Table 3. Recoveries of the MSPD method for two spiked, discrete dust samples, $n = 3$ replicates.

| Compound | Recovery \pm R.S.D. (%) | |
|----------|---------------------------|-------------|
| | Sample 1 | Sample 2 |
| TiBP | 93 \pm 5 | 80 \pm 3 |
| TBP | 107 \pm 7 | 100 \pm 1 |
| TCEP | 100 \pm 9 | 98 \pm 8 |
| TCPP | 116 \pm 17 | 99 \pm 2 |
| TDCP | 95 \pm 5 | 99 \pm 5 |
| TPP | 90 \pm 6 | 87 \pm 3 |
| TBEP | 115 \pm 14 | 109 \pm 6 |

In addition to the use of spiked samples, the extraction efficiency of the MSPD method was compared with that achieved by a previously validated MAE procedure [17], for three different real-life polluted dust samples. Obtained data are given in **Table 4**. For TiBP, TBP, TDCP and TBEP, MSPD provided similar average extraction yields as MAE; however, for TCEP, TCPP and TPP recoveries were slightly lower: between 72 and 89% of those achieved with MAE. TCEP and TCPP are the most polar of the considered OPs ($\log K_{ow}$ 1.7 and 2.6, respectively); thus, they are probably not completely extracted from the sample under the mild conditions typically used in MSPD extractions. As regards TPP, considering its strong affinity for alumina, it may remain in the layer of this sorbent placed at the bottom of the MSPD cartridge.

Table 4. Comparison of extraction efficiencies of MSPD and MAE for three non-spiked dust samples.

| Compound | Relative recovery \pm R.S.D. (%) | | | |
|----------|------------------------------------|-------------|------------|------------------|
| | Sample 1 | Sample 2 | Sample 3 | Average recovery |
| TiBP | 102 \pm 3 | 103 \pm 1 | 92 \pm 3 | 99 |
| TBP | 109 \pm 5 | 104 \pm 2 | 94 \pm 1 | 102 |
| TCEP | 112 \pm 2 | 78 \pm 1 | 77 \pm 6 | 89 |
| TCPP | 70 \pm 1 | 92 \pm 5 | 83 \pm 1 | 82 |
| TDCP | 91 \pm 1 | n.d. | 89 \pm 3 | 90 |
| TPP | 76 \pm 3 | 70 \pm 1 | 70 \pm 2 | 72 |
| TBEP | 95 \pm 3 | 92 \pm 3 | 91 \pm 2 | 93 |

Relative recoveries to those achieved using MAE, $n = 4$ replicates.

n.d., not detected

The presence of residual amounts of OPs in MSPD cartridges was investigated using the Soxhlet technique. After elution of dispersed samples with 3 mL of acetone, the contents of MSPD cartridges were subjected to Soxhlet extraction (overnight with 75 mL of acetone), followed by concentration, intensive clean-up [17] and GC-NPD determination. TCEP, TCPP and TPP were found in Soxhlet extracts at levels around 10% of those measured in primary MSPD extracts. The other OPs were not detected. Therefore, for TiBP, TBP, TDCP and TBEP the yield of the MSPD extraction can be considered as quantitative.

Fig. 3 shows GC-MS (total ionic current) and GC-NPD chromatograms for two fractions of the same dust sample, processed using the developed MSPD method and MAE. The milder conditions used in MSPD allowed a similar selectivity to be achieved, without the need for additional time-consuming clean-up steps.

Precision of the MSPD method was evaluated with the relative standard deviation (R.S.D.) data corresponding to the extraction of 0.5 g fractions of a non-spiked dust sample. Under repeatability conditions (three extractions on the same day), R.S.D. values ranged from 4 to 13%. The day-to-day precision, corresponding to 12 extractions performed on four different days, varied from 5 to 10% (**Table 5**).

Table 5. Intra-day ($n = 4$ replicates) and day-to-day ($n = 12$ extraction in four days) variability of the MSPD method.

| Compound | R.S.D. (%) | |
|----------|------------|------------|
| | Intra-day | Day-to-day |
| TiBP | 8 | 8 |
| TBP | 6 | 8 |
| TCEP | 13 | 10 |
| TCPP | 10 | 6 |
| TDCP | 5 | 5 |
| TPP | 7 | 7 |
| TBEP | 4 | 5 |

Quantification limits of the MSPD method, defined for a signal-to-noise ratio of 10 (S/N 10), ranged from 40 ng g⁻¹ for TiBP, TBP, TCEP and TCPP to 50 ng g⁻¹ for the rest of species. Procedural blanks were carried out through the entire analytical procedure to verify possible contaminations during sample preparation. None of the analytes was detected in resulting extracts.

Fig. 3. Overlay of chromatograms for fractions a non-spiked dust sample extracted by MSPD (solid line) and by microwave-assisted extraction (dotted line). (A) GC-MS detection, total ion current signal; (B) GC-NPD detection; (*) unknown compounds.

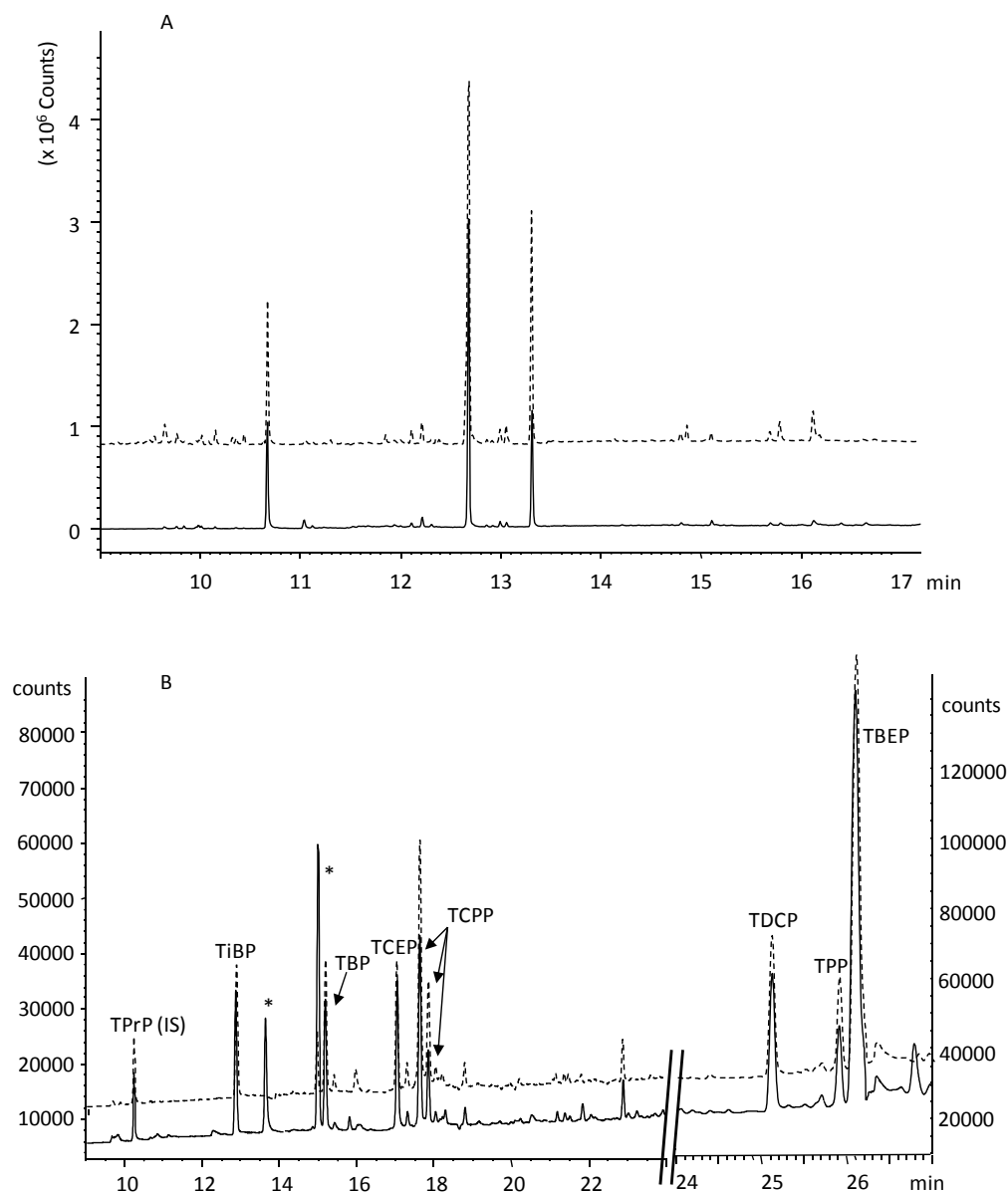


Table 6 summarizes the analytical characteristics of previously published procedures for the determination of OPs in dust samples. The proposed MSPD method provides similar quantification limits and extraction efficiencies, with the advantages of being faster and using smaller volumes of organic solvents.

Table 6. Comparison of procedures for the determination of organophosphate compounds in dust samples.

| Extraction technique | Extraction solvent | Extract Clean-up | Recoveries (%) | Detection technique | Q.L. (S/N 10) $\mu\text{g g}^{-1}$ | Ref. |
|----------------------|----------------------------------|------------------|-----------------|---------------------|------------------------------------|-----------|
| Sonication, 40 min | CCl_2H_2 (50 mL) | - | 97 ^a | GC-NPD | 0.021-0.180 ^b | [16] |
| Soxhlet, 8 hours | Hexane:acetone (8:2) | - | - | GC-MS | 0.3 ^b | [15] |
| Soxhlet, 6 hours | Acetone (75 mL) | SPE ^c | - | GC-NPD | 0.04-0.05 | [17] |
| MAE, 30 min | Acetone (10 mL) | SPE ^c | 85-104 | GC-NPD | 0.04-0.05 | [17] |
| MSPD | Acetone (3 mL) | Not required | 72-102 | GC-NPD | 0.04-0.05 | This work |

(-) not available

^a Evaluated only for triphenyl phosphate^b Estimated by multiplying the reported detection limits, defined for a S/N = 3, by a factor of 3.^c Solid-phase extraction using reversed and normal-phase sorbents.

3.5. Application to real samples

The optimised MSPD method was applied to the analysis of several samples. Found OPs concentrations are given in **Table 7**. Samples 1-4 were obtained from private residences. The rest correspond to vehicle cabins. Samples 5 and 6 were obtained by vacuuming the interior of two pairs of private cars with average ages of 2 and 7 years, respectively. For both samples, special care was taken to avoid sampling soil and sand particles from carpets. The other three samples (codes 7-9) were taken from industrial vacuum cleaners installed in car-washing areas. In this case, the sampling step was difficult to control and, in addition to dust, sand and soil were also present in the sieved samples; however, the latter three samples are more representative of the settled particulate matter that exists in car interiors. Dust from vehicles cabins provided cleaner extracts than house dust, which is consistent with the different carbon contents of both types of samples (**Table 7**).

Considering those OPs species used as plasticizers, the concentrations of TBEP were much higher than those of TiBP and TBP. The mean values measured for the other four compounds, mainly used as flame retardants, exceeded $1 \mu\text{g g}^{-1}$, with the highest levels corresponding to TCPP. Moreover, TCPP and TCEP were found in all samples at values higher than the quantification limits of the proposed method. Although the number of processed samples was not enough to establish a systematic comparison between OPs levels in dust from private houses and vehicles cabins, the concentrations of TDCP in the latter environment were clearly higher (**Table 7**). Previous data regarding levels of OPs in private cars and public transport vehicles indicated negligible or non detectable levels for this compound [4,12,29]; however, in these studies, only air samples were considered. Therefore, unlike TCEP and TCPP, TDCP appears to be basically associated with settled dust. On the whole, considering the systematic inclusion of OPs in polyurethane foam, upholstery and other polymeric materials used in car cabins, the levels of OPs in the corresponding samples were similar to those measured in dust from private houses.

Table 7. Concentrations of OPs in different dust samples.

| Sample code | TC (%) | TiBP | TBP | TCEP | TCPP | TDCP | TPP | TBEP |
|-------------|--------|---------------|---------------|---------------|-------------|-------------|-------------|-------------|
| 1 | 25.0 | 0.08 ± 0.02 | 0.17 ± 0.01 | 2.80 ± 0.03 | 3.26 ± 0.05 | n.d. | 0.36 ± 0.03 | 9.01 ± 0.76 |
| 2 | 25.1 | 0.104 ± 0.005 | 0.078 ± 0.003 | 0.099 ± 0.003 | 39.6 ± 0.3 | 0.34 ± 0.02 | 1.9 ± 0.1 | 14.0 ± 0.4 |
| 3 | 25.1 | 0.190 ± 0.008 | 0.90 ± 0.04 | 1.3 ± 0.1 | 4.5 ± 0.8 | 0.43 ± 0.06 | 1.3 ± 0.4 | 18 ± 3 |
| 4 | 28.0 | n.q. | 0.09 ± 0.01 | 0.09 ± 0.01 | 2.32 ± 0.08 | n.d. | 4.9 ± 0.3 | 3.6 ± 0.1 |
| 5 | 14.5 | 0.14 ± 0.01 | 0.040 ± 0.003 | 40 ± 2 | 1.4 ± 0.1 | 13 ± 1 | 1.0 ± 0.1 | 48 ± 2 |
| 6 | 13.7 | 0.580 ± 0.003 | 0.13 ± 0.01 | 0.55 ± 0.01 | 17.2 ± 0.1 | 7.0 ± 0.5 | 1.64 ± 0.02 | 3.21 ± 0.06 |
| 7 | 15.7 | 0.025 ± 0.003 | 0.21 ± 0.02 | 3.6 ± 0.2 | 4.2 ± 0.1 | 10.7 ± 0.2 | 2.0 ± 0.2 | 3.3 ± 0.2 |
| 8 | 15.1 | 0.12 ± 0.01 | 0.08 ± 0.01 | 0.8 ± 0.1 | 1.2 ± 0.2 | 9.5 ± 0.4 | 1.1 ± 0.2 | 1.6 ± 0.4 |
| 9 | 8.4 | 0.17 ± 0.01 | 0.047 ± 0.003 | 0.90 ± 0.01 | 1.80 ± 0.04 | 1.62 ± 0.01 | 0.5 ± 0.1 | 1.64 ± 0.01 |
| Average | | 0.16 | 0.19 | 5.6 | 8.4 | 6.1 | 1.5 | 11.4 |

Values in $\mu\text{g g}^{-1}$, $n = 3$ replicates per sample.

n.d. not detected; n.q. under quantification limits; TC, total carbon content.

4. Conclusions

The proposed procedure constitutes the first application of the MSPD technique to the determination of OPs in dust samples. The method allows extraction and clean-up to be carried out in a single step, which greatly speeds-up sample throughput. Although, for three of the investigated compounds (TCEP, TCPP and TPP) the achieved extraction yields were slightly lower than those reported for other methods based on the use of exhaustive extraction approaches, e.g. Soxhlet or MAE, the proposed alternative is far more convenient in terms of solvents and time consumption. Data obtained for non-spiked samples confirmed the ubiquitous presence of the selected OPs in dust from houses and vehicles cabins with a similar distribution pattern; however, TDCP levels were higher in the latter environment. Relevant concentrations of TBEP and of chlorinated species, which are the most concerning OPs in terms of environmental persistence and toxicity, were found in most of the samples analysed.

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1.5. PUBLICACIÓN:

***DETERMINATION OF ORGANOPHOSPHATE FLAME RETARDANTS AND PLASTICIZERS IN SEDIMENT SAMPLES USING MICROWAVE-ASSISTED EXTRACTION AND GAS CHROMATOGRAPHY WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY.**

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Talanta 79 (2009) 824

Determination of organophosphate flame retardants and plasticizers in sediment samples using microwave-assisted extraction and gas chromatography with inductively coupled plasma mass spectrometry

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Abstract

A procedure for the determination of 10 organophosphates, used as flame retardants and plasticizers, in sediment samples is presented. Microwave-assisted extraction (MAE) and gas chromatography with inductively coupled plasma mass spectrometry (GC-ICP-MS) were used for sample preparation and analytes determination, respectively. Influence of different variables on the performance of extraction and determination processes is thoroughly discussed. Temperature, type and amount of organic solvent showed a major effect on the yield of MAE. Regarding GC-ICP-MS detection, the combination of pulsed splitless injection with low radio frequency (rf) power, hard extraction conditions (referred to lens voltage) and addition of nitrogen (0.03 L min^{-1}) to the argon plasma provided the best sensitivity. Under final working conditions, recoveries between 78% and 105%, for samples spiked at different concentration levels, and limits of quantification from 2 to 4 ng g^{-1} were achieved. Analysis of un-spiked sediments confirmed the excellent selectivity of the proposed method for real-life polluted sample analysis.

Keywords: Inductively coupled plasma mass spectrometry; Microwave extraction; Organophosphate compounds; Flame retardants; Sediment.

1. Introduction

Organophosphate esters (OPs) are a heterogeneous group of compounds in terms of physical and chemical properties and applications [1]. They are commonly used as flame retardants and plasticizers and can be found in a wide variety of products, such as varnishes, polyurethane foams, upholstery and textiles. OPs are normally employed as additives, not chemically bonded; therefore, they can easily be released to the surrounding area [2]. After that, air, particulate matter, and wastewater contribute to their spread into the environment. As a result of their increasing usage, persistence of some species, particularly chlorinated OPs, and low water solubility and high adsorption to particulate matter of some of them, they can be concentrated in sediment samples [3-8]. So far, little work has been conducted for the extraction of OPs from sediments and the available procedures, which have been developed for just a few of them, are time-consuming and require large amounts of organic solvent. Soxhlet extraction using methanol-water (2:1) for 24 h [3], reflux heating with methanol-water (9:1) for 16 h [4],

ultrasound-assisted solvent extraction with ethyl acetate-acetonitrile (3:7), 2 x 30 mL, for 30 min [5], shaking with acetone for 30 min [6] or combination of sonication (20 min) and shaking (15 min) with acetone (100 mL) first and methanol (50 mL) second [7], have been proposed for OPs extraction from sediments. Further clean-up of raw extracts provided by above techniques normally involved dilution with water and partition into dichloromethane [7], and/or solid-phase extraction using normal [4,6] or reversed-phase sorbents [3]. To the best of our knowledge, the only microwave-assisted extraction (MAE) protocol for this matrix was proposed by De Geus et al. [8]. The researchers used a domestic microwave oven to perform the extraction of a few OPs in open vials, thus irradiation time was limited by the boiling point of the selected solvent mixture.

Gas chromatography (GC) with nitrogen phosphorus detection (NPD) [9,10], mass spectrometry using electron impact ionization (GC-EI-MS) [11,12] or positive chemical ionization (GC-PCI-MS) [13,14] have been proposed for the determination of OPs in environmental samples. Unsatisfactory selectivity of NPD detection, excessive fragmentation of some OPs in EI-MS, rendering ions with low m/z ratios, and limited sensitivity in PCI-MS are some of the most important drawbacks of these techniques when applied to the determination of OPs in complex environmental matrices [15]. Conversely, inductively coupled plasma mass spectrometry (ICP-MS) provides a good combination of sensitivity and selectivity, which has been exploited not only for OPs analysis in complex matrices, such as blood [16] or wastewater [17], but also for phosphorus-containing pesticides in different beverages and environmental samples [18-21].

On the other hand, as far as could be ascertained, the ICP-MS capability for the determination of triisobutyl phosphate (TIBP), tris(chloropropyl) phosphate (TCPP), tris(dichloropropyl) phosphate (TDCP) and triphenyl phosphine oxide (TPPO) has not been evaluated yet. Furthermore, published papers for the remaining OPs do not deal with the direct injection of an organic solvent in the GC-ICP-MS system but use solid-phase microextraction (SPME) as sample introduction technique [16,17]. Therefore, this work is the first approach using direct solvent injection for OPs determination by means of GC-ICP-MS.

Available data related to the presence of OPs in sediments are very limited; moreover, these compounds are usually found at low level, which poses an additional challenge to their determination. The Office of Health studies from Tokyo on its surveys from 1977 to 1979 reported levels of tributyl phosphate (TBP) between 2 and 240 ng g⁻¹ [6]. Ishikawa et al. determined levels of tris(2-chloroethyl)phosphate (TCEP) ranging from 13 to 28 ng g⁻¹ [6]. Martinez-Carballo et al. [5] analyzed several Austrian sediments determining concentrations of TBP, TCEP, tris(2-butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP) and tris(2-ethylhexyl) phosphate (TEHP) between 2.4 and 160 ng g⁻¹ and up to 1300 ng g⁻¹ for TCPP. TDCP was also detected in some of the analyzed samples. However, high levels of some OPs were measured in bottom sediment from a waste disposal site and the surrounding area in Japan: TBP, TCEP, TCPP, TDCP, TBEP and TEHP determined concentrations ranged from 2 to 7395 ng g⁻¹ [7]. The aim of this work was to develop a procedure to extract a group of 10 OPs from sediment samples. MAE was the technique of choice and GC-ICP-MS was used for determination purposes. Several variables affecting the extraction efficiency were evaluated in detail and ICP-MS parameters were

optimized. Finally, the performance of the method was demonstrated with real-life polluted sediments.

2. Experimental

2.1. Reagents, standards and samples

Acetone, ethyl acetate, *n*-hexane and dichloromethane were acquired from Pharmco-Aaper (Brookfield, CT, USA). Acetonitrile was purchased from Tedia (Fairfield, OH, USA). Tripropyl phosphate (TPrP), TiBP, TBP, TCEP, TDCP, TBEP, TPP, TEHP and TPPO were acquired from Aldrich (Milwaukee, WI, USA). TCPP, as a technical mixture of three isomers, was provided by the chemical company Dr. Ehrenstorfer (Augsburg, Germany). For quantitative purposes only the first isomer was considered. Tripentyl phosphate (TPeP) was obtained from TCI Europe (Zwijndrecht, Belgium) and used as internal standard (IS). Individual stock solutions of each analyte and the IS were prepared in acetone. Diluted standards and mixtures of target OPs were made in *n*-hexane, when used to prepare spiked samples, and in ethyl acetate, when employed as calibration standards. The stock solution of the IS was also diluted with ethyl acetate and added to calibration standards and sample extracts. Normal-phase silica cartridges (50 mg) were provided by Waters (Milford, MA, USA).

Sediment samples were obtained from rivers located in Spain and USA: Sarela (Santiago de Compostela, Spain), Asma (Chantada, Spain) and Ohio on its way through three different states: Ohio, Kentucky and Indiana (USA). Samples were dried (freeze-dried or air dried) and sieved. Fractions with particle sizes below 300 μm were considered for this study.

Preliminary assays were performed with sediment from the Ohio river spiked with 1000 ng g^{-1} for each compound. For the experimental factorial design and method performance evaluation, the sediment from the Sarela was used; the spiked levels were 1000, 200 and 50 ng g^{-1} . The spiking procedure consisted of mixing a fraction of sediment with a standard solution of OPs in *n*-hexane, using a volume of solvent which guaranteed that the sediment was completely covered. This slurry was thoroughly stirred and left in a hood until complete evaporation of the solvent. Then, it was stored in amber glass vessels at 4 $^{\circ}\text{C}$, for at least 2 weeks before extraction.

GC-ICP-MS determination conditions were optimized using a standard solution of OPs, with a concentration of 200 ng mL^{-1} , prepared in ethyl acetate.

2.2. Sample preparation

Extractions were performed using a CEM Explorer-Discoverer (Matthews, NC, USA) focused microwave extractor device, equipped with 10 mL glass vessels sealed with polytetrafluoroethylene layered septum caps. A Sorvall RC-5B Refrigerated Superspeed Centrifuge (Dupont Instruments, ON, Canada) was used to centrifuge supernatants after microwave extraction. Under optimized conditions, 0.5 g of accurately weighed sample were extracted at 150 $^{\circ}\text{C}$ in two sequential steps of 15 min duration, using 4 mL of solvent. The first extraction was performed with acetone and the second with acetonitrile. The sealed extraction

vessels were allowed to cool before being released from the microwave cavity to prevent evaporation losses. Both supernatants were combined and centrifuged at 4000 rpm, for 4 min. After that, 1 mL of ethyl acetate was added and the extract was evaporated under nitrogen stream to ca. 0.5 mL. Finally, it was purified on 50 mg silica cartridges. Analytes were recovered from the cartridge with 1 mL of ethyl acetate. The IS was added to an aliquot of the extracts. For real sample analysis, the final extract was further concentrated to 200 μ L due to the low levels of OPs found in the analyzed sediments.

Soxhlet extractions were performed with 90 mL of acetone for 16 hours. Extracts were evaporated and the solvent changed to ethyl acetate. Then, they were submitted to same clean-up procedure as microwave extracts.

2.3. Determination

Analytes were determined using an Agilent (Santa Clara, CA, USA) 6890 gas chromatograph connected, through a heated interface (Agilent Technologies, Santa Clara, CA), to an Agilent 7500cs ICP-MS system (Agilent Technologies, Santa Clara, CA, USA), equipped with shielded torch and collision/reaction cell technology. Chromatographic separations were carried out with an HP-5 capillary column (30m \times 0.32mm i.d., *df*: 0.25 μ m) purchased from Agilent (Santa Clara, CA, USA). The temperature of the column was programmed at 15 $^{\circ}$ C min^{-1} from 70 $^{\circ}$ C (held for 1 min) to 270 $^{\circ}$ C (held for 5 min). Helium (99.999%) was used as carrier gas in the GC column at a constant flow rate of 1.5 mL min^{-1} . The injector was kept at 270 $^{\circ}$ C and a pulse of 30 psi, for 0.5 min, was employed to improve the efficiency of the injection process. Aliquots (2 μ L) of standard solutions and sample extracts were injected in the splitless mode (2 min). The GC-ICP-MS interface was kept at 290 $^{\circ}$ C. In the ICP-MS, argon flow rates were 15 L min^{-1} and 0.8 L min^{-1} for the plasma and carrier gases, respectively. Nitrogen, with a flow rate of 0.03 L min^{-1} , was used as additional gas in the central argon channel. The forward radio frequency (rf) power was 700 W and the sampling depth 7 mm. The extraction lens 1 was kept at -155 V and quadrupole and octopole bias were set at -9 V and -12 V, respectively.

3. Results and discussion

3.1. Chromatographic conditions

The temperature gradient for baseline separation of target compounds was optimized in a previous work [9]. Moreover, conventional and pulsed splitless were compared as injection modes. The latter increases inlet pressure just before the beginning of a run and returns it to the set value (corresponding to the selected column flow) after a pre-established time. The pressure pulse sweeps the sample out of the inlet and into the column faster, reducing the chance of analyte decomposition and/or adsorption in the internal surface of the liner. In addition, it provides a narrow injection band in the head of the GC column.

Increasing the pressure in the injector to 30 psi led to a 2-3 fold improvement in the peak height of target species when compared to conventional splitless. Higher pressure values did not affect chromatographic responses of selected OPs and increased the risk of leaks in the injector, thus 30 psi was maintained as optimal pressure during injection.

3.2. ICP-MS detection

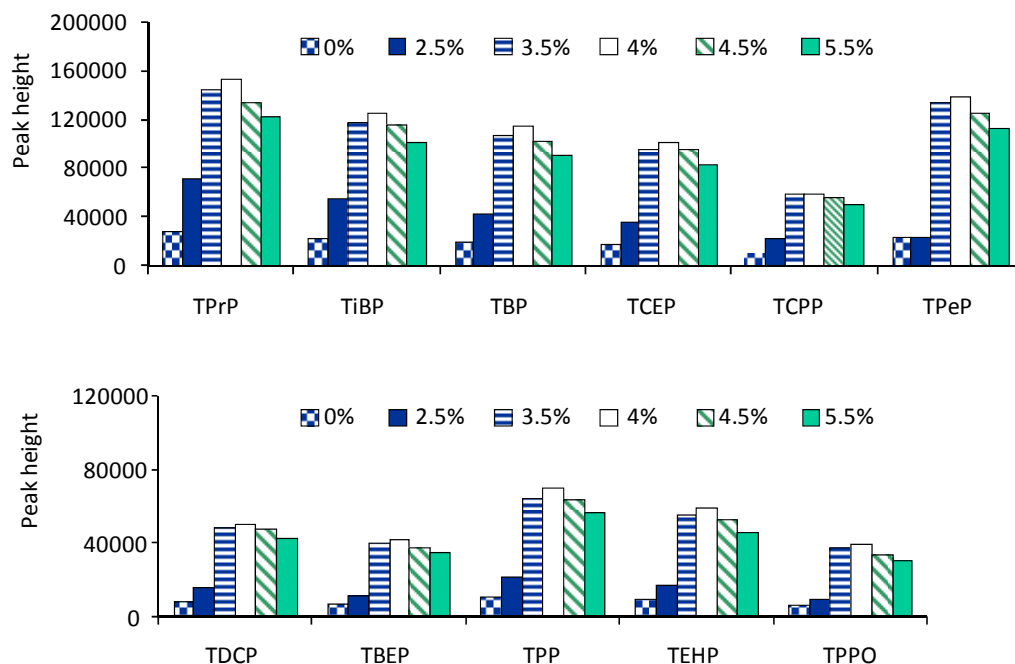
Several parameters affecting the ICP-MS performance were studied in detail. Moreover, as in previous studies, nitrogen was used as additional gas in the central argon plasma channel to enhance phosphorus ionization [16-21]. The addition of small amounts of alternate gases (N_2 , O_2 , He) is usually employed to improve the detection limits of elements with high ionization potentials such as phosphorus. Although the mechanism is not completely clear, it is attributed to changes in the bulk properties of the plasma [20].

3.2.1. Carrier gas flow, N_2 addition and collision cell conditions

The effect of the argon carrier gas flow on the response of target compounds was investigated in the range between 0.65 and 0.95 $L\ min^{-1}$. The optimum value was 0.8 $L\ min^{-1}$ (data not shown).

As previously stated, nitrogen was added to the carrier gas. **Fig. 1** shows the responses obtained with 0.8 $L\ min^{-1}$ of Ar containing percentages of nitrogen from 0% to 5.5%.

Fig. 1. Influence of nitrogen addition to the central argon plasma channel on phosphorus ionization. Percentage of nitrogen referred to an Ar flow of 0.8 $L\ min^{-1}$. Obtained response for a 200 $ng\ mL^{-1}$ OPs standard solution in ethyl acetate.



The addition of nitrogen provided an important improvement in sensitivity. Maximum peak heights were noticed when the carrier gas flow contained between 0.02 and 0.04 $L\ min^{-1}$ of N_2 , and they slightly dropped down at higher flows. On the other hand, N_2 contributed also to

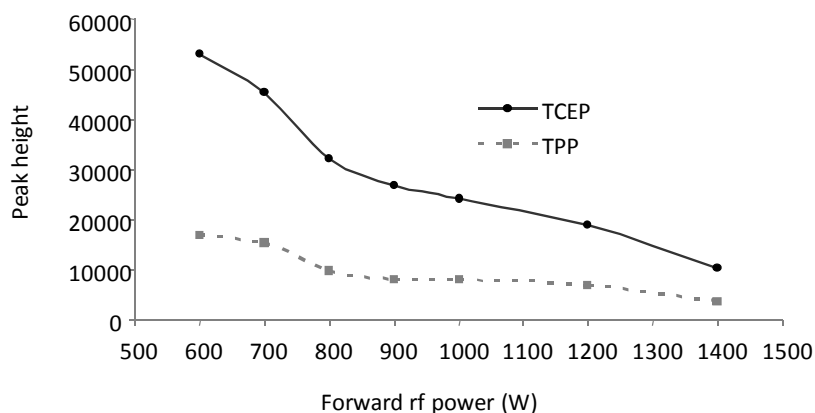
increase the baseline noise of the corresponding chromatograms. An intermediate flow of 0.03 L min^{-1} of N_2 (equivalent to a percentage of 4% relative to the carrier gas flow) was selected because it guaranteed a good signal to noise ratio and good stability versus slight changes on nitrogen flow. This trend matched with that reported in previous studies, where the addition of nitrogen was also found beneficial at low percentages [16-20].

Detection of phosphorus-containing species by ICP-MS is affected by polyatomic interferences consisting of nitrogen, oxygen and hydrogen atoms combinations. Obviously, the use of nitrogen, to enhance phosphorus ionization, worsens the spectral background at 31 m/z units. In systems furnished with collision cell technology, such interferences usually can be minimized using helium as collision gas. It was found that introducing just 0.5 mL min^{-1} (the lower flow allowed by the instrument) of this gas into the collision cell gave a similar decrease in the baseline noise and in the responses of OPs; consequently, the S/N ratio of chromatographic peaks remained unchanged and, thus, He was not used in the collision cell.

3.2.2. Forward rf power

Fig. 2 shows the effect of the rf power on the responses of TCEP and TPP. The rest of species followed a similar trend.

Fig. 2. Effect of the rf forward power on OPs response.



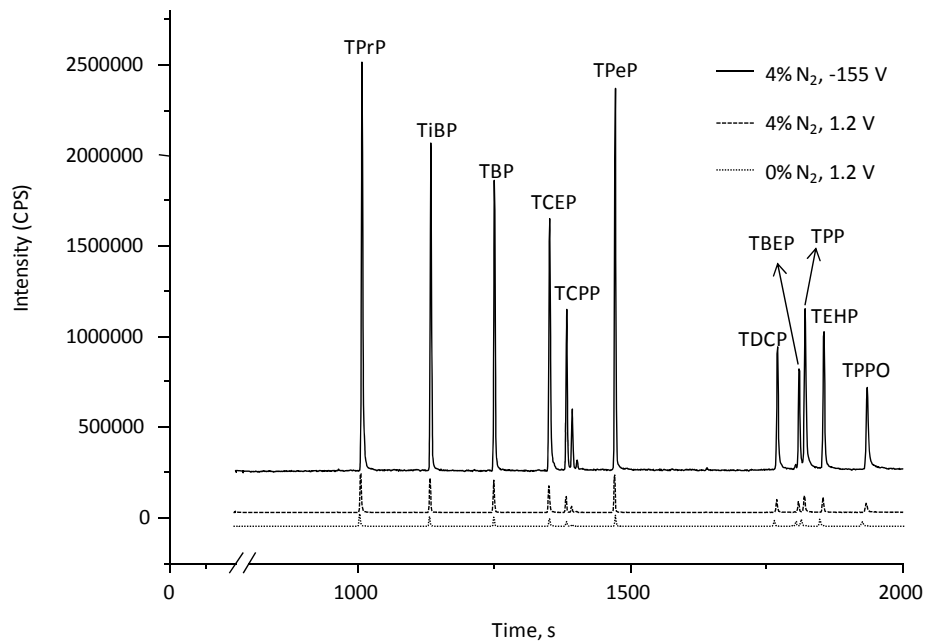
In contrast to relatively high values reported in previous articles for OPs or phosphorus-containing pesticides detection [16-20], the highest responses were obtained in the power range between 600 and 700 W, with an exponential decay of signals measured at higher powers, which matched with the trend reported by other researchers for phosphorus and halogen detection in dry plasma conditions [21]. Considering that low forward powers, 600 and 700 W, performed more or less the same, 700 W was selected as working value in order to ensure plasma stability which might be affected by the injection of complex matrices with high organic contents.

3.2.3. Extraction lens 1 voltage and makeup gas flow

As stated by Pröfrock et al. [21], the ICP-MS 7500cs system shows high dependency between its sensitivity and the extraction mode when coupled to GC. The ICP-MS instrument

used can be operated with different extraction modes, namely soft extraction (positive extraction lens 1 voltage up to +10 V) or hard extraction (highly negative extraction lens 1 voltage down to -200 V). In our research, a dramatic improvement was noticed operating the ICP-MS under hard extraction conditions (**Fig. 3**), which were not exploited in previous studies dealing with some of the OPs involved in this research [16,17].

Fig. 3. Overlapped chromatograms for a 200 ng mL⁻¹ OPs standard solution obtained with the ICP-MS operated under soft conditions (extraction lens 1 at 1.2 V) and hard (extraction lens 1 at -155 V).



The effect of the auxiliary (intermediate) argon flow in the ICP plasma was also evaluated. Flows between 0.5 and 1.3 L min⁻¹ were assayed and any noticeable difference was found neither in phosphorus response nor in the background signal with the use of makeup gas, data not shown, so it was avoided.

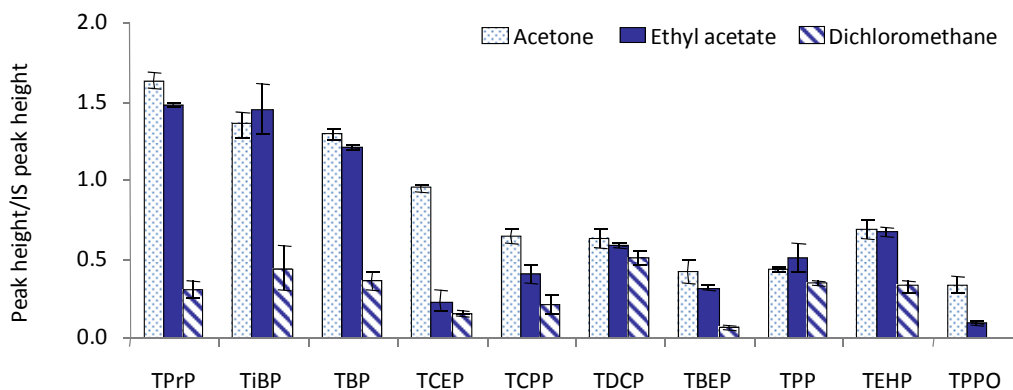
3.3. Microwave-assisted extraction optimization

3.3.1. Extraction solvent

Based on previous studies [9,10,15,2] three medium-polarity organic solvents: ethyl acetate, dichloromethane and acetone, were tested as extractants in the MAE process. Initially, extractions were performed at 80 °C for 10 minutes using 0.5 g of sediment and 5 mL of organic solvent. Obtained extracts were concentrated (ca. 0.5 mL), purified using a 50 mg silica SPE cartridge and adjusted to a final volume of 1 mL. In the case of acetone and dichloromethane extracts, 1 mL of ethyl acetate was added when they were concentrated, prior to silica purification.

In general, acetone provided better responses than ethyl acetate and dichloromethane, **Fig. 4**. For instance, TPPO was poorly extracted with ethyl acetate and not detected in dichloromethane extracts.

Fig. 4. Comparison of responses using different extraction solvents. Data for a spiked (1000 ng g^{-1}) sediment sample. MAE at $80 \text{ }^\circ\text{C}$ for 10 min using 5 mL of each solvent. Mean values with their standard deviations, $n = 3$ replicates, for 0.5 g samples.



On the basis of these results, acetone was selected for further experiments. These findings are in agreement with those reported previous works. Martínez-Carballo et al. determined 9 OPs in the Austrian aquatic environment and reported that dichloromethane was unsuitable for OPs extraction from sediment samples [5] using sonication as extraction technique. On the other hand, acetone was also the best solvent for OPs extraction from dust samples using both MAE [9] and matrix solid-phase dispersion (MSPD) [10].

3.3.2. Microwave extraction parameters

In a second step, the optimization of the extraction temperature and time, as well as the acetone volume, was simultaneously performed using an experimental factorial design type $3^1 \times 2^2$, involving a total of 12 experiments as shown in **Table 1**.

Table 1. Experimental factorial design $3^1 \times 2^2$ domain.

| Factor | Levels | | |
|----------------------------------|--------|--------|------|
| | Low | Medium | High |
| Volume (mL) | 4 | - | 8 |
| Time (min) | 5 | - | 15 |
| Temperature ($^\circ\text{C}$) | 60 | 95 | 130 |

The mass of sediment was kept at 0.5 g. Obtained extracts were processed as in the preliminary assays. Standardized values of main effects, obtained after analyzing the responses for each analyte in the experiments of the design, are presented in **Table 2**. The absolute value of

the main effect for a given compound is proportional to the variation in the efficiency of the extraction for this species, when the considered factor changes from the low to the high level established in the design domain. Its sign means whether the extraction yield increases (positive sign) or decreases (negative sign).

Table 2. Standardized values for major effects considered in the $3^1 \times 2^2$ experimental factorial design.

| Factor | Compound | | | | | | | | | |
|-------------|----------|-------|--------|--------|-------|--------|--------|-------|--------|--------|
| | TPrP | TiBP | TBP | TCEP | TCP | TDCP | TBEP | TPP | TEHP | TPPO |
| Volume (mL) | -2.67 | -2.16 | -2.90* | -4.17* | -1.50 | -4.58* | -4.28* | -1.98 | -3.35* | -3.26* |
| Time (min) | 0.41 | 0.41 | 0.33 | 2.77* | 3.05* | 4.86* | 2.51 | -2.33 | 3.74* | 3.26* |
| Temp. (°C) | 3.25* | 3.04* | 2.51 | 5.81* | 4.47* | 5.78* | 3.22* | 2.74 | 5.06* | 3.39* |

* Statistically significant effects at the 95% confidence level; Temp., temperature.

For all compounds, the volume of solvent showed a negative effect on the efficiency of the process, meaning that 4 mL provides higher extraction yields than 8 mL. Moreover, this factor was statistically significant (95% confidence level) for TBP, TCEP, TDCP, TBEP, TEHP and TPPO. On the other hand, the effect of extraction time was positive, except for TPP, and statistically significant for TCEP, TCP, TDCP, TEHP and TPPO. Finally, temperature showed a positive effect for all the compounds and was statistically significant except for TBP and TPP. According to these results, acetone volume was kept at 4 mL and the extraction time was set at 15 min. Some additional assays were carried out to ensure that the better performance of the lower volume of acetone (4 mL) compared to the higher level of the design (8 mL) was not due to evaporation losses of the analytes affecting more to the latter situation. No evaporation losses were observed in any case. The better performance of lower solvent volumes when using MAE is not new and has been already reported for other organic compounds [22,23].

On the basis of above comments, temperatures higher than those explored within the design domain were tested in further experiments. In comparison with data obtained at 130 °C, slightly higher responses were measured for TPrP, TDCP, TPP and TEHP at 150 °C, whereas further improvement was not noticed using a temperature of 170 °C (data not shown). Thus, 150 °C was selected as optimal temperature in the extraction step.

Extractions performed under above conditions provided recoveries above 84% except for TPrP and TCEP, for which a 70% recovery was achieved. Exposing the sample to the microwave radiation for a longer period was evaluated as a possible way of improvement. However, comparison between 15 and 30 min of MAE revealed no significant difference (data not shown) so 15 min was kept as extraction time.

The CEM MW system employed in this work offers the possibility of stirring the sample contained in the closed vessels, using Teflon coated stir bars, while they are exposed to the microwave field. It was observed that stirring did not improve the extraction efficiency (data not provided) therefore, this option was not further employed.

As stated before, the lower recoveries were found for TPrP and TCEP, which are two of the more polar OPs [1]. Therefore, the use of a more polar organic solvent might be a way to improve the extraction efficiency. A first extraction was performed with 4 mL of acetone under already optimized conditions (15 min, 150 °C), the supernatant removed and a second extraction with acetonitrile, under the same conditions, was carried out. Recoveries achieved for the more polar compounds rose to values above 80%. The use of acetone in both cycles was also evaluated but provided lower recoveries than acetonitrile in the case of TPrP, so the use of a different solvent for each extraction step was needed.

In an attempt to reduce the amount of solvent employed to perform the extractions, the use of 2 mL per cycle, instead of 4 mL, was evaluated (data not shown). Recoveries were the same, except in the case of TPPO, for which the extraction yield dropped to 50% when using the lower volume. Therefore, it was not possible to decrease the solvent amount.

3.4. Method performance and real sample analysis

Instrumental limits of quantification (LOQs) of the GC-ICP-MS system, calculated as the concentration of analyte giving a signal 10 times the standard deviation of the background noise of the chromatographic signal, were 10 ng mL⁻¹ for TCPP (0.9 ng mL⁻¹, as phosphorus) and 5 ng mL⁻¹ for the remaining OPs (equivalent to 0.4-0.7 ng mL⁻¹ when calculated for phosphorus). These values are in the same order of magnitude as the limit of detection previously reported for TPP [21]. Injection repeatability was evaluated at 25 ng mL⁻¹ and remained between 3% and 8%. Linearity was evaluated with standards prepared in ethyl acetate at concentrations from 5 to 3000 ng mL⁻¹. Correlation coefficients between 0.993 and 0.999 were obtained.

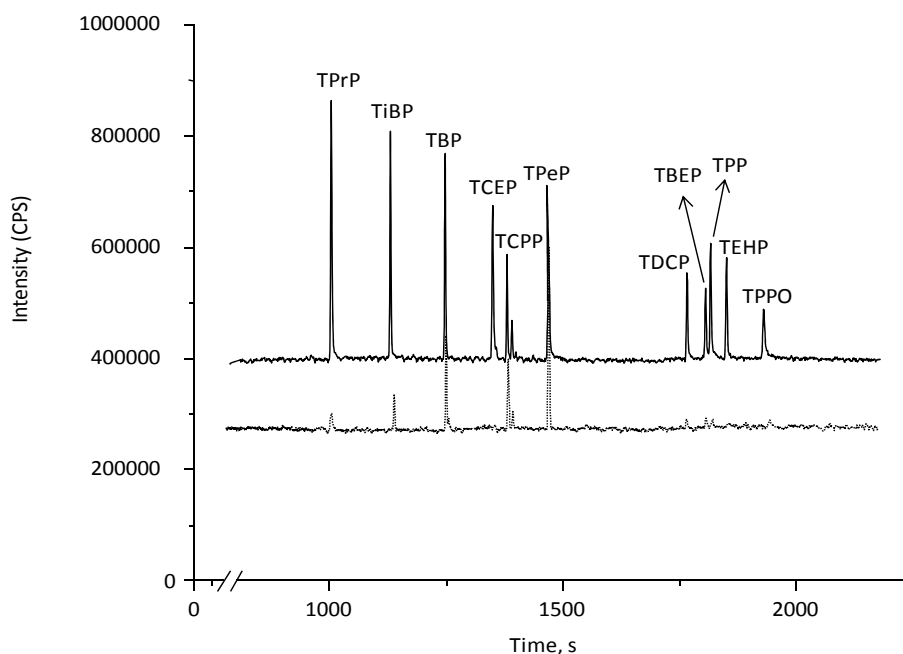
Recoveries of the proposed sample preparation method were evaluated with sediment samples spiked at three different concentration levels. Fractions of the spiked samples, as well as a non-spiked fraction of the same sediment, were extracted in triplicate under optimal conditions given in the experimental section. As shown on **Table 3**, recoveries ranged from 78% to 105%, with relative standard deviations (RSD) below 12%. Those values were calculated with standard addition over sample extracts, since different slopes were noticed for calibration graphs corresponding to solutions of OPs in ethyl acetate and matrix matched standards (spiked microwave-assisted extracts from unpolluted sediment samples). This behavior is probably related to differences in the efficiency of mass transfer processes from the body of the injector to the GC column for pure standards and sample extracts [24].

Table 3. Recoveries, as percentage, of the optimized method for three different spike levels, $n = 3$ replicates.

| | Recovery \pm SD | | |
|------|-----------------------|------------------------|-------------------------|
| | 50 ng g ⁻¹ | 200 ng g ⁻¹ | 1000 ng g ⁻¹ |
| TPrP | 100 \pm 2 | 83 \pm 1 | 89 \pm 2 |
| TiBP | 98 \pm 12 | 93 \pm 2 | 96 \pm 7 |
| TBP | 105 \pm 4 | 83 \pm 4 | 95 \pm 2 |
| TCEP | 103 \pm 7 | 78 \pm 6 | 81 \pm 4 |
| TCP | 92 \pm 9 | 81 \pm 3 | 100 \pm 2 |
| TDCP | 94 \pm 6 | 91 \pm 2 | 98 \pm 2 |
| TBEP | 94 \pm 3 | 78 \pm 3 | 96 \pm 1 |
| TPP | 103 \pm 5 | 90 \pm 6 | 97 \pm 1 |
| TEHP | 103 \pm 4 | 103 \pm 4 | 101 \pm 1 |
| TPPO | 99 \pm 1 | 100 \pm 4 | 92 \pm 5 |

Re-extraction of solid residues after MAE of the sediment spiked at 1000 ng g⁻¹, using the Soxhlet technique, confirmed the efficiency of the developed extraction method. On the other hand, procedural blanks demonstrated the absence of contamination problems. On the basis of these results, LOQs of 10 ng g⁻¹ (20 ng g⁻¹ for TCP) were obtained for the overall method. In practice, these values can be reduced until 2 and 4 ng g⁻¹, respectively, if the final extract is concentrated from 1 to 0.2 mL. A difference with other techniques employed in the determination of OPs in solid matrices, such as GC-NPD and GC-EI-MS, for which baseline level is strongly affected by the presence of co-extracted organic compounds, is the selectivity of ICP-MS detection which ensures an improvement in the S/N ratio of chromatographic peaks directly proportional to the final volume reduction of the sample extract. The above mentioned LOQs are slightly lower than those provided by Ishikawa et al. [6] using a GC-NPD system, with the advantage of a much more straightforward sample preparation procedure and similar to those reported for ultrasound-assisted extraction followed by LC-MS/MS (between 0.48 and 11 ng g⁻¹) [5]. Several sediment samples, collected from the different rivers in Spain and USA, were extracted following the optimized procedure. Chromatograms revealed the presence of traces of most OPs considered in this work, being TBP (2.8-8 ng g⁻¹) and TCP (4-10 ng g⁻¹) the most abundant species. **Fig. 5** shows the overlapped chromatograms of a 50 ng mL⁻¹ standard and a real sediment sample, concentrated to 200 μ L.

Fig. 5. Overlay of chromatograms of a 50 ng g^{-1} standard (solid line) with a concentrated sediment sample (dotted line).



4. Conclusions

MAE followed by GC-ICP-MS represents a valuable alternative for the determination of OPs in sediment samples. The proposed method presents a low consumption of organic solvents and requires a simple additional clean-up to preserve the performance of the GC column. Careful optimization of ICP-MS conditions, particularly with respect to the rf power and extraction lens voltage, resulted in quantification limits in the low ng mL^{-1} range for the OPs considered in this study. These values are similar to those reported for other techniques as GC-NPD, GC-MS and even LC-MS/MS. ICP-MS provides much less complex chromatograms, almost free of interferences, especially when compared to the above mentioned mass spectrometric techniques. These features point to GC-ICP-MS as a suitable technique for the reliable determination of OPs in samples with higher organic matter content than sediments, such as settled dust and particulate matter from indoor and outdoor environments, where OPs are ubiquitous pollutants, and sludge from sewage treatments plants, whose OPs content remains mostly unknown.

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1.6. PUBLICACIÓN:

***PRESSURIZED LIQUID EXTRACTION OF ORGANOPHOSPHATE TRIESTERS FROM
SEDIMENT SAMPLES USING AQUEOUS SOLUTIONS.**

M. García-López, I. Rodríguez, R. Cela

J. Chromatogr. A 1216 (2009) 6986

Pressurized liquid extraction of organophosphate triesters from sediment samples using aqueous solutions

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Abstract

A novel procedure for the extraction of seven organophosphate triesters (OPs), used as flame retardants and plasticizers, from sediment samples has been developed. It is based on pressurized liquid extraction of the analytes with aqueous solutions, combined with a further concentration step using solid-phase extraction (SPE) and followed by gas chromatography coupled to mass spectrometry (GC-MS) determination. The effects of different variables on the yield and selectivity of the sample preparation process are systematically evaluated. The optimal responses were observed extracting 2 g of sediment with a water:acetonitrile (75:25) solution at 90 °C and 1500 psi for 5 min. The obtained extract was made up to 200 mL with ultrapure water and passed through an OASIS HLB, 60 mg cartridge. Analytes were recovered with 2 mL of ethyl acetate and this extract concentrated to a lower volume, ca. 0.2 mL. Recoveries of the proposed extraction method ranged from 77 to 111%, with relative standard deviations below 10%, for spiked river and marine sediment samples with total carbon contents (TC) up to 4.0%. The limits of quantification (LOQs) of the method varied between 0.5 and 5 ng g⁻¹. Analysis of non-spiked sediment samples revealed the presence of low levels for some of the investigated species, with the highest concentration (47 ng g⁻¹) corresponding to tris(2-chloroethyl) phosphate (TCEP).

Keywords: Organophosphate compounds; Flame retardants; Pressurized liquid extraction; water extraction; GC-MS; Sediment samples.

1. Introduction

Organophosphate triesters (OPs) are frequently used flame retardants and plasticizers. Varnishes, hydraulic fluids, polyurethane foams and textiles are some of the products they are added to [1]. Their extensive use as additives is responsible for their occurrence in the environment, mainly in surface water, wastewater, air and dust [2-4]. This fact added to the persistence of some species, particularly the chlorinated OPs [5,6], limited water solubility and high adsorption to particulate matter of others [2] might lead to their accumulation in environmental solid samples, such as sediments. Up to the present moment, just a few studies have dealt with the determination of OPs in sediment samples [7-10]. In most of these works, sample preparation is time-consuming, requires large amounts of organic solvents and/or presents a limited selectivity, which makes necessary including further clean-up steps in the sample preparation scheme.

The use of water as extractant is an appealing alternative due to its low cost, environmental friendliness and selectivity [11]. At room temperature and atmospheric pressure,

the polarity of water is too high to efficiently extract most non-ionic organic species which are associated with soil particles. However, its dielectric constant can be lowered down to values similar to those of organic solvents by increasing the temperature at moderate pressures to keep water in the liquid state [12]. This modality has been named as subcritical water extraction (SWE) or pressurized hot water extraction (PHWE). Typical applications of this technique to recover low polar compounds from medium complexity matrices, e.g. sediments, require the use of temperatures in the range from 250 to 325 °C [11,13,14]. Practical drawbacks of operating at such high temperatures are related to the risk of analytes degradation and the lack of commercially available extraction devices [11].

Alternatively to pure water, its binary mixtures with miscible organic solvents, e.g. methanol, isopropanol, acetone and acetonitrile, usually provide acceptable extraction yields operating at temperatures below 200 °C, achieving a good selectivity and reducing the risk of analytes thermal decomposition. An additional advantage of employing aqueous solutions instead of pure water is that extractions can be automated using commercially available instrumentation, such as pressurized liquid extractors able to operate in the range of temperatures from 40 to 200 °C. Up to now, pressurized aqueous solutions have been successfully applied to the extraction of organochlorine pesticides, chlorobenzenes, chlorophenols and polycyclic aromatic hydrocarbons from soil and sediment samples [15-17].

As far as we could trace, only two methods based on the use of pressurized solvents have been applied to the extraction of OPs from sludge [18] and dust [19], respectively. In both cases, ethyl acetate was used as extractant and a very limited selectivity was obtained in the extraction process; consequently, an extra clean-up was necessary. Additionally to the above referred studies, some OPs have also been included in multi-residue methods for the determination of anthropogenic pollutants in sediments, using pressurized, heated mixtures of isopropanol:water [20]. However, in the above work the percentage of isopropanol represented up to 80% of the extraction solution and the yield of the process for several chlorinated OPs remained below 50 %.

The aim of this research work was to evaluate the possibilities and limitations of pressurized aqueous mixtures, containing moderate percentages (up to 25%) of different organic solvents, for the extraction of seven OPs compounds, showing large differences among their polarities (log K_{ow} values from 1.4 to 4.6 units), from sediment samples. The obtained extract was diluted with ultrapure water and further concentrated using the SPE technique. Alternatively, the feasibility of employing solid-phase microextraction (SPME), under previously optimized conditions for the extraction of OPs from water samples [21], to concentrate the raw extract from sediments, is also discussed. After extraction, analytes were determined by gas chromatography with mass spectrometry (GC-MS) detection.

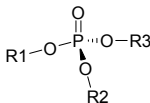







2. Experimental

2.1. Reagents, material and standards

Ultrapure water, obtained from a Milli-Q system purchased from Millipore (Bedford, MA, USA), was employed throughout this study. HPLC-grade acetonitrile, acetone and methanol,

as well as trace analysis ethyl acetate and *n*-hexane were purchased from Merck (Darmstadt, Germany). Sodium chloride was provided by VWR (Leuven, Belgium). Silicon dioxide acid-washed was acquired from Riedel-de Haën (Seelze, Germany). Tripropyl phosphate (TPrP), triisobutyl phosphate (TiBP), tributyl phosphate (TnBP), tris(2-chloroethyl) phosphate (TCEP), tris(dichloropropyl) phosphate (TDCP) and triphenyl phosphate (TPP) were acquired from Aldrich (Milwaukee, WI, USA). Tris(2-chloropropyl) phosphate (TCPP), as a technical mixture of isomers, was provided by Dr. Ehrenstorfer (Augsburg, Germany). Tripentyl phosphate (TPeP) was purchased from TCI Europe (Zwijndrecht, Belgium) and used as internal standard (IS). Chemical structures and octanol-water partition coefficients of target analytes are summarized in **Table 1**. Individual stock solutions of each species and the IS were prepared in acetone. Diluted standards and mixtures of OPs were made in *n*-hexane, when used to spike sediment samples, and in ethyl acetate, when employed to assess the performance of the GC-MS system. The stock solution of the IS was also diluted with ethyl acetate and added to calibration standards and sample extracts.

Table 1. Structures and octanol-water (K_{ow}) partition coefficients of selected species.

|  | | |
|---|--|---------------------------|
| | Substituents | ^a Log K_{ow} |
| TPrP | $R_1=R_2=R_3=$  | 1.87 |
| TiBP | $R_1=R_2=R_3=$  | 3.60 |
| TnBP | $R_1=R_2=R_3=$  | 4.00 |
| TCEP | $R_1=R_2=R_3=$  | 1.44 |
| TCPP | $R_1=R_2=R_3=$  | 2.59 |
| TDCP | $R_1=R_2=R_3=$  | 3.65 |
| TPP | $R_1=R_2=R_3=$  | 4.59 |

^a Values taken from Ref. [2].

Reversed-phase Oasis HLB (60 mg) cartridges were provided by Waters (Milford, MA, USA). Cellulose and glass fiber filters, placed at the bottom and top of PLE cells, were purchased from Restek (Bellefonte, PA, USA). A manual SPME holder and poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB, 65 μ m film thickness) fibers were obtained from Supelco (Bellefonte, PA, USA).

2.2. Samples

River and marine sediments were used throughout this study. All samples were obtained from small rivers and marine estuaries located in Galicia (Northwest Spain). Samples were air dried in a hood and sieved to increase their homogeneity. The fraction with a particle size below 0.3 mm was employed for analysis. Optimization of extraction and concentration processes was carried out with a pool of five sediments spiked at the 500 ng g⁻¹ level. For the method performance evaluation, discrete sediment samples with different total carbon (TC) contents, spiked at 200, 50 and 20 ng g⁻¹ for each OPs, were employed. The spiking procedure consisted of mixing an accurately weighed fraction of sediment with a standard solution of OPs in *n*-hexane, using a volume of solvent which guaranteed that the sample was completely covered. This slurry was thoroughly stirred and left in a hood until complete evaporation of the solvent. Then, it was stored in amber glass vessels at 4 °C, for at least 2 weeks before extraction.

2.3. Sample preparation

A Dionex (Sunnyvale, CA, USA) ASE 200 system, equipped with 11 mL capacity stainless steel cells, was used to extract OPs from sediments. A cellulose filter followed by a glass fiber one were placed at the bottom of each extraction cell, then 2 g of acid-washed silicon dioxide and the same mass of sediment were loaded into the cell. The remaining free space was filled with silicon dioxide. Finally, one cellulose filter was placed on top. Under optimized conditions, water containing a 25% of acetonitrile was employed as extractant. Extractions were performed at 90 °C and 1500 psi, using a single static extraction cycle of 5 min. Flush volume and purge time were set at 60% and 300 s, respectively.

Raw extracts provided by the PLE system (ca. 16 mL) were made up to 200 mL with ultrapure water and concentrated using a 60 mg OASIS HLB cartridge, previously pre-conditioned with ethyl acetate, methanol and water:acetonitrile (98:2), 3 mL each. Then, the sorbent was dried with a stream of nitrogen for 20 min and analytes were recovered with 2 mL of ethyl acetate. After addition of the internal standard (TPeP), the extract was either injected directly in the GC-MS system, without any additional clean-up, or concentrated with a gentle stream of nitrogen to a lower volume, ca. 0.2 mL, in case of samples fortified at low concentration levels and also to investigate the concentrations of target OPs in real life sediment samples.

2.4. Determination

Analytes were determined by GC-MS, using a Varian (Walnut Creek, CA, USA) CP 3900 gas chromatograph connected to an ion trap mass spectrometer (Varian Saturn 2100). Separations were carried out using a HP-5 MS type capillary column (30 m x 0.25 mm i.d., d_r: 0.25 μm) supplied by Agilent (Wilmington, DE, USA). Helium (99.999%) was used as carrier gas at a constant flow of 1.2 mL min⁻¹. The GC oven was programmed as follows: 70 °C (held for 1 min), at 15 °C min⁻¹ to 270 °C (held for 10 min). The GC-MS interface and the ion trap temperatures were set at 280 and 220 °C, respectively. Injections (2 μL volume) were made in the splitless mode (1 min splitless time), with the injector port at 270 °C. The mass spectrometer was operated in the electron impact ionization mode (70 eV). Electron-impact mass spectra were recorded in the range of *m/z* from 90 to 400 units. Most intense ions, used to quantify the

concentration of each compound in sediment samples are given in **Table 2**. Quantification was carried out using standard addition over final extracts obtained from SPE cartridges, in order to compensate for differences in the efficiency of mass transfer from the injector to the capillary column, in the GC system, between pure standards and sample extracts [10].

Table 2. Linearity, repeatability ($n = 5$ replicates) and instrumental limits of quantification (LOQs), defined for a S/N of 10, of the GC-MS system.

| Compound | Quantification ions (m/z) | Correlation coefficient (R^2) | Repeatability (RSD%) | | LOQ (ng mL^{-1}) |
|----------|-------------------------------|-----------------------------------|------------------------|-------------------------|-----------------------------|
| | | | 40 ng mL^{-1} | 400 ng mL^{-1} | |
| TPrP | 99 | 0.996 | 5 | 3 | 5 |
| TiBP | 99 | 0.993 | 2 | 6 | 6 |
| TnBP | 99 | 0.996 | 6 | 4 | 6 |
| TCEP | 249+251 | 0.999 | 6 | 1 | 8 |
| TCPP | 277+279 | 0.997 | 5 | 2 | 10 |
| TDCP | 379+381+383 | 0.995 | 5 | 5 | 8 |
| TPP | 325+326 | 0.994 | 6 | 4 | 4 |

3. Results and discussion

3.1. Performance of GC-MS

Table 2 summarizes some relevant data related to the determination step. The linearity in the responses of the GC-MS system was evaluated with standards, in ethyl acetate, at six different concentration levels from 10 to 2000 ng mL^{-1} . Correlation coefficients from 0.993 to 0.999 were obtained within the above interval. Relative standard deviations for five consecutive injections of standards at two different concentration levels, 40 and 400 ng mL^{-1} , ranged from 1 to 6%. Instrumental limits of quantification (LOQs) of the GC-MS system, calculated as the concentration of analyte giving a signal 10 times the standard deviation of the background noise, for chromatograms monitored using the most intense ions for each OPs, ranged from 4 ng mL^{-1} for TPP up to 10 ng mL^{-1} for TCPP.

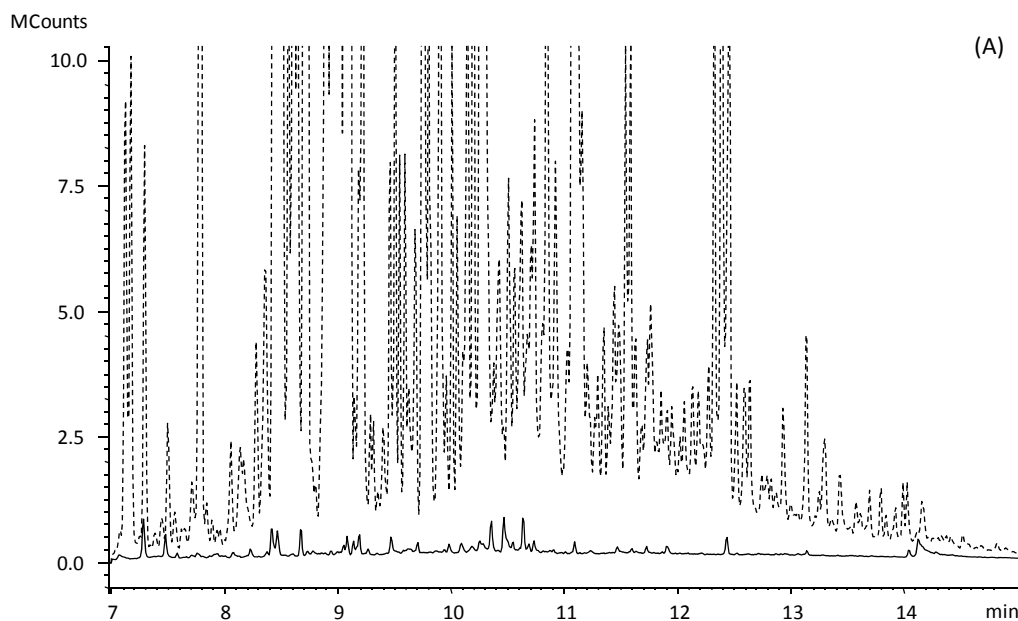
3.2. Optimization of extraction conditions

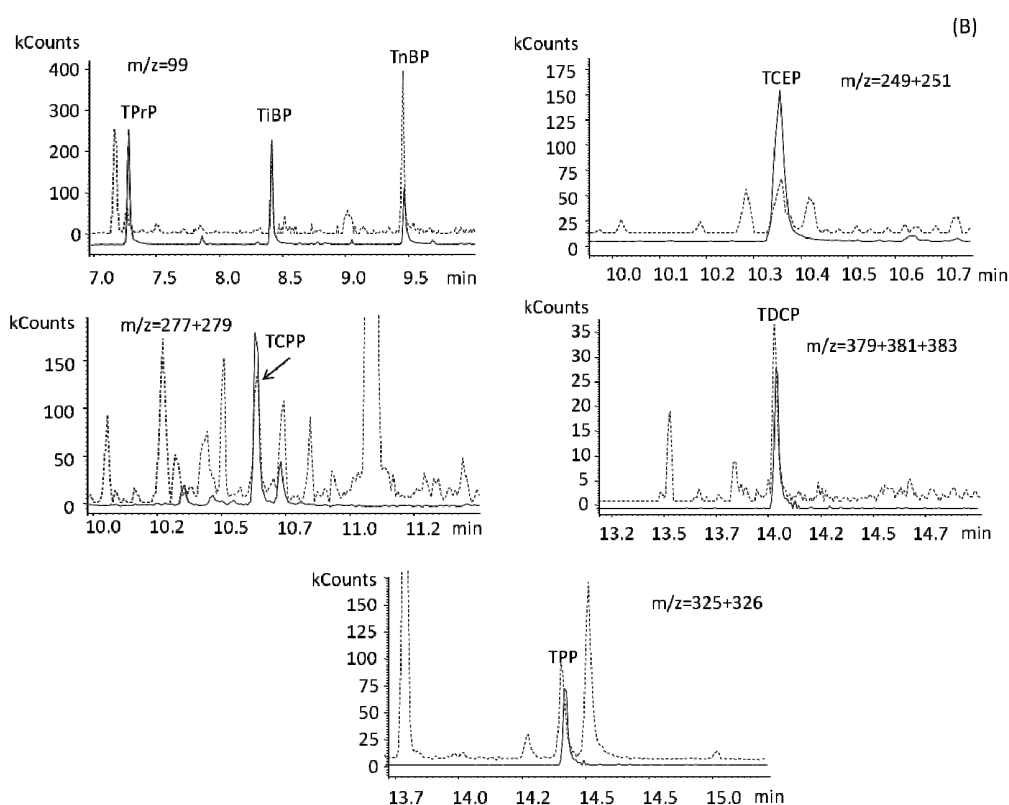
3.2.1. Preliminary assays

Initially, two spiked sediments with different total carbon (TC) contents (ca. 1 and 3%) and three extraction solutions: water, acetonitrile and water:acetonitrile (90:10) were considered. Water and water:acetonitrile extractions were carried out in the range of temperatures from 120 to 200 °C; whereas, a value of 120 °C was fixed when extractions were performed with pure acetonitrile. In all cases, a single static extraction cycle of 10 min at 1500 psi was employed. Flush volume and purge time were set at default values: 60% and 1 min, respectively. The extracts in pure water were directly concentrated using an OASIS HLB cartridge

(60 mg). Those corresponding to water:acetonitrile (90:10) were first diluted to 100 mL with ultrapure water and those obtained with 100% of acetonitrile were first evaporated to about 10 mL and then diluted to 500 mL with ultrapure water. After that, they were concentrated using same SPE conditions as those reported for water and water:acetonitrile extracts [21]. Cartridges were eluted with 2 mL of ethyl acetate and extracts injected in the GC-MS system. Operating within the above range of temperatures, water alone failed to extract TPP, the most hydrophobic of the considered OPs (**Table 1**), even from the sediment with the lower carbon content. For the most complex one, just TPrP and TCEP, the most polar of the considered compounds, were noticed in the corresponding chromatograms. On the other hand, all OPs were found in water:acetonitrile (90:10) and acetonitrile extracts from both sediments. Chromatograms corresponding to extractions performed at 120 °C are shown in **Fig. 1**. As appreciated from total ionic current (TIC), **Fig. 1A**, and selected ions chromatograms, **Fig. 1B**, acetonitrile provided much more complex extracts than its binary mixture with water. Consequently, the signal to noise (S/N) ratio of OPs peaks was considerably higher in the latter situation. On the basis of the above comments, it is evident that the mixture of water with acetonitrile provides favorable features, in terms of efficiency and selectivity, when compared to any of both solvents used individually. Therefore, extraction conditions were systematically optimized considering aqueous solutions containing up to 25% of different organic solvents as modifiers.

Fig. 1. Overlay of GC-MS chromatograms for a spiked sediment sample (3% of carbon, addition level 500 ng g^{-1}) using acetonitrile (dotted line) and water:acetonitrile 90:10 (solid line) as extractants. TIC (A) and selected ions chromatograms (B).





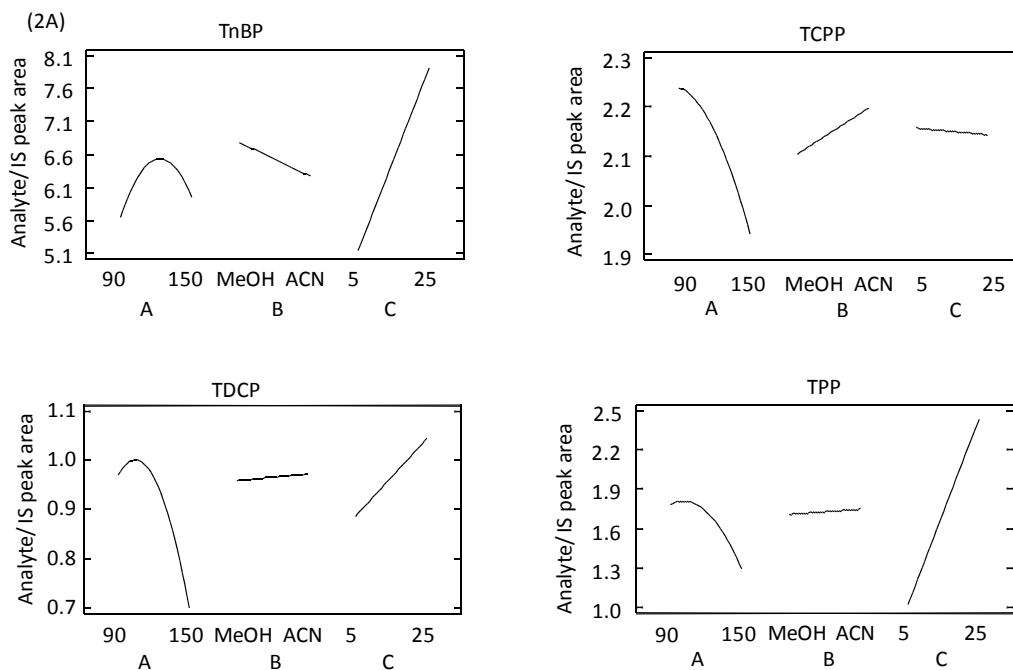
3.2.2. Pressurized liquid extraction parameters

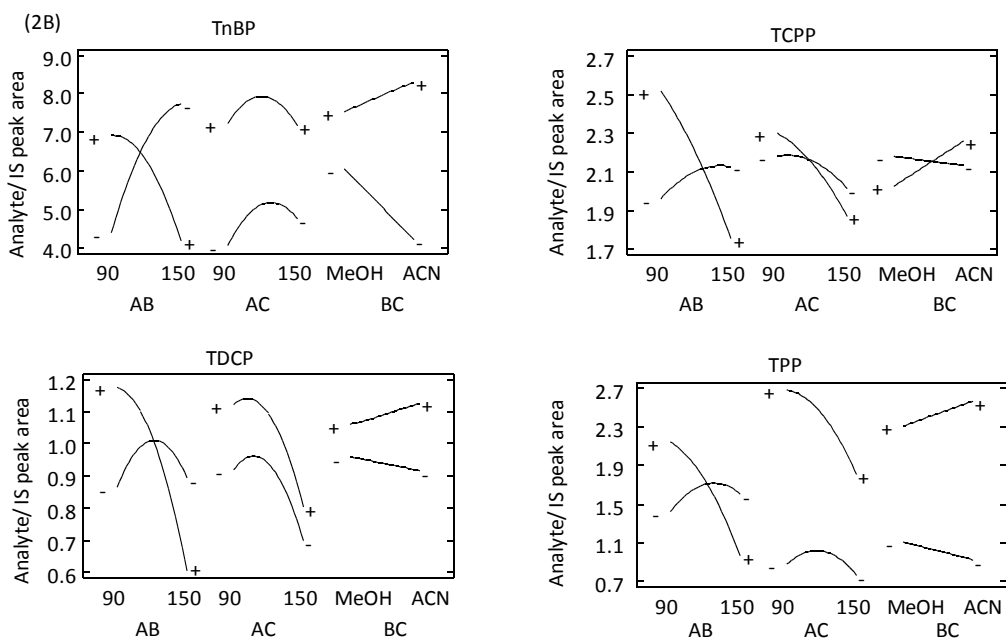
Systematic optimization of PLE extraction conditions was carried out with 2 g fractions from a pooled (river and marine) sediment sample. This matrix, showing a TC of 6.2%, was fortified with target compounds at 500 ng g^{-1} , using the procedure reported in section 2.2, and then, aged for 1 month before extraction. The effects of temperature, type and percentage of organic solvent on the efficiency of the extraction were simultaneously evaluated using a mixed level $3^1 \times 2^2$ experimental factorial design, with 2 central points. The variable temperature was evaluated at three levels in the range from 90 to 150 °C. Higher values were not considered since they are expected to reduce the selectivity of the extraction. The percentage of organic solvent was varied between 5 and 25% and two solvents with different polarities: methanol and acetonitrile were considered as modifiers, **Table 3**. Extracts were diluted, prior to the concentration step, with the amount of water necessary to maintain the percentage of organic solvent below 2%, otherwise TPrP, TCEP and TCPP might be partially lost in the SPE concentration step.

Table 3. Domain of the $3^1 \times 2^2$ experimental factorial design.

| Factor | Code | Level | | |
|-------------------------|------|----------|--------|--------------|
| | | Lower | Medium | Upper |
| Temperature (°C) | A | 90 | 120 | 150 |
| Organic modifier | B | Methanol | - | Acetonitrile |
| Modifier percentage (%) | C | 5 | - | 25 |

Responses (analyte peak area/IS peak area) obtained in the 14 experiments involved in the above design were processed with the Statgraphics Centurion XV (Manugistics, Rockville, MD, USA) software. Main effects and two-factor interactions for some of the investigated OPs are graphically depicted in **Fig. 2**. The behavior of TnBP was similar to that of TPrP and TiBP, whereas TCPP and TCEP both followed a similar trend. The length of the lines in main effect plots (**Fig. 2A**) is proportional to the variation in the efficiency of the extraction, for a given compound, when the considered factor changes from the lower to the upper level, within the domain of the design. The sign of the slope indicates whether the extraction yield increases (positive slope) or decreases (negative slope) with the investigated factor.

Fig. 2. Graphics for main effects (A) and two-factor interactions (B) obtained from the $3^1 \times 2^2$ experimental factorial design.

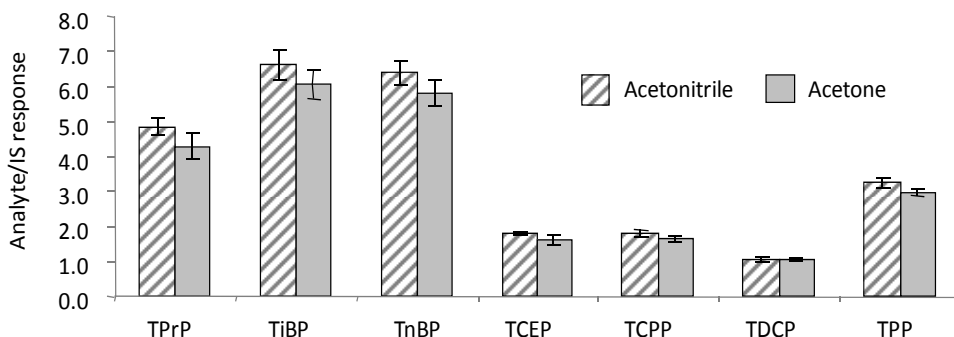


The percentage of modifier (variable C, **Table 3**) affected positively to the extraction of all species, except TCPP and TCEP. Moreover, its effect was statistically significant (95% confidence level) for those OPs showing log K_{ow} values over 3 units, **Table 1**. On the other hand, the type of modifier (methanol or acetonitrile) was the less important of the investigated factors and the sign of its effect was compound dependant. For TPP and the three chlorinated OPs, the variable temperature affected negatively to the extraction yield, being statistically significant for TDCP and TCPP. This behavior points that these compounds might be partially decomposed at high temperatures, which matches with previously reported results for some pesticides using similar extraction conditions [15]. In the case of TnBP (TiBP and TPrP followed a similar trend), the main effect associated to the extraction temperature (code A, **Table 3**) showed an important curvature, with a maximum at 120 °C (**Fig. 2A**), the medium level fixed for this variable in the experimental factor. In spite of this, neither the temperature nor the quadratic term of this variable, were of statistical significance for any of these three species.

Two factor interactions graphs (**Fig. 2B**) provided some relevant conclusions, particularly as regards temperature (code A, **Table 3**) and type of modifier (code B, **Table 3**) variables. For all compounds, a cross-shaped graph was noticed for the AB interaction, meaning that at 90 °C higher responses were achieved using acetonitrile as modifier; whereas, at 150 °C the use of methanol was more favorable. Except for TCPP and TCEP, the standardized value of the AB interaction overpassed the 95% confidence bound. Finally, the other two factor interactions (temperature-percentage of modifier, AC, and type-percentage of modifier, BC) played a negligible effect on the yield of the extraction, **Fig. 2B**.

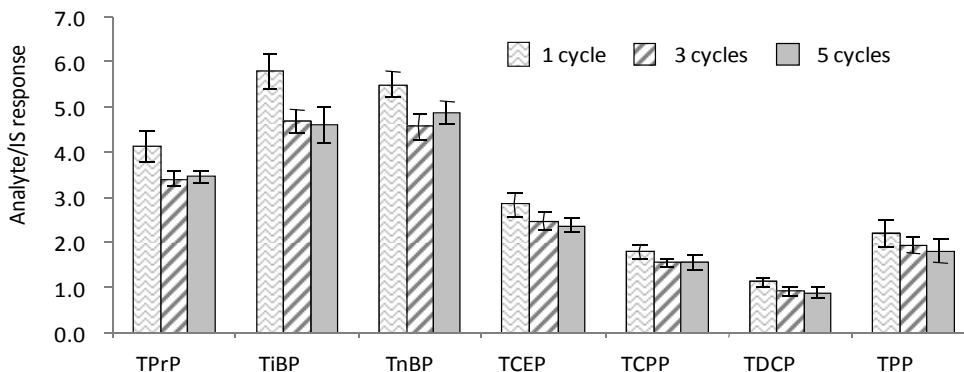
On the basis of above comments, further extractions were carried out at 90 °C using water containing a 25% of acetonitrile as organic modifier. Moreover, some additional assays were performed using acetone as alternative to acetonitrile. The former solvent had been previously employed in the extraction of OPs from dust [3,22] and it presents a lower polarity than acetonitrile. For TPrP, TiBP and TnBP slightly higher responses were observed using acetonitrile than with acetone, whereas for the rest of species no significant differences were observed between both modifiers, **Fig. 3**. Thus acetonitrile was kept as modifier in the extraction solution.

Fig. 3. Comparison of responses obtained using acetonitrile and acetone as organic modifiers (25%) in the extraction process. PLE at 90 °C and 1500 psi. A single static cycle of 10 min was used, n = 4 replicates.



Other variables, potentially affecting to the performance of PLE, such as the number of static extraction cycles, their duration, flush volume and purge time were also thoroughly investigated. **Fig. 4** shows the responses obtained for 1, 3 and 5 extraction cycles of 5 min.

Fig. 4. Comparison of responses as function of the number of extraction cycles, 5 min each, at 90 °C using water:acetonitrile (75:25) as extractant, 60% of flush volume.



For non-chlorinated, alkyl OPs (TPrP, TiBP and TnBP) obtained responses were slightly higher with one cycle than with more. For the rest of species, smaller differences were noticed;

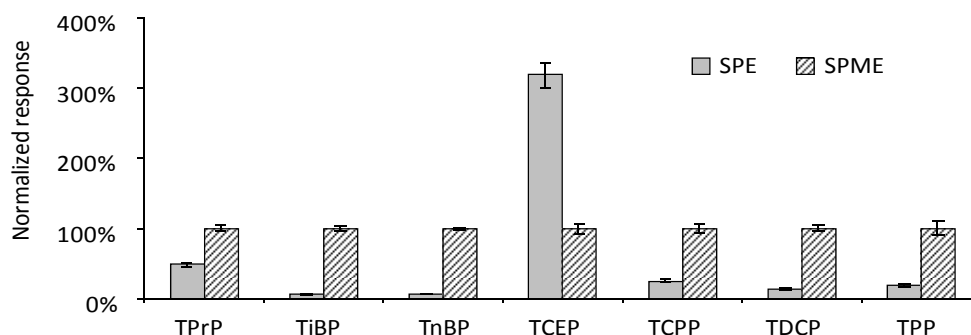
however, the trend was also a diminution in their responses with the number of extraction cycles. Therefore, one cycle was selected for further assays, which is advantageous in terms of extraction duration. After that, the duration of the static extraction cycle was varied between 5 and 25 min, data not shown. Responses obtained for triplicate extractions were independent of the extraction time, so it was kept at the lower value to speed up as much as possible the extraction process.

As regards the volume of extraction solution (flush volume, referred as a percentage of the cell volume: 11 mL) values between 60 and 150% were evaluated. Increasing the flush volume leads to an increase in the final volume of the obtained extract. Bearing this in mind, extracts were diluted, prior to the concentration step, with the amount of water necessary to maintain the percentage of acetonitrile below 2%, as previously remarked. Again, compounds responses were unaffected by this parameter (data not shown), thus 60% was selected in order to reduce the solvent consumption. The purge time controls the period during which nitrogen is passing through the stainless steel cell to sweep away all the solvent wetting the sample and the cell filling, at the end of the static extraction cycle. It can be changed between 0 and 300 s. In order to completely recover the aqueous-organic mixture employed in the extraction, the maximum allowed purge time was employed. Shorter periods were insufficient for complete solvent removal and thus, the cell filling remained wet once the extraction was concluded.

3.3. SPME versus SPE concentration

Alternatively to SPE, SPME was also considered for the concentration of OPs. In this case, extracts were made up to 25 mL with ultrapure water and an aliquot of 20 mL submitted to optimal SPME conditions previously reported for water samples [21]. In brief, a PDMS-DVB fiber was exposed to the extract, in the direct sampling mode, for 40 min at room temperature under magnetic stirring (1100 rpm), using a Teflon covered magnetic stir bar. Sodium chloride (2 g) was added to the extracts in order to improve the efficiency of the extraction, particularly for the most polar compounds. Higher salt concentrations could not be used since they promoted the separation of phases (water and acetonitrile) in the SPME vessel. Under the above conditions, responses (peak areas) obtained by SPME were between 2 and 15 times higher than those achieved using SPE as concentration technique considering a final extract volume of 2 mL, **Fig. 5**. The exception to this behavior corresponded to the highly polar TCEP, for which the response attained by SPME was 3-folds lower than using SPE, **Fig. 5**.

Fig. 5. Relative efficiencies for SPE (final extract volume 2 mL) and SPME as concentration techniques for sediment extracts, $n = 3$ replicates. Depicted values correspond to the pooled sediment matrix used in the experimental factorial design.



Taking into account that (1) TCEP is one of the most environmentally concerning organophosphorous flame retardants, (2) SPE extracts can be concentrated to a lower volume ca. 0.2 mL without noticeable losses of target species and (3) SPE extractions, at difference to SPME ones, can be performed simultaneously, the former was maintained as the concentration technique in the proposed sample preparation approach.

3.4. Method performance

Recoveries of the proposed method were evaluated with river and marine sediment samples, with different TC contents, spiked with target compounds at several concentrations in the range from 20 to 200 ng g⁻¹. Spiked fractions of each sample were aged for 2 weeks before extraction; moreover, non-fortified ones were also processed. **Table 4** summarizes the obtained results for triplicate extractions.

Table 4. Recoveries of the proposed method with their standard deviations (SD) and LOQs of the overall method, $n = 3$ replicates.

| Sediment type | Recovery (%) ± SD | | | | | LOQ (ng g ⁻¹) |
|---------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------------|
| | River (TC: 2.3%) | | River (TC: 1.2%) | | Marine (TC: 4.0%) | |
| | 50 ng g ⁻¹ | 200 ng g ⁻¹ | 20 ng g ⁻¹ | 200 ng g ⁻¹ | 50 ng g ⁻¹ | |
| TPrP | 88 ± 7 | 95 ± 7 | 104 ± 4 | 94 ± 5 | 90 ± 3 | 0.5 |
| TiBP | 87 ± 8 | 107 ± 8 | 106 ± 5 | 98 ± 4 | 77 ± 6 | 3 |
| TnBP | 85 ± 9 | 82 ± 3 | 107 ± 6 | 90 ± 6 | 87 ± 4 | 0.6 |
| TCEP | 103 ± 7 | 100 ± 7 | 109 ± 7 | 87 ± 6 | 92 ± 6 | 0.8 |
| TCPP | 98 ± 4 | 109 ± 5 | 96 ± 8 | 100 ± 4 | 81 ± 7 | 5 |
| TDCP | 95 ± 8 | 110 ± 6 | 89 ± 5 | 99 ± 7 | 98 ± 8 | 0.8 |
| TPP | 95 ± 5 | 110 ± 2 | 81 ± 5 | 79 ± 6 | 111 ± 4 | 4 |

Recoveries from 77 to 111%, with standard deviations below 10, were attained for all compounds in the three considered samples. GC-MS traces for the lower level spiked sample (20 ng g^{-1}) are presented in Fig. 6. In order to assess the yield of the proposed method with more complex matrices, an indoor dust sample, TC 25.1%, was processed using the method developed in this work and a microwave assisted extraction (MAE) protocol, specifically designed to deal with dust samples, using acetone as extractant [3]. Results obtained with both approaches are compared in Table 5. TPrP and TDCP remained under the LOQ of the method, whereas values for the rest of species ranged from 64 to 6200 ng g^{-1} . A good agreement was noticed between the measured concentrations of TiBP, TCPP and TPP using both extraction procedures; however, TnBP could not be quantified in MAE extracts due to co-elution with an interference showing the same m/z ratio. Finally, the protocol developed in this work provided a significantly higher concentration of TCEP, the most polar of the investigated species, than MAE.

Fig. 6. Selected ion chromatograms for a river sediment spiked at the 20 ng g^{-1} level.

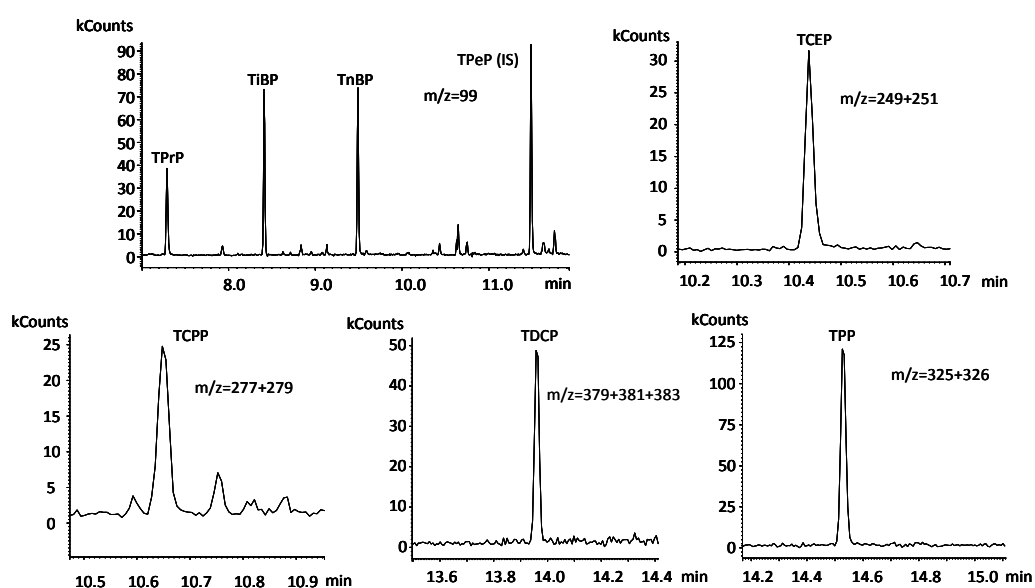


Table 5. Comparison of OPs concentrations (ng g^{-1}) found in a non-spiked dust sample (TC 25.1%) using two different extraction approaches, $n = 3$ replicates.

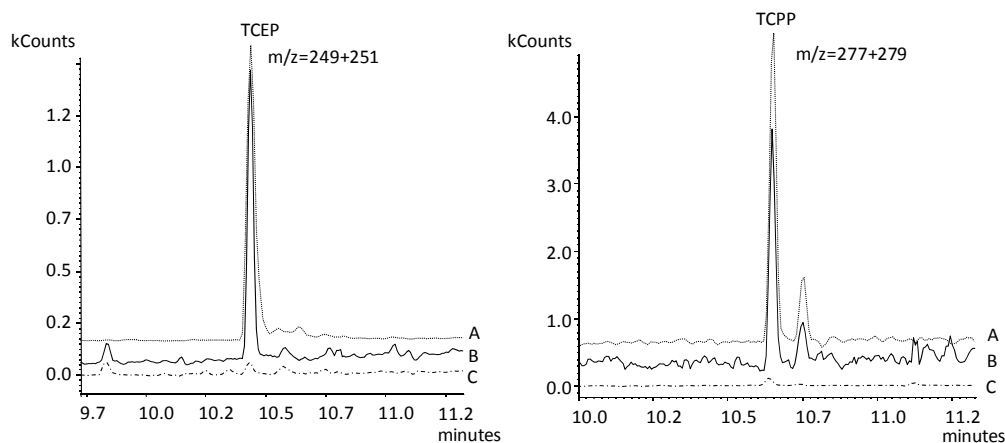
| Analyte | PLE (water:acetonitrile, 75:25) | | MAE (acetone) [Ref. [3]] | |
|---------|---------------------------------|-----|--------------------------|-----|
| | Mean | SD | Mean | SD |
| TiBP | 186 | 9 | 176 | 10 |
| TnBP | 222 | 7 | n.q. | - |
| TCEP | 6200 | 200 | 4100 | 200 |
| TCPP | 65 | 3 | 64 | 2 |
| TPP | 330 | 19 | 330 | 22 |

n.q., not quantified.

Procedural blanks often showed the presence of TiBP, TCPP and TPP at concentrations between 1 and 2 ng g⁻¹. The exact source of this contamination could not be identified; although, in case of TPP, it could be associated with the ultrapure water used in the extraction step and also to dilute the primary extract from sediment samples. Whatever the source of this contamination was, procedural blanks had to be periodically performed to avoid false positives during analysis of real, non-spiked, sediment samples. Limits of quantification (LOQs) of the proposed method were estimated considering a sample intake of 2 g and a final extract volume of 0.2 mL. Values from 0.5 to 5 ng g⁻¹ were achieved, **Table 4**. These LOQs are in the same range of values to those provided by LC-MS/MS (0.48-11 ng g⁻¹) and GC-ICP-MS (2-4 ng g⁻¹) [8,10].

Several sediments were subjected to the optimized procedure and in most of them, OPs remained below the LOQs of the method; however, relatively low levels of some target analytes were detected in two of the analyzed samples: TiBP (7.80 ± 0.05 ng g⁻¹), TCEP (45.9 ± 0.1 ng g⁻¹) and TPP (6.4 ± 0.3 ng g⁻¹) were found in one of them and TCPP (38 ± 2 ng g⁻¹) was present in the other, **Fig. 7**. Above values are significantly lower than maximum concentrations of OPs measured in sediments from highly industrialized areas in Germany [23], urban areas in Austria [8] and a waste disposal site in Japan [9]. They are also one order of magnitude lower than concentrations found in solid samples from indoor environments, e.g. dust [1-3].

Fig. 7. Overlay of GC-MS chromatograms for TCEP and TCPP in sediment samples. (A) OPs standard (20 ng mL⁻¹); (B) un-spiked sediment; (C) procedural blank.



4. Conclusions

A valuable sample preparation method for the extraction of seven OPs from sediment samples has been proposed. Pressurized liquid extraction using aqueous solutions, with a 25% of acetonitrile, provided recoveries over 77% for spiked sediment samples with different carbon contents, it required a very low consumption of organic solvents (ca. 4 mL in the extraction step) and it presented an improved selectivity versus the use of organic solvents as extractants. Optimization studies showed that maximum extraction yields were achieved at relatively low temperatures, which minimized the risk of analytes decomposition and also allowed to automate the extraction process by using commercially available PLE instrumentation. Real life sample

analysis pointed to the existence of just very low levels of OPs in environmental sediments probably due to the high water solubility of certain species and the rapid hydrolysis of the most hydrophobic ones. Preliminary results suggest the possibility of broadening the application field of the developed method to more complex matrices, such as indoor dust where OPs levels reach the μg per g level.

Acknowledgements

M. G.-L. thanks the Spanish Ministry of Science and Innovation for her FPU contract. Financial support from regional, national and FEDER funds (projects DGICT CTQ2006-03334 and PGIDIT06PXIB237039PR) is also acknowledged.

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1.7. Comentarios adicionales

Todas las metodologías recogidas en este capítulo son aptas para el análisis de los organofosforados en las matrices correspondientes, a saber: polvo y sedimentos. En ambos casos el número de trabajos publicados con anterioridad era bastante limitado. En lo que respecta al análisis de OPs en polvo, las dos técnicas de extracción utilizadas, MAE y MSPD, no se habían empleado previamente para ese fin. En el caso de los sedimentos, la extracción asistida por microondas había sido aplicada con anterioridad por De Geus y col. [1], utilizando un microondas casero. Llevaron a cabo la extracción de 6 organofosforados, entre los que el TPP era el único compuesto en común con los analitos incluidos en esta Tesis Doctoral, empleando 7.5 mL de una mezcla hexano:acetato de etilo:diclorometano (1:1:1) en viales abiertos. Por este motivo, los tiempos de irradiación evaluados estaban condicionados por el tiempo necesario para la evaporación de la mezcla de disolventes. Ésta ebullición a los 15 segundos y, como la evaporación empieza ya antes, ensayaron ciclos de 9 segundos entre los que se esperaba el enfriamiento de la mezcla. El número de ciclos óptimo resultó ser 6. La recuperación del TPP no superó el 60%. Por otro lado, la extracción presurizada con disolventes se había empleado para extraer algunos OPs pero utilizando mezclas isopropanol:agua con un 80% de disolvente orgánico y las recuperaciones obtenidas para varios OPs estaban por debajo del 50%. Así pues, las metodologías propuestas son una contribución significativa en el ámbito de la determinación de los organofosforados en muestras sólidas.

A parte de los comentarios ya recogidos en las publicaciones sobre ventajas e inconvenientes de los métodos propuestos, en este apartado se enfatiza un poco más la comparativa de los mismos en función de aspectos relevantes tales como el tiempo de preparación de muestra o el coste del análisis, que pueden resultar útiles a la hora de seleccionar una técnica u otra.

1.7.1. Polvo

La extracción asistida por microondas proporciona recuperaciones análogas a las conseguidas con Soxhlet, con la ventaja de una reducción notable del consumo de disolvente y del tiempo de extracción, además de ofrecer la posibilidad de procesar simultáneamente varias muestras [2]. Como desventaja señalar que el extracto obtenido tiene una complejidad elevada, debido a las condiciones relativamente enérgicas en las que se lleva a cabo la extracción, por lo que es necesaria la introducción de una etapa de purificación exhaustiva en el protocolo. El esquema de limpieza propuesto es bastante tedioso y la etapa que más tiempo requiere en el proceso global de preparación de muestra. Por ello, dado que los procedimientos multietapa tienen más riesgo de pérdidas y contaminación, se centraron esfuerzos en acortar esta etapa del proceso analítico. La MSPD es una alternativa atractiva que disminuye notablemente el tiempo total de preparación de muestra, reduce su manipulación e integra la extracción y la purificación en un único paso. Sin embargo, la automatización no es sencilla [3]. En la **Tabla III.1** se comparan la extracción Soxhlet, MAE y MSPD en función de datos prácticos más concretos tales como la duración de la extracción, el consumo de disolventes, el coste y algunas ventajas e inconvenientes de cada técnica. Las tres técnicas son útiles para la determinación de OPs en polvo y proporcionan unos límites de cuantificación análogos (40-50 ng g⁻¹).

Tabla III.1. Comparación de tres técnicas de extracción disponibles para la determinación de OPs en polvo.

| Técnica | Soxhlet [2] | MAE [2] | MSPD [3] |
|-------------------------------|---|---|--|
| Preparación de muestra | 20 h | 4-5 h | 1 h |
| Vol. disolvente | 75 mL acetona (extracción) + 7 mL AcOEt (purificación) | 10 mL acetona (extracción) + 7 mL AcOEt (purificación) | 2 mL hexano (limpieza) + 3 mL acetona (elución) + 1 mL AcOEt |
| Coste instrumental | Bajo | Moderado | Bajo |
| Ventajas | R% excelentes de todos los OPs | R% excelentes de todos los OPs; posibilidad de extracciones múltiples | R% buenas para todos los OPs; extracción y purificación simultáneas; bajo consumo de disolventes; rapidez; posibilidad de extracciones simultáneas |
| Inconvenientes | Tiempos de extracción prolongados; consumo elevado de disolvente; necesidad de concentración del extracto | Tiempo de espera para el enfriamiento tras la extracción; necesidad de centrifugación del sobrenadante y de purificación exhaustiva | R% de TCEP, TCPP y TPP ligeramente inferiores que con Soxhlet o MAE |

AcOEt, acetato de etilo

1.7.2. Sedimentos

Las dos metodologías propuestas en esta Tesis Doctoral para el análisis de sedimentos proporcionan límites de cuantificación del mismo orden y análogos a los de otros trabajos publicados [4,5]: dentro del intervalo de 0.5 a 5 ng g⁻¹, adecuado para la detección de estos compuestos a los niveles generalmente bajos a los que se encuentran en este tipo de matriz. Además, los protocolos propuestos en esta Tesis conllevan un consumo mucho menor de disolventes: entre 6 y 10 mL, frente a los habitualmente más de 100 mL de otras metodologías (**Tabla II.12**).

Tanto los dos métodos desarrollados en la presente Tesis como, en general, los ya existentes, constan de una etapa de purificación utilizando LLE o SPE, por lo que al tiempo de extracción hay que sumarle el de este paso posterior. En el trabajo de aplicación de MAE a sedimentos, el tiempo de extracción se ve además incrementado por los lapsos que hay que esperar para el enfriamiento de los viales de microondas, con el fin de evitar pérdidas por evaporación.

La principal ventaja del método de PLE es su mayor grado de automatización y su compatibilidad con el uso de LC-MS como técnica de determinación, sin necesidad de la etapa de concentración-extracción mediante SPE. No obstante, esta posibilidad necesita ser contrastada mediante experimentos adicionales.

Una característica común a los dos métodos desarrollados para muestras de sedimentos fue una diferencia notable en las pendientes de las curvas de calibrado correspondientes a patrones y extractos de muestras. Esta diferencia, que no había sido observada previamente con extractos de polvo, se atribuye a efectos de inyección y complica la cuantificación de los analitos en esta matriz.

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2. Muestras acuosas

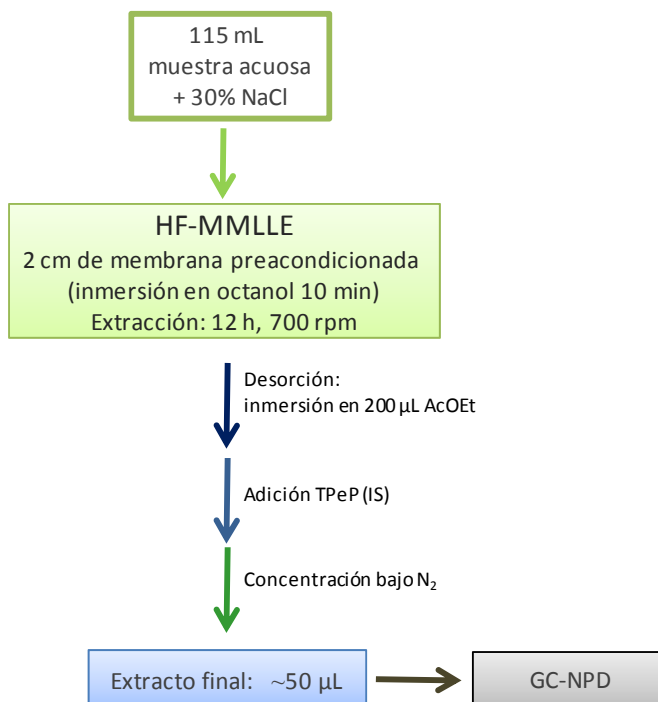
2.1. Introducción

Como ha quedado reflejado en el capítulo II, los OPs están presentes también en matrices acuosas, siendo los niveles más altos los determinados en aguas residuales. La mayoría de los trabajos que se ocupan de la cuantificación de estos compuestos en agua utilizan LLE o SPE (ver apartado II.4.2) y tan sólo dos técnicas de microextracción, SPME [1] y MASE [2], habían sido propuestas con anterioridad al inicio de nuestras investigaciones para esta matriz. La aplicación de la novedosa DLLME y de HF-MMLLE para la determinación de OPs en aguas ha sido nuestra contribución a la miniaturización de la etapa de preparación de muestra y reducción del coste y consumo de disolventes de la misma.

Finalmente, en la última etapa de esta Tesis Doctoral se incluyeron en el grupo de OPs a analizar los diésteres, que además de producirse intencionadamente, pueden generarse como resultado de la degradación de los triésteres [3]. Por la elevada polaridad de estos compuestos, se prefirió como técnica de separación y detección LC-ESI-MS/MS, ya que su separación por GC requeriría una derivatización previa, que complicaría la preparación de muestra. Además, la técnica es también adecuada para los triésteres, proporcionando unos LOQs excelentes. La metodología propuesta se basa en el uso de SPE y es la primera en la que se lleva a cabo el fraccionamiento de ambos grupos de compuestos y su determinación en la misma columna, utilizando la misma fase móvil y sin agente formador de pares iónicos.

2.2. Esquemas de los métodos desarrollados

Fig. III.5. Esquema para la determinación de OPs en muestras acuosas mediante HF-MMLLE y GC-NPD.



AcOEt, acetato de etilo

Fig. III.6. Esquema para la determinación de OPs en muestras acuosas mediante DLLME y GC-NPD.

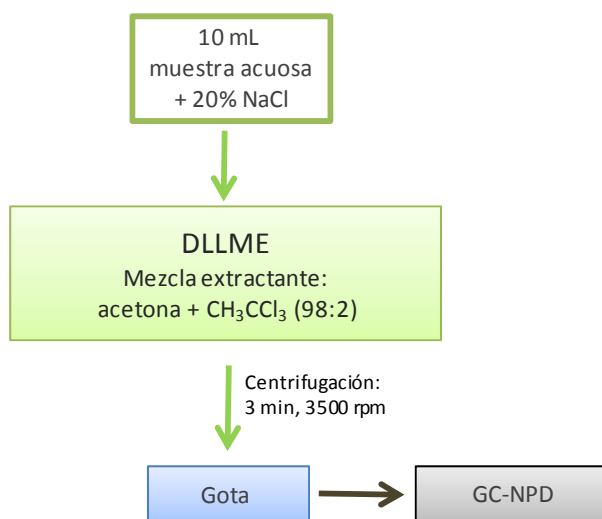
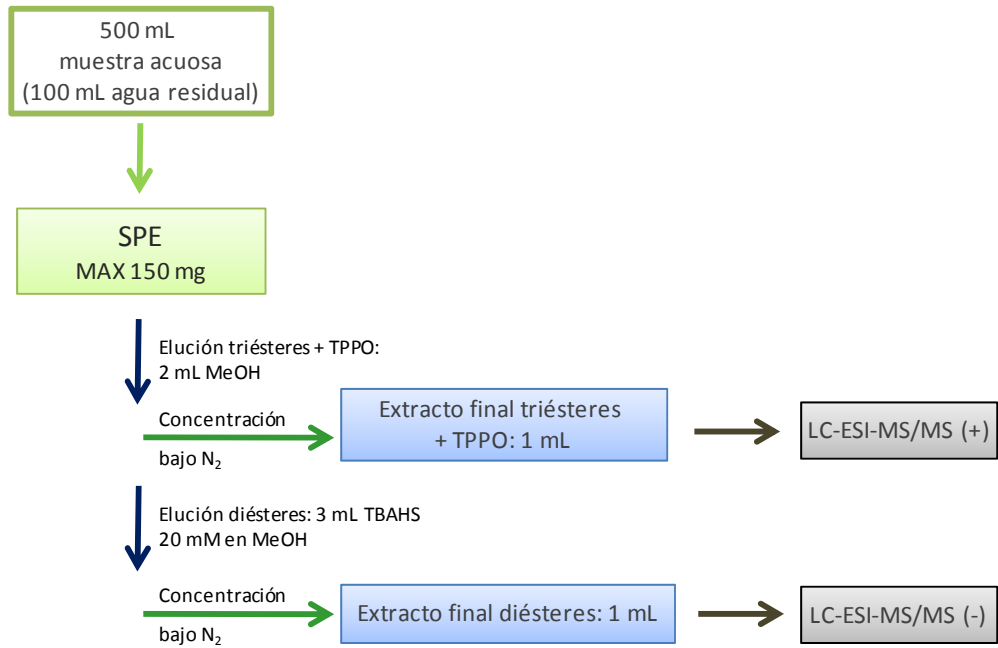


Fig. III.7. Esquema para la determinación de OPs en muestras acuosas mediante SPE y LC-ESI-MS/MS.



MeOH, metanol; TBAHS, hidrogenosulfato de tetrabutilamonio

2.3. PUBLICACIÓN:

***EVALUATION OF LIQUID-LIQUID MICROEXTRACTION USING POLYPROPYLENE MICROPOROUS MEMBRANES FOR THE DETERMINATION OF ORGANOPHOSPHORUS FLAME RETARDANTS AND PLASTICIZERS IN WATER SAMPLES.**

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Anal. Chim. Acta 625 (2008) 145

Evaluation of liquid-liquid microextraction using polypropylene microporous membranes for the determination of organophosphorus flame retardants and plasticizers in water samples.

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Abstract

In this work, the suitability of the microporous membrane liquid-liquid extraction (MMLLE) technique for the concentration of several organophosphate esters (OPs) in water samples is assessed. Analytes were first extracted into a few microlitres of an organic solvent, immobilised in the pores of a hollow polypropylene membrane, and then determined by gas chromatography with nitrogen-phosphorus detection (GC-NPD). Main parameters controlling the efficiency of the extraction step were identified and their effects on the performance of the technique discussed. Under final working conditions, 2 cm long polypropylene membranes, containing about 7 μL of octanol in the pores, were dipped in a glass vial filled with 115 mL of water with a 30% of sodium chloride. Extractions were carried out for 12 hours, at room temperature, under magnetic stirring. After that, analytes were recovered from the membrane with 0.2 mL of ethyl acetate. This extract was mixed with the internal standard (50 μL of a triphenyl phosphate solution in the same solvent) and finally reduced to ca. 50 μL . Overall enrichment factors for the optimised method ranged from 35 to 1400 times, and the achieved limits of quantification from 0.008 to 0.12 ng mL^{-1} , depending on the considered compound. Globally, the method showed an acceptable linearity and precision for all species, except for tris(2-ethylhexyl) phosphate (TEHP). Performance of the MMLLE approach is compared with that reported for other solid- and liquid-phase microextraction techniques and its suitability for the analysis of real water samples discussed.

Keywords: Microporous membrane liquid-liquid extraction; Organophosphate compounds; Flame retardants; Water samples.

1. Introduction

Organophosphate esters (OPs) constitute a heterogeneous group of chemicals extensively employed as flame retardants and plasticizers in a multitude of commodities, such as plastics, building materials, varnishes, etc. Normally, they are used as additives, instead of being chemically bound to the host materials they are intended to protect [1]; therefore, they can easily reach the surrounding environment due to volatilization, leaching and/or abrasion processes. Consequently, several OPs have been detected in different environmental and domestic matrices, e.g., air [2,3], water [4], wastewater [5,6] and particulate matter from indoor and outdoors areas [7-9].

In general, the information about the toxicological impact of OPs is still scarce; however, deleterious effects for some of them have been already reported. In terms of toxicity, persistence and mobility, triphenyl phosphate (TPP) [10], tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP) [11] and tris(2-chloro, 1-chloromethylethyl) phosphate (TDCP) [12] are the most concerning OPs. Specifically, the chlorinated ones might be considered as persistent pollutants since they are not effectively removed in conventional urban wastewater treatment plants [13, 14]; therefore, they might reach surface and drinking water supply sources. This behaviour joined to their growing usage, have promoted the interest of the authorities and thus they have been included in European lists of aquatic priority substances [15,16].

Liquid-liquid extraction (LLE) [17,18] and solid-phase extraction (SPE) [19,20] are the most resorted techniques for the concentration of OPs in aqueous samples. Despite its widespread use, LLE is tedious, multistage operation, has problems of emulsion formation and consumes large amounts of organic solvents. On the other hand, SPE defeats some of these problems; however, the cost of cartridges is not negligible and sample and solvent consumption, although lower than in LLE, are characteristic of large scale methodologies. Driven by the need to overcome these drawbacks, miniaturized scale sample preparation techniques have been developed and applied to OPs extraction [19,21,22].

As concerns miniaturization of LLE, several alternatives have been proposed [23]. The earliest, and conceptually the simplest one, is the single-drop microextraction (SDME) technique. In SDME, analytes are partitioned between the sample and a tiny drop of an organic solvent hanging from the edge of the tip of a microsyringe needle [24]. Although SDME allows achieving high enrichment factors, the low stability of the hanging drop, which can be easily lost during extraction, represents its main drawback [25]. Until now, the application of SDME to the extraction of OPs has not been reported.

Dispersive liquid-liquid microextraction (DLLME) also relies on the extraction of analytes with a small volume of organic solvent. A mixture of a high-density solvent and a polar, water-miscible one is added to the water sample in order to form a cloudy state, which guarantees an infinitely large contact surface area between donor and acceptor solutions [26]. After centrifugation, a settled drop of extractant containing the target compounds is obtained. DLLME has been applied to the extraction of OPs from water samples. Between 200- and 900-fold enrichment factors were achieved in a short-single step [22].

A third modality in liquid-phase microextraction consists of using a porous membrane to immobilize a small volume of the acceptor phase [27,28]. In practise, polypropylene microporous membranes (MM), in a hollow-fibre (HF) format, are the preferred media to support the acceptor solution, which can be contained in both, the lumen and the pores of the fibre [29,30] or just in the pores [31]. This modality has been termed as microporous membrane liquid-liquid extraction (MMLLE). Low solvent consumption, versatility provided by the extractant solvent selection, high enrichment factors, easiness of operation and possibility of simultaneously perform unattended extractions are some of its appealing features. Notwithstanding, the relatively long extraction times required are its main disadvantage [31]. For the best of our knowledge, the only application of the MMLLE to OPs determination has been developed by Jonsson et al. for blood

using porous, flat, PTFE membranes [32]. In this work, the membrane served as a barrier between the sample (12 mL) and about 0.5 mL of organic solvent, which also impregnated its pores. The acceptor organic phase was renewed several times during the extraction process, thus poor enrichment factors were attained.

In the present work, the suitability of inexpensive polypropylene microporous membranes, in a HF format, for the extraction of a group of ten OPs compounds from water samples is evaluated. Several parameters, among them nature and volume of the extraction solvent, ionic strength of the sample, exposure time and stirring rate were evaluated and their influence on the extraction process discussed. The extraction efficiency of the optimized MMLLE method is compared with those reported for DLLME [22], liquid-phase microextraction with non-porous membranes (named as MASE) [21], as well as SPME [19] when applied to same species.

2. Experimental

2.1. Solvents, standards and samples

HPLC-grade acetone, ethyl acetate, 1,1,1-trichloroethane and dibutylether were purchased from Merck (Darmstadt, Germany). Octanol and sodium chloride were provided by Aldrich (Steinheim, Germany). Standards of OPs were acquired from Aldrich and Dr. Ehrenstorfer (Augsburg, Germany). Full names, abbreviations and their octanol-water partition coefficients [33] are summarized in **Table 1**.

Table 1. Abbreviations and octanol-water (K_{ow}) partition coefficients for selected OPs.

| Compound | Abbreviation | Log K_{ow} |
|---|--------------|--------------|
| Tripropyl phosphate | TPrP | 1.87 |
| Triisobutyl phosphate | TiBP | 3.60 |
| Tributyl phosphate | TBP | 4.00 |
| Tris(2-chloroethyl) phosphate | TCEP | 1.44 |
| Tris(chloropropyl) phosphate ^a | T CPP | 2.59 |
| Tris(2-chloro, 1-chloromethylethyl) phosphate | TD CP | 3.65 |
| Tris(2-butoxyethyl) phosphate | TB EP | 3.75 |
| Triphenyl phosphate | TP P | 4.59 |
| Tris(2-ethylhexyl) phosphate | TE HP | 9.49 |
| Triphenyl phosphine oxide | TP PO | 2.83 |

^a Mixture of isomers.

Individual solutions and mixtures of target OPs were prepared in acetone. Further dilutions were made in acetone and used to prepare the spiked water samples considered during optimisation of MMLLE conditions. Tripentyl phosphate (TPeP) was acquired from TCI Europe (Zwijndrecht, Belgium) and used as internal standard (IS). A solution of this compound in ethyl acetate (ca. 0.5 $\mu\text{g mL}^{-1}$) was added to the extracts from microporous membranes to compensate

for changes in their final volume after the evaporative concentration step, as well as in the sensitivity of the GC-NPD system.

Accurel polypropylene Q3/2 hollow membranes (0.6 m x 600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) were purchased from Membrana GmbH (Wuppertal, Germany). They were cut into pieces with different lengths using a sharp blade. These pieces were accurately weighed and only those showing mass variations under 1% considered for extraction experiments. Prior to use, membranes were soaked with acetone for 30 min, the solvent allowed to completely evaporate and finally, they were stored in a closed glass vessel until being used.

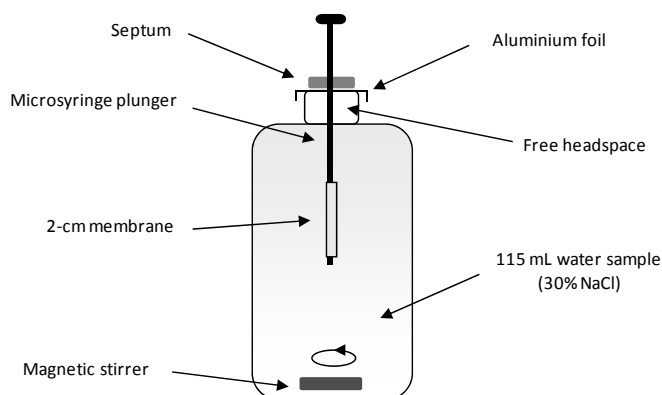
Ultrapure (Milli-Q), tap, river and urban wastewater were employed throughout this study. Samples, except ultrapure water, were passed through glass fibre filters obtained from Millipore (Bedford, MA, USA) and stored in the dark at 4 $^{\circ}\text{C}$ until analysis.

2.2. Sample preparation

The extraction assembly consisted of a hollow membrane inserted in a stainless steel microsyringe plunger (0.6 mm diameter), with a pierced septum in one of the edges. Therefore, the lumen of the membrane was not available for the acceptor phase, which was immobilized in the pores just by dipping it in the extraction solvent for 10 min. This experimental setup reduces variations in the volume of the extraction solvent leading to a better precision for the overall method and greatly simplifies loading the membrane with the acceptor phase [31]. Assuming that pores represent around 70% of the volume of the membrane [23], 7 μL of solvent (octanol under optimized conditions) were immobilised in the walls of the porous membrane.

MMLLE were accomplished submerging the impregnated membrane in a 120 mL glass vessel containing 115 mL of water sample (30% of sodium chloride), stirred at 700 rpm for 12 h, at room temperature. During extraction, vessels were covered with aluminium foil to prevent the contamination of the sample with particulate matter and to support the assembly which held the membrane (**Fig. 1**). The use of PTFE-layered silicone septa, to close the extraction vial, is not recommended because of TBEP contamination problems described elsewhere [19].

Fig.1. Setup of the MMLLE extraction step.



After finishing the extraction step, the membrane was immersed in ultrapure water for 5 min in order to remove salt residues. Then, it was dried with a soft tissue and analytes were recovered dipping the membrane into 200 μL of ethyl acetate in a 0.3 mL-insert, placed into a 1.5 mL GC vial, for 5 min. Next, the membrane was discarded and 50 μL of the IS solution were added to the extract, which was finally concentrated under N_2 stream to a volume of ca. 50 μL .

2.3. Equipment

Analytes were determined using a HP 5890 series II GC system (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a NPD detector. Separations were carried out with a HP-5MS type (5% phenyl, 95% methylpolysiloxane) capillary column (30 m x 0.25 mm i.d., d_r : 0.25 μm) purchased from Agilent (Wilmington, DE, USA). Chromatographic separations were carried out using a heating rate of 15 $^\circ\text{C min}^{-1}$ from 70 $^\circ\text{C}$ (held for 1 min) to 270 $^\circ\text{C}$ (held for 10 min). Helium (99.999%) was used as carrier gas at a constant head column pressure of 120 kPa and nitrogen as auxiliary gas in the NPD detector (25 mL min^{-1}). Synthetic air (99.995%) and hydrogen (99.999%) were also employed as detector gases at flow rates of 95 and 5 mL min^{-1} , respectively. The injected volume was 2 μL , the injector was maintained at 270 $^\circ\text{C}$ and the splitless valve was opened 1 min after injection.

Levels of OPs in MMLLE extracts were established by comparison with calibration standards in ethyl acetate, containing the same amount of TPeP (IS). Their concentrations in real water samples were determined with the standard addition methodology.

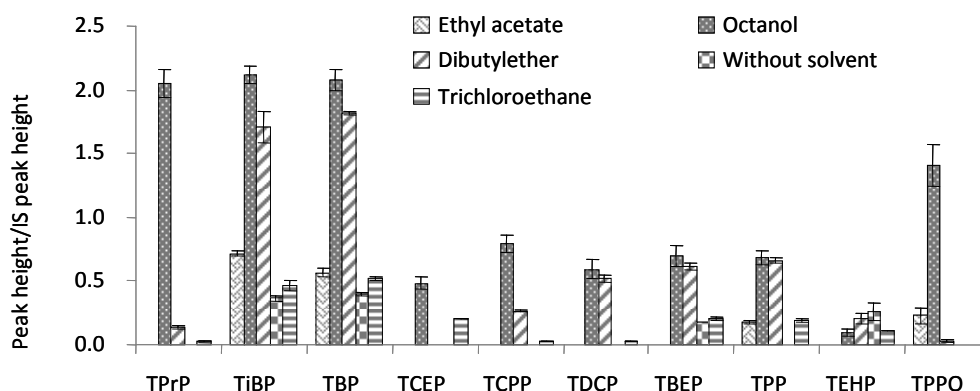
3. Results and discussion

3.1. Optimization of extraction conditions

3.1.1. Extraction solvent, fibre length and desorption efficiency

Affinity for target analytes, compatibility with the lipophilic polypropylene membrane and low water solubility are the main factors to be considered in the choice of the acceptor phase. On the basis of these criteria, and also taking into account previous applications of polypropylene membranes in two-phase liquid extractions [23], as well as the use of DLLME to extract OPs from water samples [22], four solvents, with different polarities, were tested as acceptor solutions: ethyl acetate, octanol, dibutylether and trichloroethane. The extraction capability of dry membranes was also evaluated. Assays were carried out in 22 mL vessels filled with ultrapure water, containing a 20% of NaCl and spiked with OPs at 3 ng mL^{-1} (15 ng mL^{-1} for TCEP and TEHP). Extractions were carried out in triplicate, for 2 h, with a stirring rate of 500 rpm. Obtained data are depicted in **Fig. 2**.

Fig. 2. Comparison of normalized responses for OPs using different organic solvents immobilized in the porous membrane (2 cm long), $n = 3$ replicates.



Working with dry membranes, and those impregnated with ethyl acetate, only some of the most lipophilic species (TiBP, TBP, TBEP and TEHP) were noticed in the corresponding chromatograms. On the other hand, dibutylether allowed extracting all the OPs except TCEP; meanwhile, trichloroethane provided very low extraction yields and it was unsuitable for TPPO recovery. Globally, octanol performed better than the other solvents for most OPs. This is probably due to its higher vaporization enthalpy compared with dibutylether and trichloroethane, with which shares a low and similar water solubility. The former characteristic makes octanol more appropriate for longer extraction periods with less risk of losses. The higher water solubility of ethyl acetate compared with these other tested solvents is probably the responsible for the poor results obtained with it. It is worth noting that porous polypropylene itself was able to adsorb, in a certain extension, those OPs with medium to high octanol-water partition coefficients. In fact, for TEHP ($\log K_{ow}$ 9.5) the highest response was achieved without acceptor solvent sustained in the walls of the membrane. This behavior matches with the observation of Müller et al. [34] and recent applications of microporous membranes as adsorbents of volatile, non-polar organic compounds [35].

Table 2 shows the ratios between responses obtained for 2 and 1 cm long membranes considering sampling times of 2 and 14 h. As expected, the extracted amount of OPs raised with the increase in the length of the fibre, and thus with the volume of octanol contained in the pores. In addition, for TPrP, TCPP, TDCP, TEHP and TPPO there were significant differences in the obtained ratios depending on the sampling time. These data suggest that the volume of the extractant solvent might affect not only to the efficiency but also the kinetics of the extraction process. Membranes with a length of 2 cm were selected for further experiments. Obviously, longer membranes, accommodating a larger octanol volume, are expected to improve the yield of the extraction, at the expense of also increasing the volume of solvent used in the further desorption step.

Table 2. Average ratios between responses obtained for 2 and 1 cm polypropylene membranes, impregnated with octanol, using two different sampling times.

| Compound | Response 2-cm membrane/ 1-cm one | |
|----------|----------------------------------|---------------|
| | 2 h sampling | 14 h sampling |
| TPrP | 2.5 | 1.8 |
| TiBP | 1.7 | 1.6 |
| TBP | 1.7 | 1.6 |
| TCEP | 2.6 | 2.5 |
| TCPP | 2.5 | 1.8 |
| TDCP | 2.2 | 1.7 |
| TBEP | 1.5 | 1.2 |
| TPP | 1.7 | 1.4 |
| TEHP | 1.3 | 3.1 |
| TPPO | 2.6 | 1.8 |

Data for 20 mL volume samples, spiked at 3 ng mL^{-1} and containing a 20% of NaCl. $N = 3$ replicates.

As regards the desorption step, no differences were noticed for desorption times between 5 and 20 min, neither by increasing the volume of ethyl acetate used to recover OPs from the octanol-impregnated membrane. Thus, both variables were fixed at 5 min and 0.2 mL, respectively. Also, the efficiency of the extraction remained constant when membranes were dipped in octanol for different periods (from 5 to 30 min) before being exposed to the spiked water samples, data not shown.

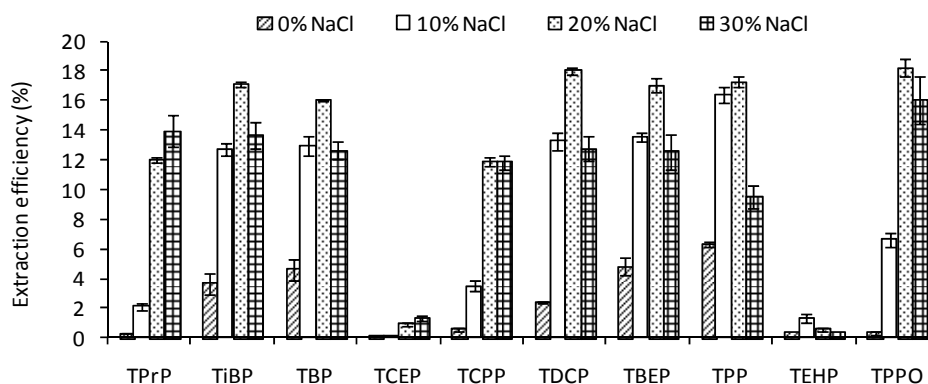
3.1.2. Sample volume

Influence of the water volume on the obtained responses was evaluated at two sampling times: 2 and 12 h. Experiments were carried out in glass vessels with different capacities (22 and 120 mL) containing 20 and 115 mL volume aliquots of the same spiked water sample (20% of sodium chloride), respectively. In general, and regardless the exposure time of the membrane, between twice and four times higher responses were attained when using the bigger vial. Taking this behavior into account, in addition to the lack of limitations regarding the volume of environmental water samples, the larger vessels were used in further experiments.

3.1.3. Ionic strength

The effect of the ionic strength was investigated using water samples spiked with OPs (3 ng mL^{-1}) and increased concentrations of sodium chloride, from 0% to 30% (**Fig. 3**).

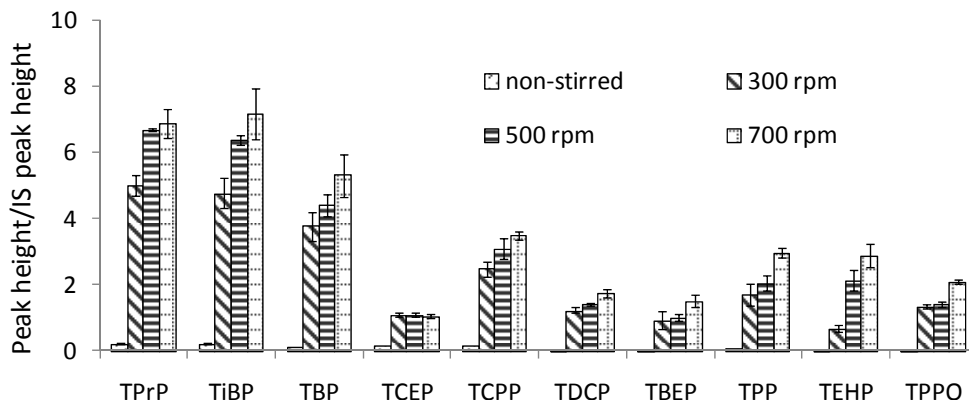
Fig. 3. Effect of the ionic strength on the efficiency of the MMLLE process. Sampling time 2 h, $n = 3$ replicates.



The addition of salt, up to 20%, significantly enhanced the extraction efficiency of all the OPs, especially for TCEP, which was not detected in the absence of NaCl. Higher salt contents, 30%, further improved the extraction yield of TCEP, whereas, for the rest of species, it remained basically the same as for 20% of salt (TPrP, TCP and TPPO), or underwent a slight to moderate reduction (TiBP, TBP, TDCP, TBEP, TPP and TEHP). These results matched with the pattern observed for SPME and DLLME when applied to same compounds [19,22]. Whatever the microextraction approach, it is assumed that, at high salt concentrations, the increase in the viscosity of the sample slows down the migration of the less polar compounds from the bulk of the sample to the surface of the solid sorbent (SPME) or the acceptor solution (DLLME and MMLLE). Considering the difficulties to extract TCEP, in further experiments a 30% of salt was added to the samples to favor the extraction of this species, at the expense of reducing the efficiency of the process for the most lipophilic OPs.

3.1.4. Stirring

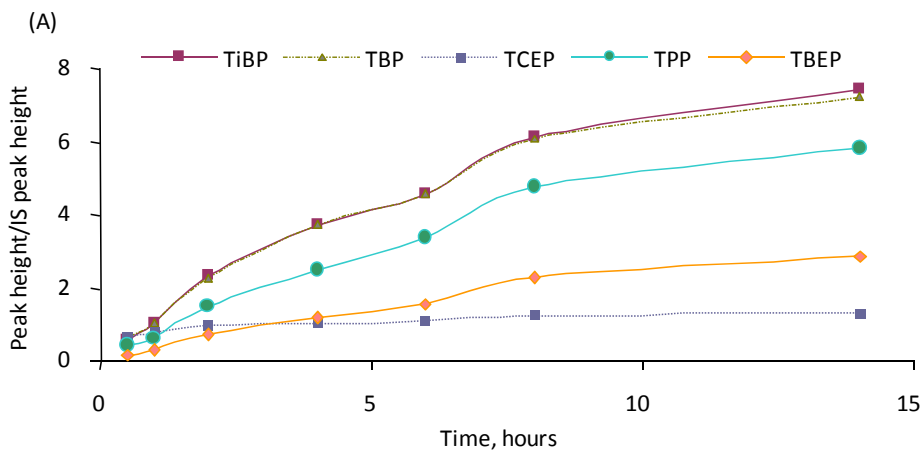
Samples were stirred using a PTFE-covered magnetic bar (20 x 6.5 mm). Stirring speeded up the diffusion of the analytes from the sample to the surface of the membrane dramatically improving the responses for all OPs (Fig. 4). Although responses obtained at 300 and 500 rpm did not differ significantly between them, they were slightly lower than those corresponding to samples stirred at 700 rpm, therefore this value was selected as optimum. Higher stirring rates are not advisable since they can promote the formation of air bubbles onto the membrane thus reducing the surface of contact with the sample.

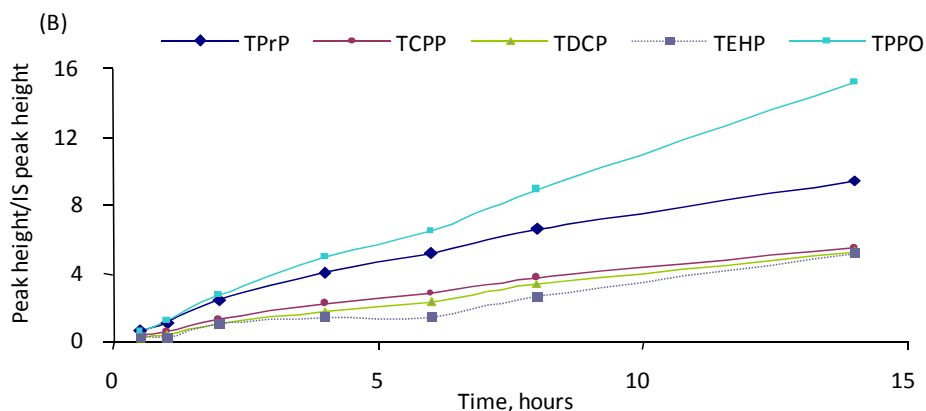
Fig. 4. Study of stirring rate influence, $n = 3$ replicates.

3.1.5. Extraction kinetics

Fig. 5 depicts the time-course of the MMLLE process for 115 mL water samples, containing a 30% of NaCl and stirred at 700 rpm. As observed, the kinetics of the MMLLE process was rather slow. For TiBP, TBP, TCEP, TPP and TBEP signals obtained for 8 and 14 h of sampling did not differ too much (**Fig. 5A**), indicating the proximity to the equilibrium; however, for the remaining OPs (**Fig. 5B**) signals kept significantly growing from 8 to 14 h. It is obvious that analytes transference from the bulk of the sample to the octanol solvent, impregnated in the pores of the HF membrane, was a slow process, which is in agreement with previous results reported for different species, using the same extraction technique and similar ratios between sample and acceptor solution [31]. In further experiments, several samples were simultaneously extracted overnight (12 h) using a multi-position magnetic stirrer.

Fig. 5. Time-course of the MMLLE for 115 mL water samples containing 30% of NaCl for (A) TiBP, TBP, TCEP, TPP and TBEP; (B) TPrP, TCPP, TDCP, TEHP, TPPO. Average data for duplicate extractions.





3.1.6. Organic solvent addition

In the extraction of apolar compounds their adsorption on the glass walls may result in lower extraction efficiencies either with SPME or SBSE. The addition of methanol was shown to effectively improve the recovery of some PAHs and organochlorine pesticides [36], PCBs [37] and PBDEs [38] from water samples. In this work, the addition of a 5% of methanol to water samples was tested. On one hand, the efficiency of the extraction for the extremely lipophilic TEHP remained unchanged. On the other, for the more polar species: TPrP, TCEP, TCPP and TPPO, a significant reduction in their extraction yields was noticed, data not shown. Consequently, the effect of adding higher percentages of methanol (up to 30%), which are expected to improve the yield of the microextraction for TEHP [38], was not investigated. Consequently, in further extractions organic modifiers were not added to water samples.

3. 2. Method performance

Table 3 summarizes some data related with the performance of the proposed method. Repeatability was investigated with water samples spiked at three different levels: 0.1, 0.5 and 1 ng mL⁻¹. Relative standard deviations (RSDs) corresponding to extractions performed in quadruplicate remained below 14% for all OPs, except for TEHP. Globally, these RSDs are slightly higher than those reported for DLLME [22], but very similar to those published for SPME [19] and MASE [21]. Linearity was investigated using ultrapure water samples spiked with increasing concentrations of OPs at six different levels between 0.02 and 7 ng mL⁻¹. Correlation coefficients between 0.990 and 0.999 were achieved within this interval. The exception was TEHP, for which poor correlation coefficients were also reported with SPME [19]. Limits of quantification of the proposed method varied between 0.008 and 0.12 ng mL⁻¹. In most cases, they were controlled by the efficiency of the extraction process; however, for TBP, its presence in procedural blanks limited the lowest quantifiable value. Reagents, solvents and the polypropylene membrane itself were discarded as potential sources of this contamination, which is likely associated with the employed ultrapure water.

Table 3. Repeatability ($n = 4$ replicates), linearity and limits of quantification (LOQs), defined for a S/N ratio of 10, of the proposed method.

| Compound | Repeatability (RSD %) | | | Linearity R^2 | LOQs (ng mL^{-1}) |
|-------------------|------------------------|--------------------------|--------------------------|--------------------|---------------------------------|
| | 1 ng mL^{-1} | 0.5 ng mL^{-1} | 0.1 ng mL^{-1} | | |
| TPrP | 4 | 7 | 5 | 0.998 | 0.020 |
| TiBP | 7 | 5 | 7 | 0.999 | 0.008 |
| TBP | 8 | 5 | 8 | 0.990 | 0.080 |
| TCEP ^a | 5 | 7 | 11 | 0.997 | 0.120 |
| TCPP | 5 | 6 | 6 | 0.998 | 0.060 |
| TDCP | 8 | 6 | 10 | 0.997 | 0.060 |
| TBEP | 8 | 14 | 14 | 0.991 | 0.050 |
| TPP | 13 | 10 | 14 | 0.996 | 0.050 |
| TEHP ^a | 16 | 29 | 37 | 0.986 | 0.100 |
| TPPO | 9 | 7 | 12 | 0.990 | 0.050 |

^aTwice higher concentrations were added for these compounds

Recoveries of the MMLLE method were defined as the ratios between the mass of each OPs in the extract from the porous membrane and that added to the sample. The first was calculated by comparing the normalized responses (peak height/IS peak height) with those measured for calibration standards containing the same amount of TPpP as IS. Obtained data are shown in **Table 4**.

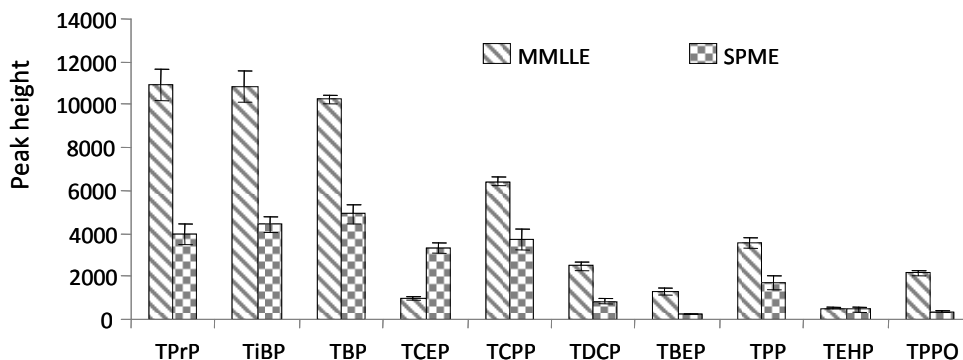
Table 4. Recoveries (%) of the MMLLE method and comparison of enrichment factors (EFs) with those reported for other liquid-phase microextraction techniques.

| Compound | Recovery (%) \pm S.D. | EFs | | |
|----------|-------------------------|-------|-------------------|------------------|
| | | MMLLE | DLLME (Ref. [22]) | MASE (Ref. [21]) |
| TPrP | 49 ± 2 | 1135 | 595 | n.a. |
| TiBP | 61 ± 4 | 1400 | 831 | 179 |
| TBP | 51 ± 4 | 1180 | 816 | 196 |
| TCEP | 2 ± 0.1 | 35 | 195 | 9 |
| TCPP | 32 ± 2 | 740 | 734 | 158 |
| TDCP | 35 ± 3 | 810 | 778 | 136 |
| TBEP | 60 ± 8 | 1384 | 906 | 182 |
| TPP | 28 ± 3 | 600 | 753 | 163 |
| TEHP | 4 ± 1 | 105 | 350 | n.a. |
| TPPO | 47 ± 6 | 1070 | 452 | 127 |

n.a. not available.

For eight of the investigated OPs recoveries between 28% and 61% were attained; however, in case of TCEP and TEHP they remained below 5%. As indicated throughout the manuscript, TCEP and TEHP are the most and the less hydrophobic OPs, **Table 1**. Their poor recoveries are probably associated with low partition coefficients and adsorption on the walls of the sample vessel, respectively. Enrichment factors (EFs), calculated multiplying the ratio between sample and final extract volumes (115 and 0.05 mL, respectively) by the recovery attained for each compound, ranged from 35 to 1400 times. These values are higher than those reported for MASE using a similar sample intake [21] and, in general, similar to those corresponding to DLLME of 10 mL samples [22] (**Table 4**). However, the latter approach clearly provided higher EFs for TCEP and TEHP. When compared with the SPME technique [19], MMLLE attained equal or higher responses, except for TCEP (**Fig. 6**).

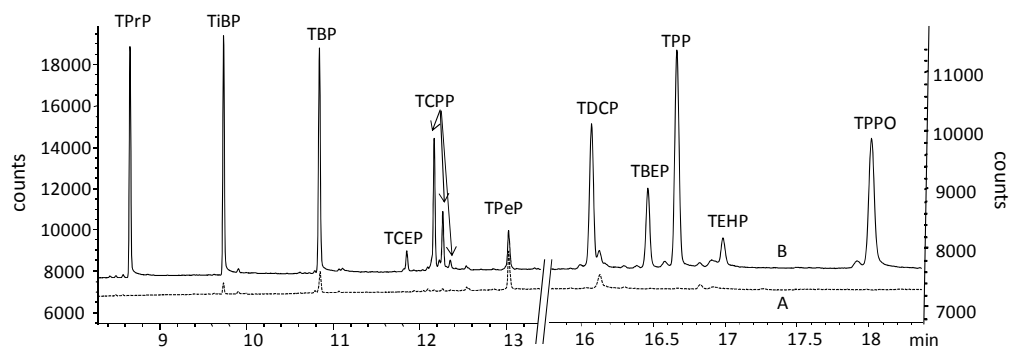
Fig. 6. Comparison of responses for aliquots of the same spiked water sample (5 ng/mL for TCEP and TEHP and 1 ng/mL for the rest OPs) processed using MMLLE and SPME under conditions given in Ref. [19]. Normalized data for $n = 4$ replicates.



3.3. Matrix effects and real samples analysis.

A well-known limitation of microextraction techniques, based on equilibrium processes, is that their efficiency might change significantly depending on the characteristics of the sample. Possible matrix effects for the optimized MMLLE method were investigated with tap, river, raw and treated wastewater. After filtration, each sample was divided in two fractions, one was considered as a blank and the other spiked with target species at 0.3 ng mL^{-1} (1.5 ng mL^{-1} for TCEP and TEHP) (**Fig. 7**).

Fig. 7. GC-NPD chromatograms for fractions of the same river sample. (A) Non-spiked aliquot. (B) Spiked at 0.3 ng mL^{-1} (1.5 ng mL^{-1} for TCEP and TEHP).



Differences between responses obtained for both fractions were normalized to those achieved for ultrapure water with the same addition level (**Table 5**). Extractions were carried out in triplicate. Except for TEHP, the yield of the MMLLE process remained basically the same for ultrapure, tap, river and treated wastewater. In case of raw wastewater, the extraction efficiency of TPrP and TPP underwent a reduction around 40%. For TCEP, the yield of the extraction for wastewater could not be assessed due to the presence of an interference, at the retention time of the compound, in the GC-NPD chromatogram. As expected, taking into account the results published for SPME [19], the efficiency of the extraction for TEHP underwent a dramatic reduction for river and wastewater, probably due to the existence of dissolved organic matter, which competed with octanol for this highly lipophilic compound.

Table 5. Evaluation of matrix effects for the MMLLE method.

| | Tap water | | River water | | Treated wastewater | | Raw wastewater | |
|------|-----------|------|-------------|------|--------------------|------|----------------|------|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| TPrP | 109 | 7 | 100 | 6 | 94 | 8 | 64 | 9 |
| TiBP | 102 | 7 | 105 | 6 | 98 | 4 | 106 | 11 |
| TBP | 110 | 13 | 102 | 6 | 99 | 4 | 93 | 6 |
| TCEP | 100 | 9 | 83 | 15 | ^a | | ^a | |
| TCPP | 103 | 4 | 99 | 4 | 108 | 6 | 83 | 5 |
| TDCP | 112 | 7 | 99 | 5 | 109 | 6 | 105 | 6 |
| TBEP | 100 | 7 | 97 | 9 | 101 | 7 | 95 | 12 |
| TPP | 101 | 15 | 92 | 8 | 111 | 6 | 62 | 1 |
| TEHP | 87 | 10 | 25 | 4 | 12 | 18 | 14 | 9 |
| TPPO | 104 | 5 | 113 | 7 | 107 | 7 | 110 | 14 |

Addition level 0.3 ng mL^{-1} (1.5 ng mL^{-1} for TEHP and TCEP), $n = 3$. Relative recoveries, as percentages, to those obtained for ultrapure water.

^aTCEP peak overlapped with an interference.

The optimized methodology was used to determine the levels of OPs in three different treated wastewater samples from urban treatment plants. TiBP, TBP, TCPP were present in all the samples while TDCP, TBEP and TPP were, at least, quantified in one of them (**Table 6**). TPrP, TCEP, TEHP and TPPO were not detected in any of the samples. The concentration of the quantified OPs did not exceed the 0.5 ng mL^{-1} level.

Table 6. Concentrations (ng mL^{-1}) of OPs in treated urban wastewater samples.

| | Sample code 1 | Sample code 2 | Sample code 3 |
|------|-----------------|-------------------|-----------------|
| TiBP | 0.34 ± 0.04 | 0.31 ± 0.03 | 0.03 ± 0.03 |
| TBP | 0.26 ± 0.05 | 0.22 ± 0.02 | nq |
| TCPP | 0.46 ± 0.06 | 0.21 ± 0.01 | 0.12 ± 0.02 |
| TDCP | nq | 0.078 ± 0.006 | nd |
| TBEP | nd | nd | 0.16 ± 0.02 |
| TPP | nd | 0.065 ± 0.01 | nd |

nd, not detected; nq, below quantification limits.

4. Conclusions

Possibilities and limitations of the MMLLE technique for the concentration of several OPs in water samples have been evaluated. Globally, the proposed sample preparation approach provided satisfactory precision, LOQs and linearity for eight of the ten evaluated species. The setup of the extraction is simple, the process consumes just a few microliters of organic solvent and its cost remains very low (about € 0.05 for a 2 cm membrane). Additionally, the disposable nature of the membranes avoids carryover problems, usually associated with SPME procedures. Moreover, for these OPs, MMLLE led to higher EFs, or responses, than DLLME, MASE and SPME. Its only drawback versus these techniques is the slowness of the extraction. In practice, this limitation can be attenuated by simultaneous extraction of several samples in a multi-position magnetic stirrer.

On the other hand, the developed method (MMLLE followed by GC-NPD determination) is unsuitable for the determination of TCEP and TEHP in water samples. For the first compound, the obtained LOQ was not low enough for the analysis of environmental water samples; moreover, in wastewater, its GC-NPD signal was affected by the co-elution of interfering species. Some of these limitations could be partially alleviated replacing the NPD detector by a more selective and sensitive technique, e.g. mass spectrometry detection [9]. In case of TEHP, whatever the determination technique, the poor achieved precision and linearity, added to the strong dependence between the yield of the extraction and the type of matrix, impeded the application of the MMLLE approach to its determination in water samples.

Acknowledgements

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2.4. PUBLICACIÓN:

***DEVELOPMENT OF A DISPERSIVE LIQUID-LIQUID MICROEXTRACTION METHOD FOR ORGANOPHOSPHORUS FLAME RETARDANTS AND PLASTICIZERS DETERMINATION IN WATER SAMPLES.**

M. García-López, I. Rodríguez, R. Cela

J. Chromatogr. A 1166 (2007) 9

Development of a dispersive liquid-liquid microextraction method for organophosphorus flame retardants and plasticizers determination in water samples

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Abstract

A fast, inexpensive and efficient sample preparation method for the determination of 10 organophosphorus compounds in water samples is presented. Analytes were extracted using the dispersive liquid-liquid microextraction (DLLME) technique and determined by gas chromatography with nitrogen-phosphorous detection (GC-NPD). The influence of several variables (e.g. type and volume of dispersant and extraction solvents, ionic strength, shaking time and mode, etc.) on the performance of the sample preparation step was carefully evaluated. Under final working conditions, 1 mL of acetone containing a 2% of 1,1,1-trichloroethane (20 μ L) was added to 10 mL of water with a 20% of sodium chloride. The ternary mixture was centrifuged at 3500 rpm to allow phases separation. After removing the aqueous supernatant, an aliquot of the settled extract was injected in the GC-NPD system. Under the above conditions, the method provided enrichment factors between 190 and 830 times (depending on the considered compound), relative standard deviations below 10%, except for tris(2-ethylhexyl) phosphate (TEHP), and quantification limits between 0.01 and 0.08 ng/mL. Matrix effects were assessed using different water samples and accuracy was evaluated by comparison with solid-phase microextraction.

Keywords: Dispersive liquid-liquid microextraction; Organophosphorus compounds; Water analysis; Gas chromatography.

1. Introduction

Organophosphate esters (OPs) are high volume production chemicals, widely employed as flame retardants and plasticizers additives in polymers, lubricants, varnishes, etc. [1,2]. Usually, they are just dispersed, not chemically bound, in host materials, therefore, they can be released into the surrounding environment [3]. OPs have been detected in several matrices such as air [4,5], indoor dust [3,6-8], sludge from sewage treatment plants [9], and different water samples [10-16]. The presence of OPs in the last compartment is a matter of concern because of the high mobility of the most polar species, particularly tris(2-chloroethyl) phosphate (TCEP) and tris(chloropropyl) phosphate (TCPP), which seemed to pass through conventional urban wastewater treatment plants without undergoing significant removal rates [10,15-17]. Although the medium-term effects of OPs on the environment are unknown, the European Union has included some of them in the aquatic priority pollutants lists [18,19].

Procedures for the determination of OPs in water samples involve an enrichment step followed by chromatographic analysis. Traditionally, liquid-liquid extraction (LLE) [14,20] and solid-phase extraction (SPE) [10,11,15,21] have been the most often employed extraction methods; moreover, as in the case of many other organic compounds, microextraction techniques are playing an increasing importance in the determination of OPs. Miniaturization, reduction of organic solvents consumption and improvement in the selectivity of the extraction are some of the potential advantages of microextraction techniques. In a previous paper, the usefulness of solid-phase microextraction (SPME) for the determination of OPs in water samples was demonstrated [16]. Quintana et al. have also showed the feasibility of employing a non-porous polypropylene membrane to improve the selectivity in the liquid-liquid extraction of OPs from water samples. The technique, named membrane-assisted solvent extraction (MASE), provided enrichment factors between 100 and 200 times, except for TCEP, using 1 mL of cyclohexane as acceptor solution [22]. Both approaches, SPME and MASE, achieved detection limits in the low pg/mL region, however, they required relative long extraction periods. In the case of SPME, the kinetics of the extraction is limited by the small surface of the coated fibre. The same restriction affects single drop microextraction (SDME), although, to the best of our knowledge, this technique has not been applied to the extraction of OPs from water samples, yet. In case of MASE, mass transference processes are even slower since the non-porous membrane reduces the rate of analytes diffusion from the water sample to the acceptor solution.

Very recently, Rezaee et al. have introduced a novel modality of liquid-liquid microextraction, referred as dispersive liquid-liquid microextraction (DLLME), which overcomes the aforementioned problems [23]. DLLME employs a mixture of a high-density solvent (extractant) and a water miscible, polar solvent (disperser). Acetone, methanol and acetonitrile can be used as dispersers, whereas chlorinated solvents (eg. chlorobenzene, carbon tetrachloride, tetrachloroethylene) are useful as extractants. In practice, the extractant represents only around 1-3% of the total volume of the extraction mixture. When this solution is added to a water sample, a cloudy state, consisting of fine droplets of the extractant dispersed in the aqueous matrix, is formed. The large contact surface between the sample and the droplets of the extractant speeds up mass transference processes. After centrifugation, the latter settles at the bottom of the vial. Up to now, DLLME has been successfully applied to the concentration of several families of organic [23-29] and inorganic species [30], as chelates, in water samples.

The goal of this work is to assess whether DLLME can be used as a valuable sample concentration approach for the determination of a group of several OPs, exhibiting large differences among their polarities, in water samples using gas chromatography analysis. Influence of extractant and disperser solvents, as well as ionic strength, on the performance of the method is discussed in detail. Moreover, some operational details related to the way of mixing the sample and the extraction mixture are also evaluated.

2. Experimental

2.1. Reagents, standards and material

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Trace analysis quality acetone and chlorinated solvents: dichloromethane (CH_2Cl_2), chloroform (CHCl_3), carbon tetrachloride (CCl_4), chlorobenzene ($\text{C}_6\text{H}_5\text{Cl}$), and 1,1,1-trichloroethane (CH_3CCl_3), considered for OPs extraction were obtained from Merck and Aldrich (Milwaukee, WI, USA). Tripropyl phosphate (TPPrP), tributyl phosphate (TBP), triisobutyl phosphate (TiBP), TCEP, tris(2-chloro-, 1-chloromethylethyl) phosphate (TDCP), tris(butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), TEHP and triphenyl phosphine oxide (TPPO) were acquired from Aldrich. TCP, as a technical mixture of isomers, was provided by Dr. Ehrenstorfer (Augsburg, Germany). Sodium chloride was also obtained from Aldrich. Individual solutions and mixtures of target species were prepared in acetone. Further dilutions were also made in acetone and used to prepare the spiked water samples considered during optimisation of DLLME conditions. Calibration standards were dissolved in each of the chlorinated solvents considered for analytes extraction.

Glass tubes (12 mL volume) with a conic bottom and a screw cap, furnished with a PTFE-lined septum, were acquired from Afora (Barcelona, Spain). A manual SPME holder and poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB, 65 μm film thickness) fibres were obtained from Supelco (Bellefonte, PA, USA).

Ultrapure (Milli-Q), tap, river and urban wastewater samples were employed throughout this study. Samples, except ultrapure water, were passed through glass fibre filters obtained from Millipore (Bedford, MA, USA) and stored in the dark at 4 $^\circ\text{C}$ until analysis.

2.2. Sample preparation

Optimization of DLLME conditions was performed using ultrapure water samples spiked with OPs at concentrations between 1 and 4 ng/mL, depending on the considered species. Under final conditions, 10 mL of filtered water containing a 20% of sodium chloride were poured in conical bottom glass tubes. Then, 1 mL of the extraction mixture (acetone: CH_3CCl_3 , 98:2) was added using a micropipette. The tube was closed, allowed to stand for 1 min and then centrifuged at 3500 rpm for 3 min. During centrifugation, the dispersed droplets of CH_3CCl_3 settled at the bottom of the vessel, building-up a drop with a volume of 12 μL . After removing most of the supernatant with a Pasteur pipette, the volume of the trichloroethane extract was measured using a microsyringe with a bevel tip needle. An aliquot of this extract (2 μL) was injected in the GC-NPD system. Performance of the DLLME method was investigated in terms of recoveries and enrichment factors (EFs). Recoveries were defined as the ratio between the mass of each OP in the sedimented phase and that added to the sample. The first was calculated as the product of analytes concentration and drop volume. EFs were determined multiplying the ratio between sample and extract volumes, by the recovery achieved for each compound.

Pre-concentration capability and accuracy of the proposed method were compared with those achieved using SPME under conditions optimized in an earlier work [16]. In brief, SPME

extractions were carried out at room temperature for 40 min, using a PDMS-DVB fibre exposed directly to water samples (20 mL) saturated with sodium chloride. Fibres were desorbed at 270 °C for 5 min in the splitless mode.

2.3. Determination conditions

Analytes were determined using a HP 5890 series II GC system (Hewlett-Packard, Avondale, MA, USA) equipped with a split/splitless injector and a NPD system. Separations were carried out with a DB-5 type (5% phenyl, 95% methylpolysiloxane) capillary column (30 m x 0.32 mm I.D., d_f : 0.25 μ m) purchased from Agilent (Wilmington, DE, USA). The oven temperature program was the following: 70 °C (1 min), first ramp at 15 °C/min until 200 °C (3 min), second ramp at 5 °C/min to 250 °C (held for 5 min). For extracts in chlorobenzene, the initial temperature of the oven had to be increased to 100 °C, otherwise split peaks were obtained for some compounds. Nitrogen (99.999%) was used as carrier gas at a constant head column pressure of 96 kPa, and also as auxiliary gas in the NPD system (25 mL/min). Synthetic air (99.995%) and hydrogen (99.999%) were used as detector gases at flow rates of 95 and 5 mL/min, respectively. The injected volume was 2 μ L, the injector temperature was maintained at 270 °C and the splitless valve opened 1 min after injection.

Concentrations of OPs in the extracts from water samples, obtained with the DLLME technique, were determined by external calibration. Standards at seven different concentration levels (from 0.01 to 4 μ g/mL) were prepared in each of the chlorinated solvents considered for analytes extraction. Correlation coefficients for the corresponding calibration curves ranged from 0.990 to 0.999.

3. Results and discussion

3.1. Extraction solvent and ionic strength

Selection of the extractant is a key step in the optimization of DLLME conditions. Five chlorinated solvents, CH₂Cl₂, CHCl₃, CCl₄, C₆H₅Cl and CH₃CCl₃, showing densities above 1 g/mL, low water solubilities and different polarities were initially considered for the dispersive liquid-liquid microextraction of Ops (**Table 1**).

Table 1. Properties of the extractants considered in this study.

| Solvent | Density (g/mL) | Water solubility (10 ⁻³ M) | Log K _{ow} |
|----------------------------------|----------------|---------------------------------------|---------------------|
| CCl ₄ | 1.59 | 2.0 | 2.89 |
| C ₆ H ₅ Cl | 1.10 | 0.79 | 2.81 |
| CH ₂ Cl ₂ | 1.25 | 67 | 1.19 |
| CH ₃ CCl ₃ | 1.34 | 7.3 | 2.10 |
| CHCl ₃ | 1.48 | 16 | 1.76 |

Formation of a sedimented phase was investigated with 10 mL water samples. Extraction mixtures, consisting of 1 mL of acetone with variable percentages (up to 10%) of the above solvents, were rapidly injected in the water sample using a 1 mL gastight syringe [23]. After that, vials were closed and centrifuged for 5 min at 3500 rpm. For CH_2Cl_2 , phases separation was never observed. In case of CHCl_3 , a sedimented phase could be obtained when the percentage of this solvent in the extraction mixture exceeded the 7%, however, its volume was not repeatable. On the other hand, for CCl_4 , $\text{C}_6\text{H}_5\text{Cl}$ and CH_3CCl_3 , which present lower water solubilities than CH_2Cl_2 and CHCl_3 (Table 1), well-defined, settled drops were obtained. On the basis of these results, the first three solvents were considered in further experiments.

Recoveries of the DLLME process, as function of the extractant and the ionic strength of the sample, are summarized in Table 2. The percentage of each chlorinated solvent in the extraction mixture was adjusted to obtain settled drops with volumes comprised between 12 and 20 μL . In absence of sodium chloride, similar results were observed for the three extractants. Analytes with $\log K_{ow}$ values around four units (TiBP, TBP, TDCP, TBEP and TPP) were recovered in an extension higher than 84%, whereas much lower efficiencies were obtained for the most polar OPs: TPrP, TCEP, TCPP and TPPO ($\log K_{ow}$ values from 0.5 to 2). Finally, TEHP was recovered in an extension around 50%. Taking into account its strong lipophilic behaviour ($\log K_{ow}$ 10), it seems feasible that adsorption on the walls of the extraction tube might compete with the extraction process.

Table 2. Recoveries of the DLLME as function of the extractant and the ionic strength of the water samples.

| Compound | 0% NaCl | | | 10% NaCl | | | 20% NaCl | |
|--------------------------------------|---------------------------------------|--|--|---------------------------------------|--|--|---------------------------------------|--|
| | CCl_4 (26 μL) | $\text{C}_6\text{H}_5\text{Cl}$ (26 μL) | CH_3CCl_3 (30 μL) | CCl_4 (26 μL) | $\text{C}_6\text{H}_5\text{Cl}$ (26 μL) | CH_3CCl_3 (30 μL) | CCl_4 (26 μL) | CH_3CCl_3 (20 μL) |
| TPrP | 14 ± 4 | 28 ± 3 | 25 ± 8 | 43 ± 1 | 46 ± 5 | 56 ± 8 | 77 ± 3 | 71 ± 3 |
| TiBP | 108 ± 7 | 108 ± 4 | 88 ± 8 | 111 ± 3 | 92 ± 9 | 108 ± 6 | 116 ± 5 | 100 ± 1 |
| TBP | 107 ± 8 | 109 ± 3 | 86 ± 5 | 109 ± 5 | 85 ± 7 | 97 ± 2 | 112 ± 4 | 98 ± 4 |
| TCEP | 6 ± 13 | 12 ± 3 | 7 ± 4 | 12 ± 6 | 18 ± 5 | 16 ± 12 | 25 ± 9 | 23 ± 6 |
| TCPP | 33 ± 5 | 64 ± 1 | 44 ± 4 | 75 ± 3 | 58 ± 4 | 74 ± 3 | 94 ± 1 | 88 ± 2 |
| TDCP | 95 ± 6 | 87 ± 8 | 100 ± 5 | 106 ± 4 | 59 ± 6 | 117 ± 14 | 105 ± 2 | 93 ± 7 |
| TPP | 103 ± 4 | 92 ± 8 | 106 ± 5 | 105 ± 6 | 60 ± 5 | 124 ± 6 | 103 ± 2 | 90 ± 6 |
| TBEP | 101 ± 3 | 100 ± 9 | 84 ± 8 | 118 ± 10 | 66 ± 4 | 97 ± 6 | 105 ± 5 | 109 ± 7 |
| TEHP | 44 ± 18 | 50 ± 1 | 69 ± 10 | 52 ± 12 | 36 ± 1 | 76 ± 15 | 37 ± 12 | 42 ± 26 |
| TPPO | 12 ± 13 | 37 ± 4 | 12 ± 12 | 34 ± 9 | 42 ± 3 | 32 ± 12 | 56 ± 7 | 54 ± 15 |
| Vol. settled phase (μL) | 12 μL | 15 μL | 12 μL | 16 μL | 14 μL | 20 μL | 17 μL | 12 μL |

Data for 10 mL samples using 1 mL of acetone as disperser, $n = 4$ replicates. Vol., volume.

Addition of sodium chloride to water samples affected to analytes and extractant solubilities as well as to the density of the sample. In case of $\text{C}_6\text{H}_5\text{Cl}$, the effect of sodium chloride was assessed only at one level, 10%. For higher concentrations of salt, sample and extractant densities were very similar, therefore phases separation became impossible. Moreover, using

C_6H_5Cl , the addition of a 10% of salt to water samples did not improve extraction recoveries (**Table 2**). For CCl_4 and CH_3CCl_3 , the extraction yields of TPrP, TCEP, TCPP and TPPO improved significantly with the ionic strength of water samples, whereas for the rest of analytes (except TEHP) they remained constant and higher than 85% (**Table 2**). For CH_3CCl_3 , the volume of the settled phase was also dramatically affected by the concentration of NaCl. In fact, for samples containing a 20% of NaCl, the percentage of this solvent in the extraction mixture was reduced from 3 to 2% to maintain the volume of the sedimented drop under 20 μL . Taking into account that CH_3CCl_3 is less toxic than CCl_4 and it provided similar extraction efficiencies, even when the volume of the sedimented phase was lower (see data on the last two columns of **Table 2**), the first was selected as extractant for the rest of this study.

Additional experiments with samples containing a 30% of salt only showed a further improvement in the recovery of TCEP, whereas for the rest of species extraction yields decreased in comparison with values attained for a 20% of NaCl, data not given.

3.2. Volume of extraction solvent

Recoveries and EFs obtained in the extraction of 10 mL water samples (20% in NaCl), using 1 mL of acetone with increasing volumes of CH_3CCl_3 , from 20 to 45 μL , are summarized in **Table 3**.

Table 3. Recoveries and enrichment factors (EFs) achieved for different volumes of CH_3CCl_3 using acetone (1 mL) as disperser, $n = 3$ replicates.

| Compound | 20 μL^a (12 μL^b) | | 30 μL^a (26 μL^b) | | 45 μL^a (38 μL^b) | |
|----------|------------------------------|-----|------------------------------|-----|------------------------------|-----|
| | Recovery (%) \pm SD | EFs | Recovery (%) \pm SD | EFs | Recovery (%) \pm SD | EFs |
| TPrP | 71 \pm 3 | 595 | 113 \pm 8 | 435 | 101 \pm 4 | 263 |
| TiBP | 100 \pm 1 | 831 | 99 \pm 6 | 381 | 115 \pm 6 | 298 |
| TBP | 98 \pm 4 | 816 | 103 \pm 6 | 396 | 110 \pm 6 | 287 |
| TCEP | 23 \pm 6 | 195 | 51 \pm 7 | 196 | 55 \pm 2 | 143 |
| TCPP | 88 \pm 2 | 734 | 119 \pm 7 | 458 | 111 \pm 5 | 289 |
| TDCP | 93 \pm 7 | 778 | 94 \pm 9 | 362 | 115 \pm 5 | 300 |
| TPP | 90 \pm 6 | 753 | 101 \pm 6 | 388 | 116 \pm 4 | 302 |
| TBEP | 109 \pm 7 | 906 | 95 \pm 6 | 365 | 114 \pm 8 | 295 |
| TEHP | 42 \pm 26 | 350 | 66 \pm 11 | 254 | 60 \pm 13 | 157 |
| TPPO | 54 \pm 15 | 452 | 70 \pm 12 | 269 | 85 \pm 6 | 221 |

^aVolume of CH_3CCl_3 in the extraction mixture.

^bVolume of the settled phase.

Recoveries for TCEP, TPPO, TCPP and TPrP improved with the volume of extractant, however, even for these compounds, EFs dropped significantly due to the increase in the volume of the sedimented phase (**Table 3**). Obviously, this diminution was more evident for TiBP, TBP,

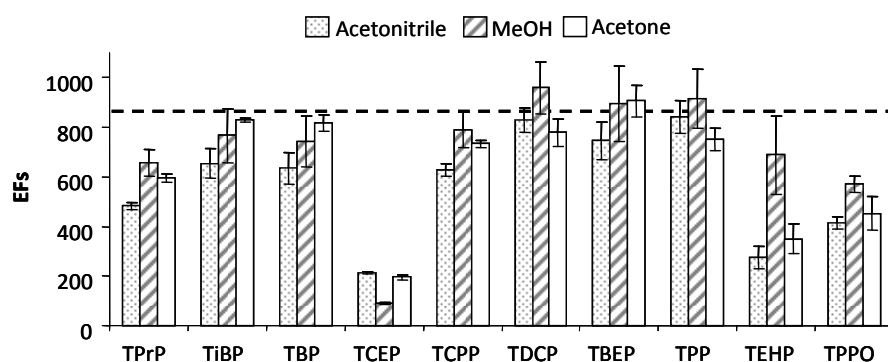
TDCP, TBEP and TPP, which were already quantitatively extracted with 20 μL of CH_3CCl_3 . In practice, this means more diluted extracts and thus higher quantification limits. Consequently, the volume of CH_3CCl_3 was limited to 20 μL . Although the use of lower volumes of extractant might lead to higher EFs, at least for the most lipophilic OPs, this option is not recommended since it is extremely complicate to handle extracts with volumes below 10 μL .

To confirm that residual amounts of OPs in the sample, after the DLLME, were in agreement with recoveries given in **Table 3**, some re-extraction assays were carried out. After performing the first DLLME, the upper aqueous phase was taken and submitted to second extraction in a clean tube. Responses for TiBP, TBP, TDCP, TBEP and TPP in second extracts represented less than 3% of those obtained in the first ones, meanwhile for TCPP this percentage rose to 7% and it was around 15% for TPrP, TEHP and TPPO. Finally, the recovered amount of TCEP in the second extract was about half of that measured in the first drop.

3.3. Disperser solvent

Acetonitrile and methanol were evaluated as alternative dispersers to acetone in the DLLME process. Extraction mixtures (1 mL volume) of each of these solvents and CH_3CCl_3 (20 μL), were added to 10 mL of spiked water samples with a 20% of NaCl. In all cases, 12 μL of settled phase were measured after vials centrifugation, therefore, variations in the yield of the dispersive liquid-liquid microextraction can be directly related to the nature of the disperser solvent. Obtained data are shown in **Fig. 1**. The horizontal, dotted line in this graph is the ratio between sample and extract volumes and represents the maximum allowable EF value.

Fig. 1. Influence of the disperser solvent on the EFs of the DLLME method. Data for 10 mL of water with a 20% of NaCl using CH_3CCl_3 (20 μL) as extractant. Mean values for $n=4$ replicates.



The highest differences among the three dispersers were noticed for TCEP. For this compound, acetone and acetonitrile provided twice higher enrichment factors than methanol. From an environmental perspective TCEP is one the most concerning OPs, therefore, the use of methanol as disperser was discarded. EFs for acetone and acetonitrile were very similar, although the first performed slightly better for TPrP, TiBP, TBP, TCPP and TBEP, therefore it was maintained as disperser solvent for the rest of the study. When compared with MASE, DLLME provided at least 4 times higher EFs for the same analytes [22].

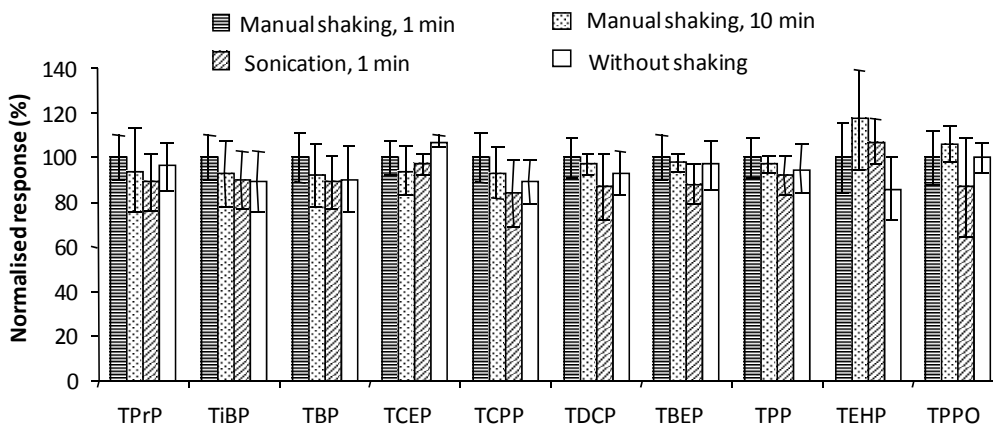
3.4. Operational parameters

3.4.1. Addition of the extraction mixture and shaking

When extractant and disperser were added separately to the water sample formation of a settled drop was not observed, making clear the need of mixing both solvents previously. To avoid cross-contamination problems, it was investigated whether high speed injection of the extraction mixture into the water sample with a gastight syringe, as recommended in previous papers [23-29], was indispensable to achieve high-extraction yields or if direct addition, e.g. with a micropipette, without contact with the sample, sufficed. Experimental data did not show any difference between both series of experiments, data not given.

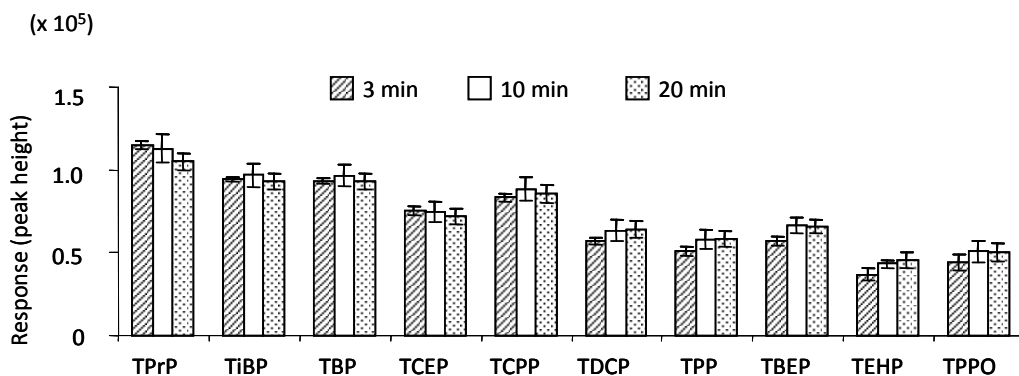
It would be also expected that shaking the extraction tube, after addition of the extraction mixture and before centrifugation, could have some influence in the extraction efficiency because it allows a more intimate and prolonged contact between aqueous and organic phases. **Fig. 2** compares the responses obtained for aliquots of the same spiked water sample, with and without shaking. In addition to manual shaking, sonication of the ternary solution, in a ultrasound bath, was also considered. As observed, neither manual nor ultrasound shaking played a significant effect on the efficiency of the DLLME. In further experiments, the extraction mixture was added to samples using a micropipette and tubes were just allowed to stand for 1 min before being centrifuged.

Fig. 2. Normalized responses as function of shaking time and mode, $n = 3$ replicates.



3.4.2. Centrifugation

Centrifugation was essential in order to obtain two distinguishable phases in the extraction tubes. **Fig. 3** shows that similar results were achieved using centrifugation times comprised between 3 and 20 min. Thus, the lower value was selected to speed up sample preparation.

Fig. 3. Influence of centrifugation on the responses achieved for OPs, $n = 3$ replicates.

3.5. Method performance

Precision of the DLLME method was examined using samples spiked at three different concentration levels. Relative standard deviations (RSDs) in the response of each analyte, for quadruplicate extractions, are shown in **Table 4**.

Table 4. Repeatability ($n = 4$ replicates), linearity and quantification limits ($S/N = 10$) of the DLLME method.

| Compound | Repeatability (RSD %) | | | Linearity R^2 | LOQ (ng/mL) |
|----------|------------------------|------------------------|----------------------|--------------------|-------------|
| | 0.1 ng/mL ^a | 0.2 ng/mL ^a | 1 ng/mL ^a | | |
| TPrP | 6 | 4 | 2 | 0.993 | 0.01 |
| TiBP | 6 | 3 | 2 | 0.997 | 0.04 |
| TBP | 3 | 6 | 2 | 0.997 | 0.01 |
| TCEP | 7 | 6 | 4 | 0.993 | 0.04 |
| TCPP | 7 | 4 | 3 | 0.993 | 0.03 |
| TDCP | 6 | 8 | 3 | 0.998 | 0.06 |
| TPP | 8 | 9 | 4 | 0.997 | 0.05 |
| TBEP | 7 | 8 | 4 | 0.998 | 0.05 |
| TEHP | 17 | 14 | 9 | 0.996 | 0.08 |
| TPPO | 9 | 6 | 9 | 0.997 | 0.06 |

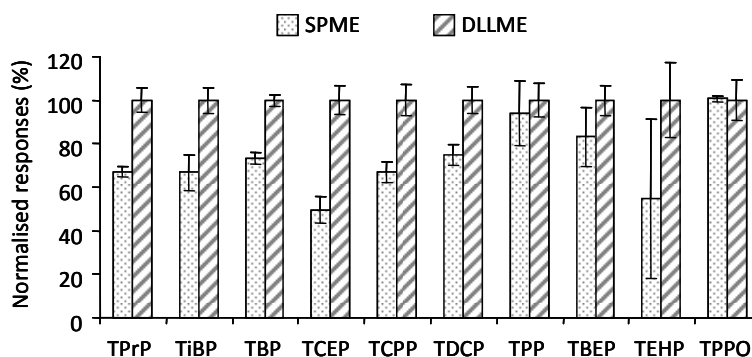
^a Concentrations of TiBP, TBP and TPP were half of this value; in case of TCEP it was twice higher.

For most compounds, values between 3 and 9% were achieved. The exception was TEHP, for which the corresponding RSDs remained between 9 and 17%. Globally, the obtained RSDs are slightly lower or very similar to those reported for SPME and MASE, respectively [16, 22]. Linearity was investigated using ultrapure water samples spiked with increasing concentrations of OPs at seven different levels between their quantification limits and 10 ng/mL. Acceptable correlation coefficients, even for TEHP which could not have been determined by SPME [16], were achieved within this interval, **Table 4**. Quantification limits of the method,

defined for a signal to noise (S/N) ratio of 10, ranged from 0.01 to 0.080 ng/mL (**Table 4**), with the lowest values corresponding to those species eluting in the first part of the chromatogram.

In order to obtain a direct comparison between extraction capabilities of the proposed sample preparation method and SPME, under identical instrumental detection conditions, aliquots of the same spiked sample were processed using both approaches. As depicted in **Fig. 4**, DLLME provided similar or higher responses for all species than SPME, with the additional advantages of being much faster (total sample preparation time about 5 min versus 40 min for SPME) and presenting a lower cost.

Fig. 4. Comparison of responses for aliquots of the same spiked water sample (from 0.4 to 1.5 ng/mL) processed using DLLME and SPME under conditions given in Ref. [16]. Normalized data for $n = 4$ replicates.



Procedural blanks showed the absence of contamination problems except in case of TiBP, which was normally presented at levels around 0.010 ng/mL in the chromatograms corresponding to non-spiked samples. Organic solvents used in the dispersive liquid-liquid microextraction process, sodium chloride and material (extraction tubes and caps) were discarded as potential sources of TiBP. Blanks of the SPME procedure also showed the presence of low TiBP signals, therefore, the compound could be presented in the ultrapure water. The quantification limit reported for this compound in **Table 4** was estimated taking into account blanks contribution.

Possible matrix effects of DLLME were evaluated by extracting samples of ultrapure, tap, river, treated and raw wastewater spiked (after filtration) with target analytes (0.4 ng/mL of TiBP, TBP and TPP, 1.8 ng/mL of TCEP and 0.8 ng/mL for the rest of OPs). Except in case of ultrapure water, the response for each compound was corrected with that corresponding to non-spiked aliquots of the same sample. Spiked and non-spiked fractions were processed in triplicate and responses for each compound in the different samples normalized to those measured for ultrapure water. In case of tap, river and treated wastewater, the efficiency of the DLLME remained the same as that achieved for ultrapure water, therefore, quantification can be performed by external calibration, **Table 5**. For raw wastewater, a 30% diminution in the responses of TiBP and TBEP was noticed; consequently, the standard addition method is recommended for this type of matrix. As expected, the extraction yield of TEHP underwent a progressive and significant reduction (up to 60%) with the increase in the complexity of the

samples, therefore, its quantification always requires to use the standard addition method. This pattern is in agreement with the behaviour described for TEHP using SPME as concentration technique [16].

Table 5. Evaluation of possible matrix effects of the DLLME method.

| Compound | Tap water | | River water | | Treated wastewater | | Raw wastewater | |
|----------|-----------|----|-------------|----|--------------------|----|----------------|----|
| | Mean (%) | SD | Mean (%) | SD | Mean (%) | SD | Mean (%) | SD |
| TPrP | 100 | 5 | 103 | 5 | 106 | 7 | 103 | 7 |
| TiBP | 95 | 7 | 100 | 9 | 84 | 7 | 66 | 6 |
| TBP | 98 | 8 | 104 | 10 | 100 | 9 | 94 | 7 |
| TCEP | 105 | 11 | 104 | 5 | 103 | 4 | 107 | 8 |
| TCPP | 100 | 9 | 105 | 5 | 93 | 11 | 89 | 8 |
| TDCP | 106 | 12 | 94 | 5 | 95 | 11 | 96 | 10 |
| TPP | 104 | 10 | 91 | 7 | 93 | 12 | 85 | 8 |
| TBEP | 107 | 12 | 94 | 10 | 82 | 12 | 71 | 9 |
| TEHP | 114 | 4 | 57 | 4 | 51 | 21 | 40 | 20 |
| TPPO | 107 | 15 | 97 | 7 | 95 | 8 | 101 | 10 |

Normalized responses (as percentage) to those achieved for ultrapure water. Addition level 0.4-1.8 ng/mL, $n = 4$ replicates.

3.6. Application to real samples

The proposed method was used to determine the levels of OPs in four different treated wastewater samples. Two of them were also processed using SPME (**Table 6**). A reasonable agreement was observed between values determined by both techniques.

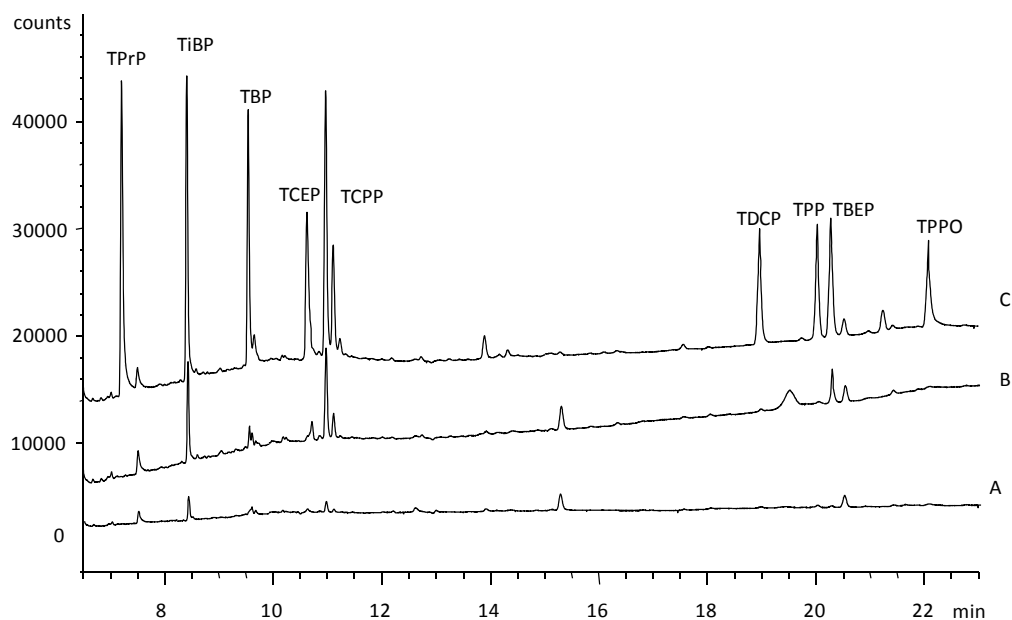
Table 6. Concentrations (ng/mL) of OPs in treated urban wastewater samples.

| Compound | Sample code 1 | | Sample code 2 | | Sample code 3 | Sample code 4 |
|----------|---------------|-------------|---------------|-------------|---------------|---------------|
| | DLLME | SPME | DLLME | SPME | DLLME | DLLME |
| TiBP | 0.14 ± 0.01 | 0.12 ± 0.01 | 0.129 ± 0.004 | 0.14 ± 0.01 | 0.27 ± 0.03 | nq |
| TBP | 0.06 ± 0.01 | 0.06 ± 0.01 | nq | nq | 0.020 ± 0.002 | nq |
| TCEP | nq | nq | nq | nq | 0.30 ± 0.05 | nd |
| TCPP | 0.30 ± 0.03 | 0.27 ± 0.02 | 0.293 ± 0.008 | 0.25 ± 0.01 | 0.09 ± 0.02 | 0.15 ± 0.01 |
| TBEP | nq | nq | 0.31 ± 0.02 | 0.21 ± 0.01 | 0.6 ± 0.1 | 0.12 ± 0.01 |

Mean values with their standard deviations, $n = 4$ replicates. nq: under quantification limits.

TCPP was found in all processed samples, and TiBP, TBP, TBEP and TCEP in at least one of them (**Fig. 5**). Quantified values remained below the 1 ng/mL level and were similar to those reported for treated wastewater samples in Germany [14, 31]. On the other hand, TPPO, TEHP, TPP, TDCP and TPrP could not be detected in any of the four samples.

Fig. 5. Overlay of GC-NPD chromatograms for water samples. (A) procedural blank, (B) non-spiked treated wastewater (code 2, **Table 6**) and (C) same sample spiked at 0.4 ng/mL.



4. Conclusions

The suitability of DLLME for the extraction of 10 OPs from water samples has been demonstrated. The developed procedure is a valuable alternative to classical large-scale methods, such as conventional liquid-liquid extraction, since it drastically reduces organic solvents consumption. Besides, DLLME allows obtaining a directly analysable extract in a short-single step, which is very advantageous compared to SPME or MASE. The type of extractant and the ionic strength of the sample were the variables which played a more relevant effect on achieved recoveries and enrichment factors. From the best of our knowledge, this work describes the first application of 1,1,1-trichloroethane as an effective extractant in DLLME. Performance (quantification limits, precision and accuracy) of the proposed method fitted the requirements for the determination of OPs in real-polluted water samples.

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2.5. PUBLICACIÓN:

***MIXED-MODE SOLID-PHASE EXTRACTION FOLLOWED BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF TRI- AND DI-SUBSTITUTED ORGANOPHOSPHORUS SPECIES IN WATER SAMPLES.**

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Mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry for the determination of tri- and di-substituted organophosphorus species in water samples

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Abstract

A procedure for the determination of three phosphoric acid diesters, eight triesters and triphenylphosphine oxide (TPPO) in water samples is presented. Analytes were simultaneously concentrated using a mixed-mode (reversed-phase and anionic-exchange) solid-phase extraction (SPE) sorbent and then sequentially eluted with methanol (triesters and TPPO) followed by a 20 mM tetrabutylammonium hydrogen sulphate (TBAHS) methanolic solution, case of diesters. After that, they were determined, in two different runs, by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS), operating the ESI source in the positive (triesters and TPPO) and negative (diesters) ionization modes. The efficiency of the extraction step varied between 70 and 105%, except in the case of tris(2-ethylhexyl) phosphate (TEHP), and it was barely affected by the type of water sample. Moreover, low signal suppression effects were noticed in the ESI ionization of extracts obtained from different environmental water samples. As a result, the standard addition methodology was only required for the accurate quantification of tri-substituted organophosphorus (OPs) species in wastewater samples. Limits of quantification of the optimized method ranged from 0.2 to 10 ng L⁻¹, depending on the sample matrix and the considered compound. The analysis of river and wastewater samples confirmed the occurrence of several tri- and di-substituted OPs in the aquatic environment, with the highest concentrations corresponding to tris(butoxyethyl) phosphate (TBEP) and tris(chloropropyl) phosphate (TCPP).

Keywords: Organophosphorus compounds; Flame retardants; Wastewater; Mixed-mode solid-phase extraction; LC-MS.

1. Introduction

Tri-substituted organophosphorus (OPs) compounds are employed as plasticizers and flame retardant additives in textiles, wallpapers, varnishes and polymeric materials. In most cases, these species are not chemically bonded to the host materials; therefore, they can be easily emitted to the surrounding areas [1]. As a result, several OPs have been found in different environmental matrices [2], such as air [3], dust [4] and wastewater [5]. The presence of tri-substituted OPs in the latter compartment is a matter of concern because of the high mobility and poor removal rates of the most polar species, particularly tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloroisopropyl) phosphate (TCPP), which pass through conventional urban wastewater treatment plants without undergoing significant removal rates [5,6].

Phosphoric acid diesters, particularly dibutyl phosphate (DBP) and diethylhexyl phosphate (DEHP), are often employed as extractants of radioactive elements [7,8]. Apart of being intentionally produced, diesters are also formed as biodegradation intermediates and metabolites [9,10] of triesters. In addition, DBP and monobutyl phosphate (MBP) might be the decomposition products of tributyl phosphate (TBP) when used as extractant in the nuclear industry [11].

During last years, several studies have addressed the determination of OPs triesters in water samples using different sample preparation approaches and mainly gas chromatography (GC) as separation technique. GC combined with nitrogen-phosphorus detection (NPD) [12,13], mass spectrometry using electron impact (EI-MS) [14] or positive chemical ionization (PCI-MS) [15], atomic emission detection (AED) [16] or inductively coupled plasma (ICP)-MS [17,18] have been used for tri-substituted OPs determination. However, none of these techniques alone meets the requirements of high selectivity, sensitivity and affordable cost. On the other hand, triesters are also amenable to liquid chromatography (LC) determination [19,20]; multiple reaction monitoring (MRM) tandem mass spectrometry (MS/MS) detection combines low limits of quantification (LOQs) and a high degree of selectivity. In addition, LC-MS/MS allows the analysis of polar molecules such as phosphoric acid diesters [21] that can only be subjected to GC analysis if derivatized [22], with all the disadvantages that entail. Up to this date, only a few works have dealt with the analysis of organophosphate diesters and, bearing in mind the above information, LC-MS has been the preferred choice [23-26].

Triesters extraction from water samples has been accomplished with liquid-liquid extraction (LLE) [27] or solid-phase extraction (SPE) [20] but also with microextraction approaches such as solid-phase microextraction (SPME) [28], dispersive liquid-liquid microextraction (DLLME) [29] or microporous membrane liquid-liquid extraction (MMLLE) [30]. Nevertheless, in the few papers reporting diesters extraction from water [25,26] or urine [22,24] samples, SPE has been the selected technique. Quintana et al. determined phosphoric acid diesters in wastewater samples utilizing ion-pair solid-phase extraction (IP-SPE) with tributylamine (TrBA) through Lichrolut RP18 cartridges [25]. Möller et al. [23,24] developed a molecularly imprinted solid-phase extraction (MISPE) method for extracting diphenyl phosphate (DPP) from urine samples and compared its performance with several commercially available SPE sorbents, showing that Oasis MAX cartridges were also suitable for DPP extraction, providing higher recoveries than the molecularly imprinted polymer (MIP) but lower selectivity. Finally, Shindler et al. [22] determined some diesters in urine samples by means of SPE, with Isolute Env+, followed by a derivatization step and a further clean-up prior to the determination by GC-MS/MS.

To the best of our knowledge, up to the moment, there is only one work where some triesters and diesters have been simultaneously extracted [26]. They were included in a multi-residue method for the determination of emerging organic pollutants in water samples using SPE with Oasis HLB. After elution of the SPE cartridge with methanol, OPs were determined in two different runs considering reversed-phase (triesters) and ion-pairing (diesters) LC separation mechanisms. Two different LC columns using different modifiers in the mobile phase were also

employed [26]. Unfortunately, in the above work, a detailed evaluation of potential matrix effects during electrospray ionisation (ESI) of tri- and di-substituted OPs is missed.

The aim of the present investigation was to develop an improved methodology for the determination of tri- and di-substituted OPs in water samples using SPE and LC-ESI-MS/MS as extraction and determination techniques, respectively. Efforts were mainly focussed on (1) increasing the selectivity of the extraction step, which resulted in lower signal suppression effects during ESI and (2) developing common LC separation conditions for both families of OPs, allowing the use of the same column for their determination. The effects of different factors on the efficiency of both steps are thoroughly evaluated and the performance of the method evaluated using environmental water samples with different complexities.

2. Experimental

2.1. Reagents, standards and material

HPLC-grade methanol and ammonia, 25% aqueous solution, were purchased from Merck (Darmstadt, Germany). Triethylamine (TEA) and tetrabutylammonium hydrogen sulphate (TBAHS) were provided by Aldrich (Steinheim, Germany), whereas acetic and formic acid were supplied by Riedel-de Haën (Seelze, Germany). Ultrapure water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) system. Tripropyl phosphate (TPPrP), TBP, triisobutyl phosphate (TiBP), TCEP, tris(1,3-dichloroisopropyl) phosphate (TDCP), tris(butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), tris(2-ethylhexyl) phosphate (TEHP), triphenyl phosphine oxide (TPPO), DPP and DEHP were acquired from Aldrich (Milwaukee, WI, USA). TCPP, as a technical mixture of isomers, was provided by Dr. Ehrenstorfer (Augsburg, Germany) and DBP was obtained from Fluka (Steinheim, Germany). Chemical structures and some relevant properties of the above compounds have been compiled in a recent review [2]. Individual standards and mixtures of tri- and di-substituted OPs were prepared in methanol and 20 mM TBAHS in methanol, respectively.

Oasis HLB (60 mg) and MAX (60 and 150 mg) SPE cartridges were obtained from Waters (Milford, MA, USA).

2.2. Samples

Ultrapure water, river water and urban wastewater, obtained from a sewage plant equipped with primary and secondary (activated sludge) treatment units, were employed throughout this study. Samples, except ultrapure water, were passed through a combination of glass fiber pre-filters and 0.45 μm pore size nitrocellulose filters (Millipore), both 47 mm in diameter, and concentrated immediately after arriving to the laboratory.

2.3. Solid-phase extraction

2.3.1. Breakthrough and elution studies

Optimization of SPE conditions was performed with aliquots (100-500 mL) of different environmental water samples, fortified with target compounds and passed through the

considered SPE sorbent (ca. 10 mL min⁻¹). Breakthrough volumes were evaluated by passing the spiked samples through two cartridges connected in series. After the extraction step, they were disconnected and processed independently. Elution volumes were determined by collecting consecutive 1 mL fractions of the SPE cartridge eluate.

2.3.2. Sample extraction conditions

Under final conditions, 500 mL of river water, or 100 mL in the case of wastewater, were concentrated using the mixed-mode Oasis MAX sorbent (150 mg), previously conditioned with a 20 mM methanolic solution of TBAHS, methanol and water, 5 mL each. After finishing the extraction step, the SPE cartridge was rinsed with 10 mL of ultrapure water and then dried under nitrogen stream. Analytes were eluted in two different fractions using 2 mL of methanol (triesters and TPPO) followed by 3 mL of a 20 mM TBAHS methanolic solution (diesters). Finally, extracts were concentrated with a gentle stream of nitrogen and made up to 1 mL using methanol or 20 mM TBAHS in methanol for triesters and diesters, respectively.

2.4. Liquid chromatography-mass spectrometry

Compounds were determined by LC-MS/MS using a Varian (Walnut Creek, CA, USA) system. The LC instrument comprised two ProStar 210 high-pressure mixing pumps (Varian), a vacuum membrane degasser (Metachem Technologies, Bath, UK) and a ProStar 410 module (Varian) consisting of an autosampler and a thermostated compartment for the LC column. The LC system was interfaced to a triple-quadrupole 1200L mass spectrometer fitted with a ESI source (Varian).

Compounds were separated using a Luna C18 column (100 mm x 2 mm; 3 µm), acquired from Phenomenex (Torrance, CA, USA), and connected to a C18 (4 mm x 2 mm) guard cartridge from the same supplier. Five millimolar ammonium acetate in ultrapure (Milli-Q) water (A) and in methanol (B) were used as mobile phases for the separation of both groups (tri- and di-substituted) of OPs, which were determined in two different runs operating the ESI source in the negative (di-) and positive (tri-substituted species) ionization modes. For the former group of compounds the optimized gradient was: 0-2 min, 50% B; 17-22 min, 100% B; 23-27 min, 50% B. Regarding triesters and TPPO, the composition of the mobile phase was varied accordingly to the following program: 0-2 min, 50% B; 16-30 min, 100% B; 32-35 min, 50% B. In both cases, the mobile phase flow was set at 0.2 mL min⁻¹ and the column maintained at 30 °C. The injection volume for standards and sample extracts was 10 µL, filling the remaining free space in the loop (total volume 100 µL) with ultrapure water. This injection mode avoids broadening of the injection band without dilution of methanolic extracts, from SPE cartridges, previously to their injection in the LC system.

Nitrogen (99.999%), used as nebulising (50 psi) and drying gas (200 °C, 20 psi) in the ESI source, was provided by a high purity generator (Domnick Hunter, Durham, UK). The temperature of the ESI housing was maintained at 50 °C and the voltage of the ESI needle fixed at 5000 V and 4500 V in the positive and negative ionization modes, respectively. Argon (99.999%) was employed as collision gas (2.9 x 10⁻⁶ psi) for MS/MS measurements.

Search for the most intense MS/MS transitions was performed by infusion of individual standards of each OPs (ca. $2 \mu\text{g mL}^{-1}$ in methanol:water, 1:1) at a constant flow of $20 \mu\text{L min}^{-1}$, in the positive and negative ionization modes for tri- and diesters, respectively. Firstly, the intensity of the signal for each parent ion, corresponding to protonated ($[\text{M}+1]^+$) or de-protonated ($[\text{M}-1]^-$) species, was optimized changing the capillary voltage. After that, they were subjected to collision induced dissociation (CID), optimizing the collision energy to maximize the responses of product ions. Two transitions were recorded per compound: the most intense one was used to quantify the response of each species and the other transition was employed for qualifying purposes.

2.5. Yields of SPE, matrix effects and overall recoveries

The yield of the SPE process was assessed by comparison of signals attained for samples fortified before and after extraction. In this way, the obtained responses remained unaffected by potential changes in the efficiency of the ESI ionization, depending on the complexity of SPE extracts. On the other hand, potential matrix effects related to changes in the efficiency of the ESI ionization were evaluated through a series of experiments as reported in other works [31,32]. In brief, responses (peak areas) obtained for extracts from real water samples, spiked after the SPE extraction step, were compared with those measured for a standard with the same concentration, prepared in methanol (triesters and TPPO) or in 20 mM TBAHS methanolic solution (diesters). Non-spiked aliquots of same samples were also processed in order to compensate for the presence of target species in environmental matrices.

Overall recoveries for the whole method, accounting for the efficiency of the SPE extraction and matrix effects, were evaluated using external calibration as quantification technique. The difference between peak areas obtained for spiked (before extraction) and un-spiked samples were compared with those measured for standards.

2.6. Method performance

Figures of merit corresponding to the LC-MS/MS determination step and the proposed procedure (SPE followed by LC-MS/MS) were estimated with pure standards and environmental water samples fortified at different concentration levels. The linearity in the response of the LC-MS/MS system was investigated by injection of standard mixtures at eight different concentration levels from 2 to 1000 ng mL^{-1} . Instrumental limits of quantification (LOQs) were calculated as the concentration of each species giving a signal 10 times the standard deviation of the background noise in the LC-MS/MS chromatogram. LOQs of the proposed procedure were evaluated taking into account (1) the instrumental LOQs, (2) the enrichment factor provided by the SPE procedure for each matrix (500 and 100 mL for river and wastewater, respectively) and (3) the presence of compounds in procedural blanks corresponding to the concentration of 500 mL aliquots of ultrapure water.

3. Results and discussion

3.1. Optimization of LC-ESI-MS/MS conditions

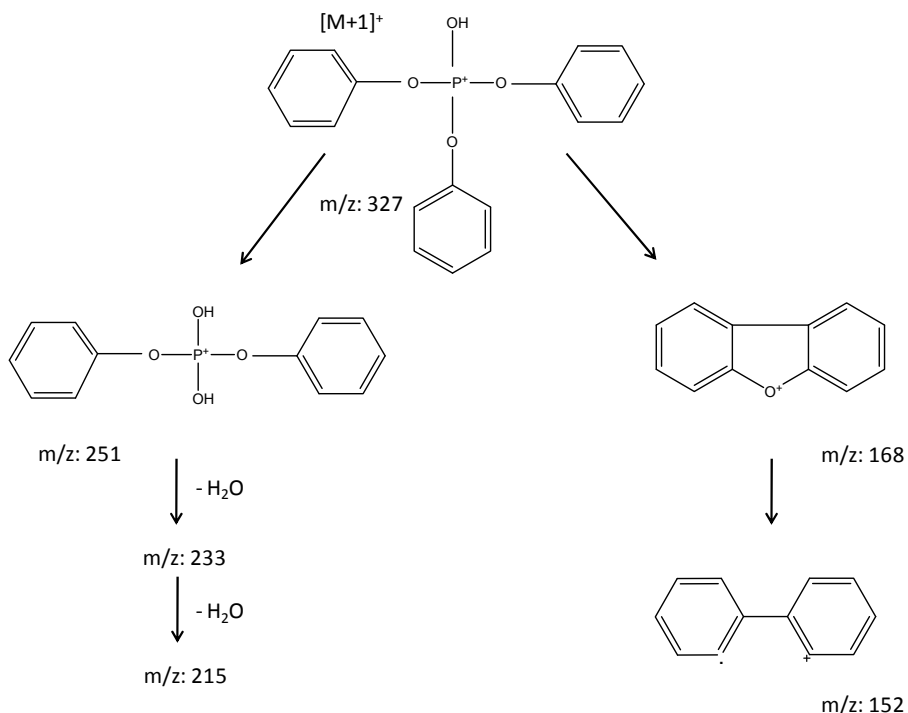
Table 1 summarizes the ionization modes (positive or negative), capillary voltages, collision energies and the two most intense transitions for each species with their relative intensities.

Table 1. Optimized ESI-MS/MS parameters for the MRM determination of target compounds.

| Compound | Capillary Voltage (V) | MRM transitions (m/z) | Collision energy (eV) | Relative signal intensity (%) |
|----------|-----------------------|-----------------------|-----------------------|-------------------------------|
| ESI + | | | | |
| TCEP | 56 | 285>99 | 17 | 100 |
| | | 285>223 | 7 | 91 |
| TPPO | 96 | 279>201 | 17.5 | 100 |
| | | 279>219 | 14.5 | 6 |
| TPrP | 30 | 225>99 | 13.5 | 100 |
| | | 225>141 | 7 | 44 |
| TCPP | 40 | 327>99 | 15 | 100 |
| | | 329>99 | 15.5 | 98 |
| TPP | 96 | 327>152 | 23.5 | 100 |
| | | 327>215 | 19 | 80 |
| TDCP | 72 | 431>99 | 15 | 100 |
| | | 433>99 | 15.5 | 79 |
| TiBP | 30 | 267>99 | 11.5 | 100 |
| | | 267>155 | 5.5 | 37 |
| TBP | 30 | 267>99 | 11.5 | 100 |
| | | 267>155 | 5.5 | 38 |
| TBEP | 48 | 399>199 | 9 | 100 |
| | | 399>299 | 7.5 | 91 |
| TEHP | 40 | 435>99 | 8 | 100 |
| | | 435>323 | 5 | 19 |
| ESI - | | | | |
| DPP | -76 | 249>93 | 26 | 100 |
| | | 249>155 | 20.5 | 12 |
| DBP | -56 | 209>79 | 20 | 100 |
| | | 209>153 | 13 | 58 |
| DEHP | -30 | 321>79 | 25 | 100 |
| | | 321>209 | 20 | 36 |

In the case of alkylated OPs, reported transitions corresponded to the replacement of alkylated moieties by hydrogen, or their elimination as neutral fragments, rendering as later products the $[H_4PO_4]^+$ or $[PO_3]^-$ ions, for tri- and di-substituted species, respectively. The most intense transitions in the MS/MS spectra of TPPO also reflected the elimination of an aromatic ring (279>201) or its replacement by a hydroxyl substituent (279>219). The $[M-1]^-$ parent ion corresponding to DPP underwent the loss of a phenolic group, as a neutral fragment (249>155) or as a negatively charged phenolate (249>93). Finally, TPP presented a more complex fragmentation pathway (**Fig. 1**), with rearrangements between phenolic moieties (327>168), followed by a further removal of oxygen (168>152). Another mechanism consisted of the replacement of a phenyl group by hydrogen (327>251) followed by two successive losses of water, **Fig. 1**.

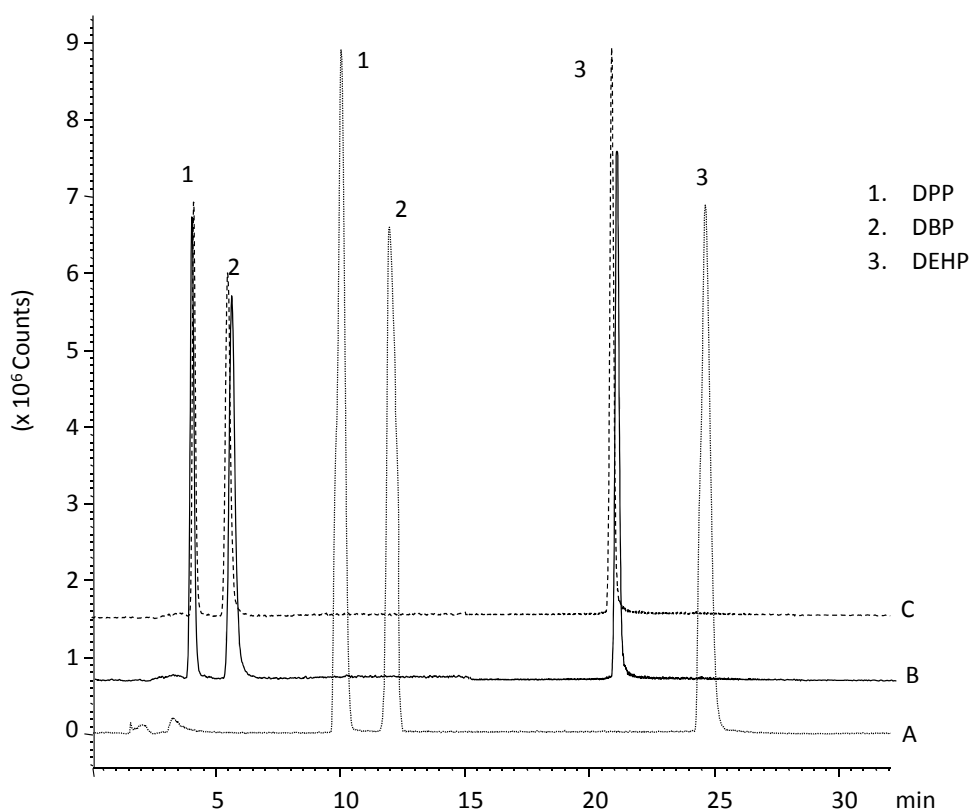
Fig. 1. Proposed MS/MS fragmentation pathways for TPP in the positive ionization mode.



On the basis of previous works [19,20,25,33] two mobile-phase additives, ammonium acetate (0-10 mM) and formic acid (0-0.1%), were considered in order to improve compounds separation and the sensitivity of the method. The evaluation of their influence was performed by injection of a $0.5 \mu\text{g mL}^{-1}$ standard of each group of compounds (di- and tri-substituted ones). OPs diesters are acidic species with estimated pKa values under 2 units [2]; moreover, two of the three species considered in this study (DBP and DPP) show log Kow values under 3 units, consequently they are expected to be poorly retained in reversed-phase LC column. In fact, in previous works tri-n-butylamine was added to the mobile phase as ion-pairing reagent to

increase their affinity by the stationary phase of the column [25,26]. However, accordingly to our experiments, the three compounds were retained by the stationary phase of the LC column using just methanol and water, without any modifier, as mobile phases. Anyhow, broad peaks were noticed for all species. Addition of formic acid, at a 0.1% concentration, to the mobile phase (pH 2.7 units) led a slight reduction in the peak widths, without modifying the retention times of target species. Ammonium acetate produced a further reduction in the peak widths of OP diesters. Moreover, it also shortened their retention times, **Fig. 2** (chromatograms A and B). This later effect is explained by the higher ionic strength of the mobile phase, which increases the solubility of the anionic diesters. Considering a 5 mM concentration of ammonium acetate, similar retention times were noticed for standards in methanol and those prepared in a 20 mM TBAHS methanolic solution, **Fig. 2** (chromatograms B and C). As further described in this study, this salt was necessary to elute these species from the mixed-mode SPE sorbent.

Fig. 2. Total ionic current (TIC) chromatograms for a standard of diesters ($0.5 \mu\text{g mL}^{-1}$, in methanol) using the gradient reported in the experimental section and different concentrations of ammonium acetate (NH_4AcO) as mobile phase modifier. (A) 1 mM (pH 5.5). (B) 5 mM (pH 5.3). (C) 5 mM, standard in a 20 mM methanolic solution of TBAHS.



As regards the tri-substituted OPs, none of the above modifiers showed a significant effect on the performance of their LC separation; therefore, ammonium acetate, the same modifier as for diesters, was selected as additive in the mobile phase. In general, increasing the amount of this salt (from 1 to 10 mM) caused a reduction in the efficiency of their ESI ionization, **Fig. 3**. Nevertheless, a 5 mM concentration was chosen in order to operate the LC column under similar conditions to those reported for diesters.

Fig. 3. Comparison of responses (average values with their standard deviations) obtained for a standard of tri-substituted OPs ($0.5 \mu\text{g mL}^{-1}$) as function of the concentration of ammonium acetate (NH_4AcO) added to the mobile phase, $n = 3$ replicates.

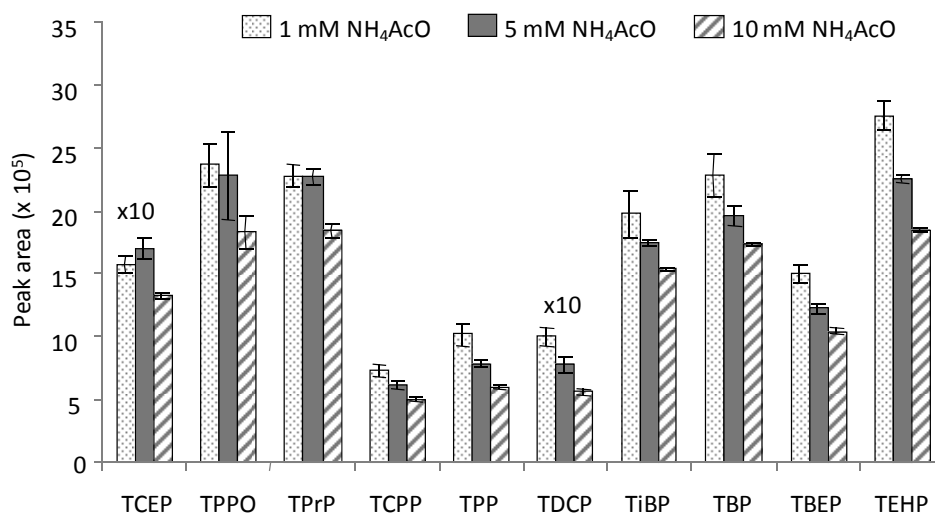


Fig. 4 depicts the chromatograms corresponding to a standard of the ten tri-substituted OPs (100 ng mL^{-1} per compound), acquired under conditions reported in the experimental section. Although two pairs of compounds (TPrP-TCPP and TPP-TDCP) co-eluted, they can be quantified individually using their characteristics MRM transitions. On the other hand, a baseline separation was achieved between the isomeric tri-butyl phosphates, **Fig. 4**.

Fig. 4. TIC and MRM chromatograms for a standard of tri-substituted OPs ($0.1 \mu\text{g mL}^{-1}$) under optimized LC-ESI-MS/MS conditions.

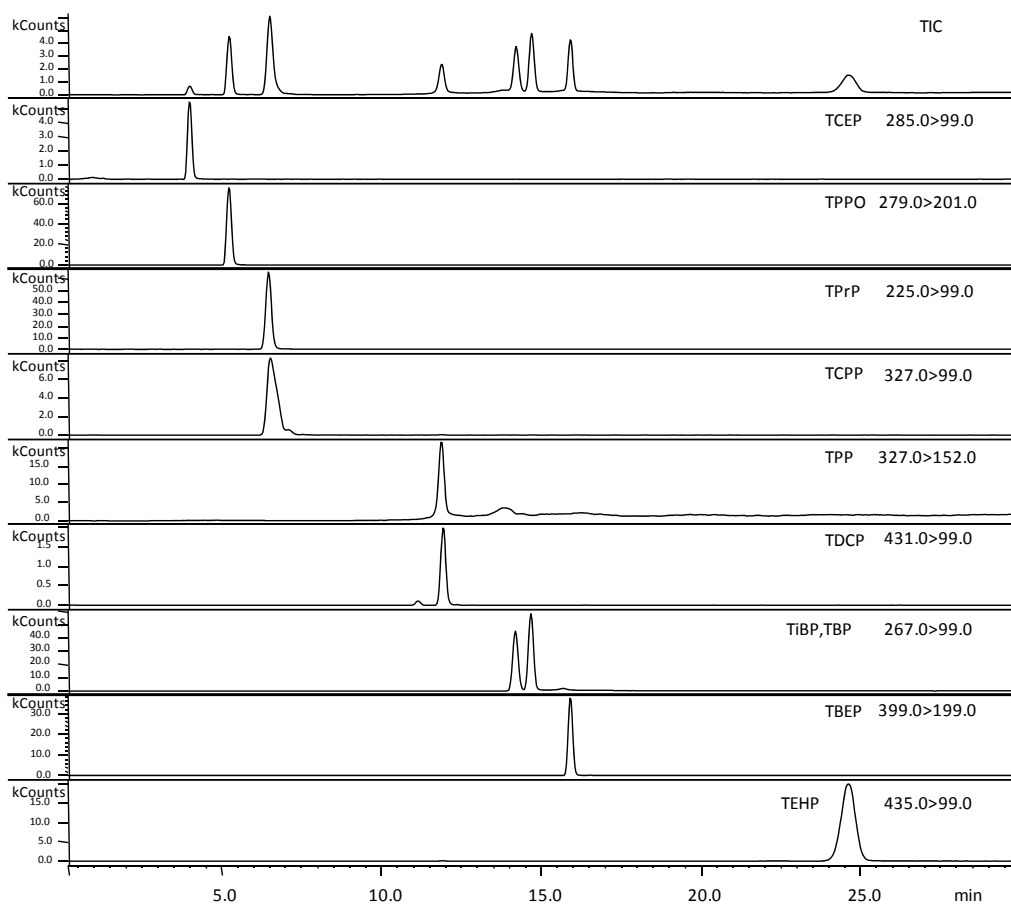


Table 2 summarizes the performance of LC-ESI-MS/MS system for target species using the above optimized conditions. The plot of peak areas versus the concentration of each compound fitted a linear trend with correlation coefficients (R^2) ranging from 0.991 to 1.000. Intra- and inter-day precision was evaluated at two concentration levels (25 and 100 ng mL^{-1}). The relative standard deviations (RSDs), obtained under reproducibility conditions, stayed below 9 and 8% for di- and tri-substituted compounds, respectively. Instrumental LOQs varied between 0.3 and 1.0 ng mL^{-1} in the case of diesters, and they ranged from 0.1 to 1 ng mL^{-1} for the triesters. Overall, the above values are slightly lower than those reported by Martínez-Carballo et al. [33] for OPs triesters.

Table 2. Linearity, inter- and intra-day precision and instrumental limits of quantification (LOQs) of the LC-ESI-MS/MS system.

| Compound | Retention time (min) | Linearity, R ² (2-1000 ng mL ⁻¹) | Precision (RSD %) | | | LOQs (ng mL ⁻¹) |
|----------|----------------------|--|---------------------------------|-------------------------|---|--------------------------------|
| | | | Intra-day (n = 5 replicates) | | ^a Inter-day (n = 15 replicates) | |
| | | | 25 ng mL ⁻¹ | 100 ng mL ⁻¹ | 100 ng mL ⁻¹ | |
| TCEP | 4.13 | 0.996 | 7 | 2 | 8 | 0.4 |
| TPPO | 5.48 | 0.993 | 3 | 3 | 6 | 0.1 |
| TPrP | 6.93 | 0.992 | 2 | 2 | 6 | 0.1 |
| TCPP | 7.06 | 0.993 | 3 | 2 | 7 | 0.5 |
| TPP | 12.07 | 0.995 | 6 | 3 | 8 | 1.0 |
| TDCP | 12.14 | 0.998 | 7 | 4 | 8 | 0.3 |
| TiBP | 14.38 | 0.992 | 3 | 2 | 4 | 0.1 |
| TBP | 14.86 | 0.991 | 4 | 2 | 4 | 0.1 |
| TBEP | 16.08 | 0.993 | 4 | 3 | 4 | 0.1 |
| TEHP | 25.48 | 0.997 | 7 | 3 | 4 | 1.0 |
| DPP | 4.76 | 1.000 | 7 | 6 | 5 | 1.0 |
| DBP | 6.61 | 0.999 | 6 | 3 | 4 | 0.6 |
| DEHP | 21.74 | 1.000 | 3 | 5 | 9 | 0.3 |

^a Injections were performed on 3 consecutive days.

3.2. Optimization of solid-phase extraction conditions

The earlier series of experiments aiming to evaluate the performance of the SPE step were performed using aliquots of ultrapure water (from 50 to 500 mL), spiked with target compounds at 10 ng mL⁻¹. Breakthrough studies were carried out without acidification of the samples in order to limit the co-extraction of humic acids during analysis of environmental samples. Using the Oasis HLB sorbent (60 mg cartridges), the tri-substituted OPs and DEHP were retained quantitatively in the first cartridge; however, DBP and DPP showed a lower affinity by this reversed-phase polymer, appearing in the extract from the second cartridge, even when the sample intake was limited to 50 mL. On the other hand, considering the same mass of MAX sorbent, breakthrough volumes over 500 mL were achieved for all OPs, when dealing with aliquots of ultrapure water. The same behavior was observed for river water; however, when experiments were repeated using 250 mL of raw wastewater, around 10% of triesters passed to the second cartridge, whereas diesters remained quantitatively in the first one. Breakthrough studies were repeated with larger cartridges, containing 150 mg of the MAX sorbent. In this case, up to 250 mL of raw wastewater and 500 mL of river water could be concentrated without noticeable breakthrough problems for any compound.

HLB and MAX sorbents also showed different features as concerns the selectivity of the elution step. With the first polymer, all OPs were recovered with a small volume of methanol

(2 mL in case of 60 mg cartridges). The same volume of methanol sufficed to elute the tri-substituted OPs from the mixed-mode sorbent; however, the diesters remained attached to the sorbent by ionic interactions. In order to favor the desorption of these latter compounds, methanol modified with formic acid (2%), TEA (20 mM), ammonium acetate (20 mM) and TBAHS (20mM) were tested. The two latter salts resulted effective to break the electrostatic interactions between the anionic diesters and the positively charged groups in the sorbent. Using TBAHS, the three diesters were recovered with the first three fractions (1 mL each) of eluent. In the case of ammonium acetate, a considerable amount of DPP was still noticed in the 4th fraction; thus, TBAHS was selected as modifier. Taking above considerations into account water samples were concentrated using the 150 mg MAX cartridges. After drying the sorbent with a gentle stream of nitrogen, the triesters and TPPO were first eluted with 2 mL of methanol and then the diesters were recovered with 3 mL of a 20 mM TBAHS solution in methanol. Both fractions were evaporated to 1 mL, and injected directly in the LC-MS/MS system.

Table 3 summarizes the efficiency of the SPE extraction for river (500 mL) and wastewater (100 mL) samples, spiked at two different concentrations.

Table 3. Percentages of recovery (mean values with their standard deviations, SD) of the SPE step with MAX 150 mg cartridges for river (500 mL) and wastewater samples (100 mL), spiked at 1 and 5 ng mL⁻¹, respectively.

| | Recovery % (SD) | | |
|------|-----------------|--------------------|----------------|
| | River water | Treated wastewater | Raw wastewater |
| TCEP | 84 (2) | 82 (3) | 70 (4) |
| TPPO | 94 (2) | 104 (2) | 99 (3) |
| TPrP | 87 (3) | 97 (2) | 92 (2) |
| TCPP | 91 (7) | 88 (2) | 87 (2) |
| TPP | 89 (5) | 93 (3) | 86 (2) |
| TDCP | 86 (7) | 89 (3) | 80 (5) |
| TiBP | 82 (2) | 98 (3) | 95 (3) |
| TBP | 84 (2) | 99 (2) | 93 (2) |
| TBEP | 75 (3) | 94 (1) | 96 (2) |
| TEHP | 26 (8) | 27 (2) | 16 (4) |
| DPP | 101 (5) | 98 (2) | 91 (4) |
| DBP | 105 (6) | 97 (2) | 78 (5) |
| DEHP | 94 (3) | 91 (10) | 82 (5) |

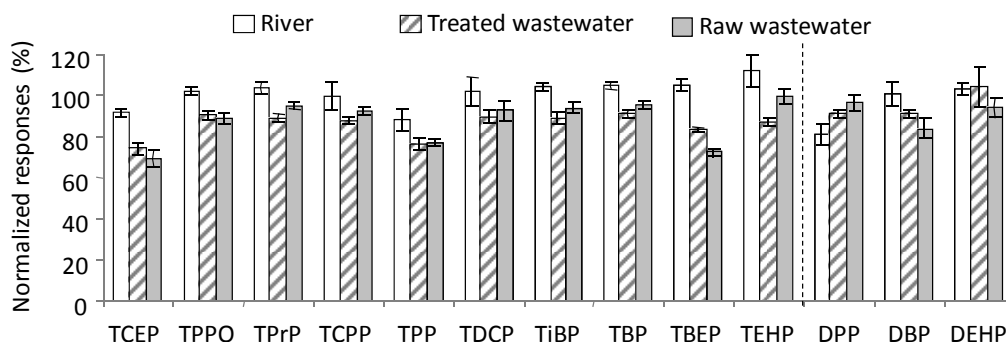
In general, the SPE process rendered recoveries from 70 to 105% for all compounds in the three tested matrices. The only exception was TEHP, which is a highly lipophilic compound (log Kow 9.49) with a strong trend to remain attached to glass material and PTFE pipes connecting the water sample with the SPE cartridge, which led to unsatisfactory recoveries. This

problem can be overcome by rinsing these connections with methanol, which is further mixed with the fraction eluted from the SPE cartridge. The obvious drawback of the above strategy is that the volume of the final extract increases significantly, up to 20-30 mL [26]. Considering that, to the best of our knowledge, the occurrence of TEHP in environmental water samples has never been reported, it was preferred to exclude it from the list of target OPs than to raise the consumption of methanol in the SPE process. Globally, the above extraction efficiencies are similar to those reported for OP triesters in wastewater using the OASIS HLB sorbent [20] and diesters concentrated on C18, after formation of the corresponding ion-pairs in the aqueous sample [25]. The major advantage of the methodology optimized in this work is that both families of OPs are concentrated simultaneously and then fractionated during elution of SPE cartridges.

3.3. Matrix effects and other figures of merit

A well-known shortcoming of LC-MS based methods, particularly when ESI sources are used, is that the yield of the ionization process might change significantly between standards and sample extracts. Thus, the presence of salts and other organic species in the extracts from SPE cartridges may produce signal enhancement or suppression effects. In previous works, signal attenuation effects up to 50 and 70% have been reported for the determination of tri- and di-substituted OPs, respectively, in raw wastewater samples concentrated 100 times over reversed-phase sorbents [20,25]. On one hand, such strong matrix effects increase the LOQs of the method; on the other one, the time-consuming standard addition method needs to be used for an accurate evaluation of analytes' levels in environmental water samples. **Fig. 5** shows the responses obtained for spiked extracts from river and wastewater (concentrated 500 and 100 times, respectively) after normalization to a standard of the same concentration ($0.5 \mu\text{g mL}^{-1}$). Maximum signal suppression effects, around 35%, were noticed for a few triesters (e.g. TCEP), in the most complex of the investigated matrices: raw wastewater. For diesters, matrix effects were even less important, with a maximum of signal suppression around 20%. On the basis of these data, it appears that mixed-mode SPE cartridges provided more selective extractions than reversed-phase ones used in previous works [20,25].

Fig. 5. Matrix effects evaluation. The differences between responses obtained for spiked and non-spiked extracts from river and wastewater samples (concentrated 500 and 100-folds, respectively) were normalized to those measured for a standard with the same concentration ($0.5 \mu\text{g mL}^{-1}$). Mean values with their standard deviations, $n = 3$ replicates.



The overall recoveries of the whole analytical procedure for tri-substituted OPs ranged from 47 to 109%, with standard deviations below 10%, **Table 4**.

Table 4. Overall recoveries (determined with external calibration) and estimated LOQs of the proposed method for river and wastewater samples.

| | % Recovery with standard deviation, $n = 3$ replicates | | | | LOQs (ng L^{-1}) | |
|------|--|--------------------------|---------------------------------|-----------------------------|-----------------------------|------------|
| | ^a River water | ^b River water | ^c Treated wastewater | ^c Raw wastewater | River | Wastewater |
| TCEP | 108 ± 6 | 77 ± 5 | 60 ± 8 | 47 ± 6 | 0.8 | 7 |
| TPPO | 89 ± 5 | 96 ± 3 | 94 ± 2 | 88 ± 3 | 0.2 | 1 |
| TPrP | 85 ± 1 | 90 ± 3 | 86 ± 2 | 88 ± 5 | 0.2 | 1 |
| TCPP | 86 ± 1 | 90 ± 2 | 86 ± 4 | 79 ± 8 | 1 | 5 |
| TPP | 85 ± 7 | 77 ± 6 | 70 ± 6 | 65 ± 8 | 2 | 10 |
| TDCP | 109 ± 8 | 88 ± 5 | 80 ± 7 | 74 ± 7 | 0.6 | 3 |
| TiBP | 85 ± 2 | 85 ± 6 | 87 ± 4 | 89 ± 5 | 10 | 10 |
| TBP | 88 ± 3 | 88 ± 5 | 90 ± 3 | 89 ± 4 | 10 | 10 |
| TBEP | 96 ± 3 | 78 ± 9 | 76 ± 7 | 67 ± 4 | 10 | 10 |
| DPP | 77 ± 8 | 86 ± 2 | 77 ± 4 | 83 ± 4 | 2 | 10 |
| DBP | 79 ± 5 | 73 ± 5 | 73 ± 9 | 70 ± 5 | 1 | 5 |
| DEHP | 79 ± 3 | 75 ± 7 | 90 ± 1 | 77 ± 6 | 0.7 | 4 |

^a Addition level 0.1 ng mL^{-1} .

^b Addition level 1 ng mL^{-1} .

^c Additon level 5 ng mL^{-1} .

The lowest value corresponded to TCEP, the species showing the shorter retention time and the highest signal suppression effects during ESI ionisation (**Fig. 5**). Although for the rest of tri-substituted compounds the global efficiency of the sample preparation methodology remained over 65%, the standard addition method is recommended to assess their concentration in sewage water samples, whereas external calibration can be used when dealing with river water. For the three diesters involved in this study, global recoveries over 70%, with standard deviation values from 2 to 9%, were attained, **Table 4**. Consequently, external calibration was used to quantify their levels in river and wastewater samples.

Procedural blanks often showed traces of TiBP, TBP and TBEP (ca. 2-3 ng L^{-1}). Probably, these compounds arise from polymeric materials used in the purification water system. Moreover, the presence of TBEP in the blanks could be related with the high levels of this species in dust from indoor environments [4], and/or being due to the presence of this plasticizer in septa used to close the autosampler vials [28] or any other plastic connector used in the SPE process. Obviously, procedural blanks have to be run periodically in order to avoid the report of false positives during analysis of environmental water samples.

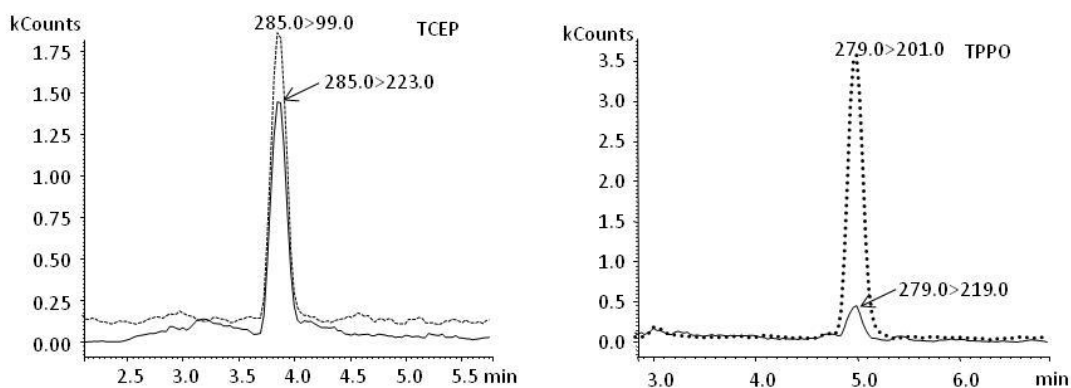
LOQs from 0.2 to 2 ng L⁻¹ and ranging from 1 to 7 ng L⁻¹ were achieved for those compounds not affected by contamination problems in river water and wastewater, respectively, **Table 4**. In the case of TiBP, TBP and TBEP a common LOQ of 10 ng L⁻¹ was estimated on the basis of the above discussion related with procedural blanks.

3.4. Real samples analysis

The optimized methodology was applied to determine the levels of target species in grab samples corresponding to sewage and river water. The obtained concentrations are summarized in **Table 5**. Codes 1-3 correspond to pairs of samples simultaneously collected in the inlet and outlet of the same sewage plant, equipped with primary and activated sludge treatments, which receives the discharges from a 100,000-inhabitants city. The highest concentrations found in these samples corresponded to TBEP, followed by TCPP, with levels ranging from 300 up to more than 3000 ng L⁻¹. On the other hand, TPP and TPrP stayed below the detection limit of the method. As regards diesters, their concentrations in wastewater samples varied between 50 and 200 ng L⁻¹. The presence of significant concentrations of several tri- and di-substituted OPs in the effluent of the plant confirms the contribution of urban wastewater to their introduction in the aquatic environment. Sample code 4 was collected in the river which receives the effluent of the above referred sewage plant, ca. 5 km downstream. As for the wastewater samples, TBEP and TCPP were the species showing the highest concentrations in this matrix, which indicates a certain mobility of both OPs in the aquatic environment. Chromatographic signals of compounds detected in this sample are shown in **Fig. 6**. Accordingly to the pioneer work of Quintana et al. [25], the peak showing the same transitions as DBP and a shorter retention time may correspond to the diester of TiBP; however, this assumption could not be confirmed due to the lack of commercially available standards.

Samples 5-7 correspond to three different rivers without known discharges of urban wastewater; however, the first two ones flow through highly industrialized areas. In these samples the concentrations of OPs were significantly lower than in the rest of specimens, being TCPP the only compound which surpassed the 50 ng L⁻¹ level. Globally, data on **Table 5** confirm the ubiquity of TCPP, TBEP, TBP, TiBP and TDCP in river and wastewater samples.

Fig. 6. Quantification (dotted line) and confirmation (solid line) MRM traces for species detected in a non-spiked river water sample (code 4, **Table 5**).



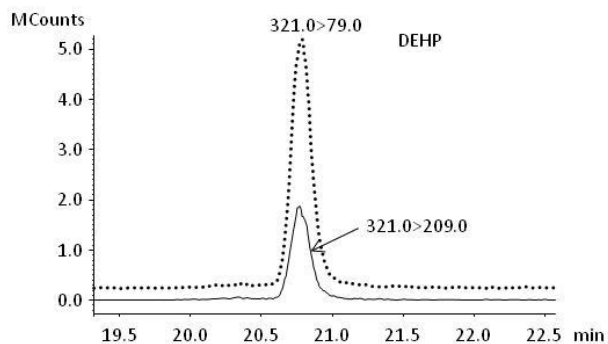
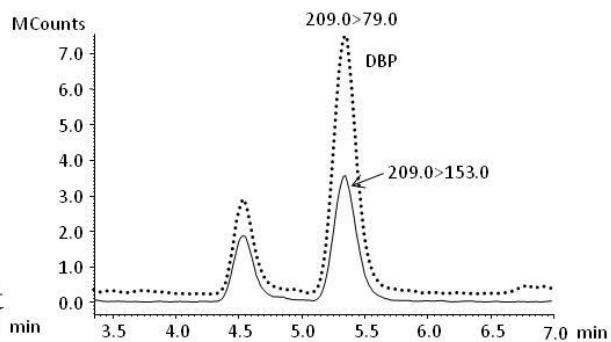
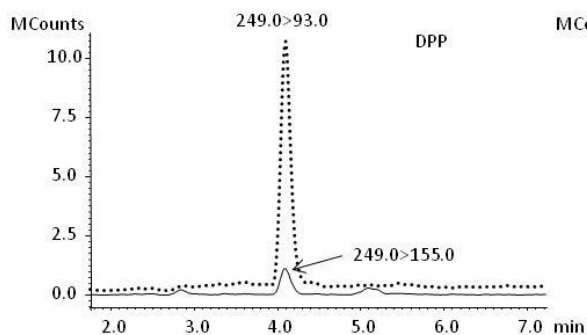
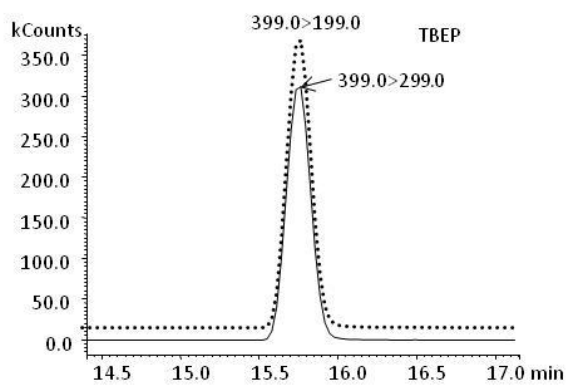
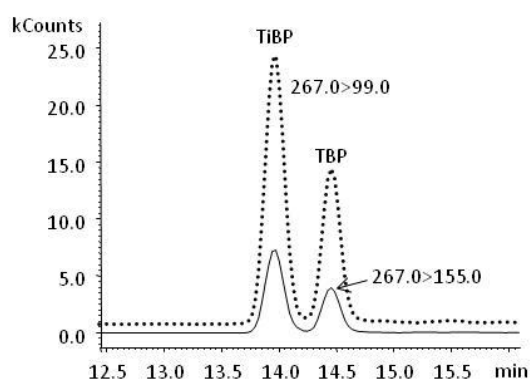
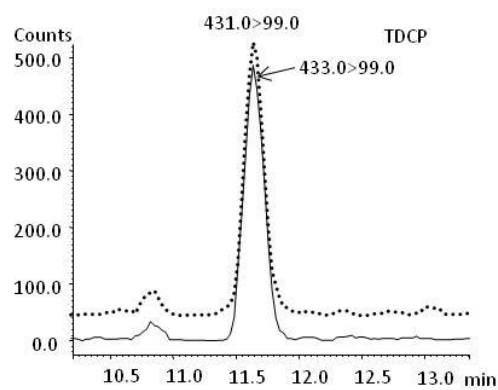
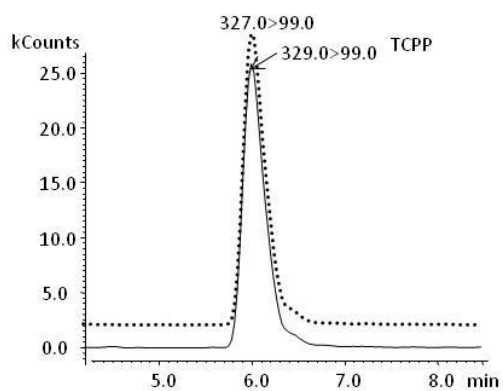


Table 5. Concentrations of OPs (ng L^{-1} , with their standard deviations) in environmental water samples, $n = 3$ replicates.

| Code | Sampling date | Type | TCEP | TPPO | TPRP | TCPP | TPP | TDCP | TIBP | TBP | TBEP | DPP | DBP | DEHP |
|------|---------------|-----------------------|--------|----------|------|--------|------|--------|--------|--------|----------|---------|---------|----------|
| 1 | 09/7/2009 | Raw Wastewater | nd | 17.2±0.8 | nd | 290±10 | nd | 68±2 | 188±14 | 82±4 | 2200±200 | 73±2 | 133±6 | 200±10 |
| 1 | 09/7/2009 | Treated Wastewater | 61±3 | 22.1±0.4 | nd | 510±30 | nd | 103±4 | 206±9 | 82±3 | 1300±80 | 137±2 | 137±8 | 47±8 |
| 2 | 27/7/2009 | Raw Wastewater | nd | 5.6±0.2 | nd | 401±15 | nd | nd | 39±3 | nq | 680±50 | 77±4 | 74±7 | 120±10 |
| 2 | 27/7/2009 | Treated Wastewater | nd | 6.1±0.5 | nd | 303±24 | nd | nd | 60±5 | 16±1 | 1050±20 | 97±4 | 84±7 | 150±20 |
| 3 | 11/09/2009 | Raw Wastewater | 70±10 | 13±2 | nd | 540±30 | nd | 100±10 | 115±5 | 47±2 | 3100±100 | 50±20 | 102±7 | 201±30 |
| 3 | 11/09/2009 | Treated Wastewater | 210±30 | 21±2 | nd | 680±50 | nd | 140±10 | 960±60 | 230±20 | 3400±200 | 105±5 | 82±2 | 140±20 |
| 4 | 11/09/2009 | River | 85±5 | 13±1 | nd | 430±30 | nd | 70±10 | 89±6 | 50±3 | 2700±400 | 70±2 | 65±6 | 92±9 |
| 5 | 27/7/2009 | River | nd | 2.0±0.2 | nd | 24±3 | 35±4 | nd | 10±1 | nq | nq | 7±1 | 8.0±0.6 | nq |
| 6 | 27/7/2009 | River | nd | 2.8±0.3 | nd | 64±8 | 18±3 | nd | 11±1 | nq | 10±1 | nd | 30±1 | 14±1 |
| 7 | 27/7/2009 | River | nd | nd | nd | 28±3 | nd | nd | 12±2 | nq | 34±2 | 9.6±0.2 | 8.6±0.4 | 16.9±0.4 |

nd, not detected; nq, under limit of quantification.

4. Conclusions

Mixed-mode Oasis MAX cartridges are an interesting alternative to reversed-phase sorbents for the simultaneous extraction of nine tri-substituted OPs and three diesters from environmental water samples. Analytes can be recovered in an extension higher than 70%, without the need of adjusting the pH of the samples and without addition of ion-pairing agents. Tri- and di-substituted compounds are fractionated during the elution step and further determined by LC-MS/MS in two consecutive injections, operating the ESI source in the positive and negative modes, respectively. In comparison with previous works, devoted to the determination of either triesters or diesters, less signal attenuation was observed during ESI ionization, which points to a higher selectivity in sample preparation process. In fact, the three diesters involved in this study can be quantified using external calibration even in raw wastewater samples. To the best of our knowledge, this is the first time that tri- and di-substituted OPs have been determined by LC-MS/MS, using the same chromatographic column as well as the same additive in the mobile phase.

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2.6. Comentarios adicionales

Las tres metodologías desarrolladas y recogidas en este capítulo son apropiadas para el análisis de los organofosforados en muestras acuosas. Los protocolos de preparación de muestra propuestos son sencillos y se pueden implementar con facilidad en cualquier laboratorio analítico por no requerir instrumentación específica. Siguiendo las tendencias actuales en preparación de muestra, se desarrollaron dos métodos basados en técnicas de microextracción (DLLME y HF-MMLLE), que consituyen una alternativa atractiva a las habituales LLE y SPE y, por otro lado, se optimizó el primer método para el fraccionamiento de diésteres y triésteres en una única etapa, utilizando SPE.

A efectos prácticos, cuando tenemos que enfrentarnos al análisis de triésteres en muestras de agua disponemos de varios métodos, ¿Cuál debemos escoger? A la hora de seleccionar una técnica debemos ser conscientes de sus ventajas y limitaciones para poder decantarnos por la más adecuada con criterio, en función de las necesidades. Las ventajas de las técnicas de microextracción frente a LLE y SPE y los inconvenientes de estas últimas ya se han comentado en la sección II.3, por lo que la preferencia por utilizar una técnica de microextracción es obvia. Ahora bien, para el análisis de triésteres en aguas, además de DLLME y MMLLE, presentadas en esta Tesis Doctoral, se han desarrollado métodos basados en SPME [1] y MASE [2], cuyas condiciones óptimas, además de algunas de sus características analíticas, se recogen en la **Tabla II.15**, en el apartado II.4.2. En la **Tabla III.2** se comparan estas cuatro técnicas considerando aspectos prácticos relevantes tales como la duración de la extracción o el consumo de disolvente y muestra, el grado de dificultad, el coste, etc.

Tabla III.2. Comparativa de técnicas de microextracción disponibles para el análisis de triésteres en aguas.

| Técnica | SPME [1] | DLLME [4] | MASE [2] | HF-MMLLE [5] |
|--------------------------|----------|----------------------------|----------------------------------|-------------------------------|
| Tiempo extracción | 40 min | Instantánea | 3 h | 12 h |
| Vol. disolvente | - | 1 mL acetona+ 20 µL TCE | 1 mL ciclohexano+ 250 µL MeOH | 7 µL octanol+ 200 µL AcOEt |
| Vol. muestra | 20 mL | 10 mL | 100 mL | 115 mL |
| Dificultad | baja | media | media | baja |
| Coste | moderado | muy bajo | bajo | muy bajo |
| Purificación | no | no | no | no |
| Automatización | posible | difícil | posible | difícil |

Vol., volumen; TCE, tricloroetano; MeOH, metanol; AcOEt, acetato de etilo

En lo que respecta al tiempo de extracción, la DLLME es la más rápida de todas ya que el equilibrio se alcanza instantáneamente por ser enorme la superficie de contacto entre muestra y extractante. En el otro extremo se encuentra MMLLE, que presenta una cinética bastante lenta por lo que requiere tiempos de extracción largos para alcanzar límites de cuantificación aceptables. Esta es una de sus principales limitaciones, aunque puede ser superada llevando a

cabo extracciones simultáneas en un agitador multiposición. De forma análoga, se puede proceder con las extracciones MASE. Por otro lado, el tiempo de muestreo de la SPME es sólo ligeramente más largo que la duración del programa cromatográfico, por lo que no compromete demasiado la capacidad de procesar muestras; sin embargo, si no se dispone de un dispositivo automatizado para llevar a cabo la SPME limita el número de muestras procesables, ya que requiere la presencia del operador. El consumo de disolvente es bajo para todas las técnicas, nulo en el caso de la SPME. La cantidad de muestra necesaria por extracción es baja, del orden de los mililitros, siendo MMLLE y MASE las que requieren volúmenes mayores. De todas formas, esto no suele ser un problema porque normalmente, en el caso de matrices acuosas, se dispone de gran cantidad de muestra. En términos de simplicidad todas son técnicas sencillas, aunque DLLME requiere un poco de destreza para el manejo de la gota y su recuperación. Los problemas de efecto memoria están descartados en DLLME, por razones obvias, y en MMLLE ya que el bajo coste de la membrana permite su descarte tras cada extracción. En el caso de SPME y MASE se reutilizan la fibra y la membrana, respectivamente.

En lo que concierne a los efectos de matriz, excluyendo el TEHP, la eficacia de la extracción en SPME y MASE no se ve afectada por la complejidad de la matriz, siendo posible la cuantificación de muestras acuosas de diversa complejidad (agua de grifo, de río o residual) mediante calibración externa, utilizando [2] o no [1] patrón interno. Sin embargo, en el caso de DLLME y HF-MMLLE la cuantificación mediante adición estándar puede evitarse en el caso de matrices acuosas de complejidad baja a moderada, pero es necesaria para el análisis de influente, ya que la eficacia de extracción de algunos compuestos (TiBP, TBEP, TPP) se ve comprometida. Además, en DLLME, la complejidad de las muestras de influente puede hacer necesaria la utilización de volúmenes de extractante mayores que el optimizado en el trabajo, ya que puede ser posible la formación de una interfase que dificulte la recuperación de gotas con un volumen tan reducido. En la **Tabla III.3** se recogen las respuestas normalizadas de muestras de influente con adición, expresadas como porcentaje, respecto a agua ultrapura. Las muestras se extrajeron con cuatro técnicas de microextracción diferentes bajo condiciones óptimas y el nivel de adición estaba comprendido entre 0.3 y 5 ng mL⁻¹.

Tabla III.3. Comparación de efectos de matriz para varias técnicas de microextracción aplicadas al análisis de influente.

| Compuesto | SPME [1] | DLLME [4] | MASE [2] | HF-MMLLE [5] |
|-----------|----------|-----------|----------|--------------|
| TiBP | 102 | 66 | 89 | 106 |
| TBP | 101 | 94 | 106 | 93 |
| TCEP | 118 | 107 | 108 | * |
| TCPP | 99 | 89 | 83 | 83 |
| TDCP | 98 | 96 | 95 | 105 |
| TBEP | 98 | 71 | 88 | 95 |
| TPP | 85 | 85 | 99 | 62 |
| TEHP | 19 | 40 | - | 14 |
| TPPO | 102 | 101 | 100 | 110 |

-, no incluido; *, evaluación imposible por coelución con una interferencia. Leer en el texto para más detalles.

Los factores de enriquecimiento proporcionados por HF-MMLLE son mayores que los obtenidos con MASE, para un volumen de muestra análogo, y similares, en general, a los correspondientes a la DLLME para muestras de 10 mL [4]. Esta última proporciona EFs claramente superiores a los de HF-MMLLE para TCEP y TEHP y también mejores para el TPP. En comparación con la SPME, la DLLME y la HF-MMLLE proporcionan, en general, respuestas superiores, con la excepción del TCEP en el caso de la HF-MMLLE [4,5].

En lo tocante al trabajo desarrollado para la determinación de diésteres y triésteres, lo más destacado es la posibilidad de fraccionar ambos grupos de compuestos y su separación cromatográfica con la misma columna y fase móvil, sin necesidad de agente formador de pares iónicos ni control de pH. Además, el método de SPE con cartuchos de modo mixto permitió reducir los efectos de matriz y cuantificar los tres diésteres mediante calibración externa, incluso en muestras tan complejas como influentes de depuradoras. Teniendo en cuenta su capacidad para la separación de tri- y diésteres del ácido fosfórico, sus excelentes LOQs y su selectividad, LC-MS/MS es, con seguridad, la técnica más potente para la determinación de OPs en muestras complejas. Su compatibilidad con el uso de surrogados marcados isotópicamente, cuya disponibilidad actual es aún muy limitada, le confiere ventajas adicionales sobre GC-EI-MS y frente a GC-ICP-MS para todos los compuestos considerados. Por otro lado, laboratorios que poseen un número elevado de muestras seguramente se decanten por el uso de SPE como técnica de concentración debido a sus posibilidades de automatización, total o parcial, y su amplia aceptación en los laboratorios de control ambiental.

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IV. CONCLUSIONES

Las metodologías desarrolladas en la presente Tesis Doctoral son adecuadas para la determinación de los organofosforados seleccionados en muestras medioambientales de complejidad variable y constituyen, además, una alternativa atractiva a los métodos ya disponibles para el análisis de esas matrices. A parte de su utilidad práctica, cabe destacar también la novedad de algunos de los procedimientos optimizados ya que técnicas como la MSPD, DLLME o HF-MMLE no se habían aplicado con anterioridad a estos analitos.

Los métodos propuestos son, en general, sencillos y con un bajo consumo de disolventes. Sus características analíticas tales como recuperaciones, precisión o LOQs mejoran las de otros métodos disponibles o están al mismo nivel.

La aplicación de los métodos optimizados al análisis de muestras reales evidencia su validez y ha confirmado la ubicuidad de los compuestos organofosforados analizados, sobre todo en agua y polvo de atmósferas interiores.

A continuación, se destacan las conclusiones más relevantes que se desprenden de los resultados obtenidos en cada uno de los trabajos.

Microwave-assisted extraction of organophosphate flame retardants and plasticizers from indoor dust samples.

Las recuperaciones obtenidas con MAE son análogas a la proporcionadas por Soxhlet con las ventajas adicionales de un consumo de disolvente mucho menor, un tiempo de extracción notablemente reducido y la posibilidad de llevar a cabo extracciones simultáneas, hasta 12 muestras con el extractor utilizado en este trabajo. Dado que las recuperaciones obtenidas son cuantitativas, se puede cuantificar las muestras mediante calibración externa.

A pesar de la selectividad del NPD, la elevada complejidad de los extractos de MAE hace necesaria la inclusión de una etapa de limpieza exhaustiva para prolongar la vida útil de la columna cromatográfica. Precisamente, esta etapa es la limitante de la velocidad de los análisis y, por ello, se centraron esfuerzos en desarrollar un método alternativo para disminuir el tiempo total de preparación de muestra. MSPD fue la solución a esta limitación.

Optimisation of a matrix solid-phase dispersion method for the determination of organophosphate compounds in dust samples.

El método propuesto es la primera aplicación descrita de MSPD a la determinación de estos organofosforados. Las principales ventajas frente a otras técnicas disponibles para el análisis de polvo es el hecho de que permite llevar a cabo la extracción y la purificación en una única etapa, conlleva un consumo menor de disolventes y reduce el tiempo dedicado a la etapa de preparación de muestra de forma notable. A pesar de que las recuperaciones para tres de los organofosforados seleccionados (TCEP, TCPP y TPP) son ligeramente inferiores a las proporcionadas por técnicas de extracción más exhaustivas como MAE o Soxhlet, no se requiere la utilización de la adición estándar con fines cuantitativos, lo cual favorece la obtención de resultados rápidos. Otra ventaja inherente a la MSPD es el bajo coste de las extracciones. La

precisión de la técnica es buena y los LOQs análogos a los proporcionados por la combinación MAE-GC-NPD.

Determination of organophosphate flame retardants and plasticizers in sediment samples using microwave-assisted extraction and gas chromatography with inductively coupled plasma mass spectrometry.

Este trabajo constituye la primera aplicación de GC-ICP-MS para la determinación de cuatro de los organofosforados seleccionados (TiBP, TCPP, TDCP y TPPO). Además, es también pionero en la determinación de los organofosforados incluidos en el estudio mediante inyección directa de un disolvente orgánico en el sistema.

El método desarrollado es una alternativa valiosa a los métodos existentes para el análisis de sedimentos, conlleva un consumo bajo de disolventes, requiere un proceso de limpieza posterior simple, proporciona LOQs equiparables a los de otras técnicas como GC-NPD o LC-MS/MS y cromatogramas menos complejos. Esto convierte a GC-ICP-MS en una técnica tremendamente útil para el análisis de matrices más complejas como el polvo o los lodos. Sin embargo, las determinaciones son costosas por el elevado consumo de argón, varios litros por minuto, el diseño de la interfase GC-ICP es crítico y la imposibilidad de utilizar patrones marcados isotópicamente para estos compuestos, en caso de disponibilidad, es una limitación a tener en cuenta.

Pressurized liquid extraction of organophosphate triesters from sediment samples using aqueous solutions.

La metodología propuesta requiere un consumo bajo de disolventes y es adecuada para la extracción de los organofosforados de muestras de sedimento de complejidad variable. En comparación con aplicaciones previas de PLE a la extracción de OPs, proporciona una mayor selectividad por la utilización como extractante de una mezcla hidroorgánica con un contenido acuoso elevado (un 75% de la mezcla). Las recuperaciones del método son superiores al 77%, la precisión buena (RSD < 10%) y los LOQs equiparables a los de otros métodos para el análisis de sedimentos utilizando GC-NPD o LC-MS/MS.

Evaluation of liquid-liquid microextraction using polypropylene microporous membranes for the determination of organophosphorus flame retardants and plasticizers in water samples.

La metodología desarrollada es simple, requiere una manipulación mínima de la muestra, conlleva un consumo reducido de disolvente y su coste es muy asequible. Aunque los tiempos de muestreo son largos, esta limitación puede compensarse llevando a cabo extracciones simultáneas y desatendidas en un agitador multiposición. La naturaleza desechable de las membranas evita los problemas de efecto memoria asociados a SPME. La técnica proporciona una precisión y LOQs buenos para la mayoría de los OPs estudiados (excepto para TCEP y TEHP) y factores de enriquecimiento, en general, superiores a los de DLLME y MASE. La eficacia de la extracción es análoga para agua ultrapura, de grifo, de río y efluente pero menor para muestras de influente. La calibración externa utilizando patrones que han sido sometidos al

mismo proceso de extracción que las muestras es un método de cuantificación válido para matrices acuosas de complejidad baja a moderada y la cuantificación mediante adición estándar es inevitable para muestras de influente.

Development of a dispersive liquid-liquid microextraction method for organophosphorus flame retardants and plasticizers determination in water samples.

El método desarrollado es una alternativa tremendamente valiosa a los métodos existentes para la determinación de estos compuestos en agua ya que reduce drásticamente el consumo de disolventes, la manipulación de muestra y el volumen necesario para el análisis, proporcionando un extracto directamente analizable en una única etapa en la que se consigue un elevado grado de concentración de los analitos. Otra de sus características diferenciadoras es la rápida cinética de extracción. Este trabajo constituye la primera aplicación de DLLME a la determinación de los organofosforados seleccionados y de la utilización del 1,1,1-tricloroetano como extractante, cuya toxicidad es inferior a la de otros disolventes empleados.

La precisión del método (RSD < 10%) es ligeramente mejor o análoga a la proporcionada por SPME o MASE. La comparación directa de señales obtenidas bajo condiciones optimizadas de SPME y DLLME revela una sensibilidad análoga o superior de la última respecto a la primera. Excepto en el caso del TEHP, la calibración externa utilizando patrones que han sido sometidos al mismo proceso de extracción que las muestras es un método de cuantificación válido para matrices acuosas de complejidad baja a moderada. De nuevo, la cuantificación mediante adición estándar es inevitable para muestras de influente.

Mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry for the determination of tri- and di-substituted organophosphorus species in water samples.

El procedimiento desarrollado permite, utilizando los cartuchos de modo mixto Oasis MAX, la concentración simultánea y elución secuencial (empleando metanol puro primero y una disolución metanólica 20 mM de TBAHS después) de los organofosforados trisustituídos y los tres diésteres del ácido fosfórico seleccionados (los únicos disponibles comercialmente). Los Oasis MAX, en comparación con adsorbentes de fase reversa previamente utilizados, no requieren el ajuste del pH de la muestra ni la adición a la misma de agentes formadores de pares iónicos. Además, proporcionan una mayor selectividad en la etapa de preparación de muestra, que se traduce en una reducción de los efectos de matriz durante la ionización en ESI.

El método propuesto es el primero que permite la determinación de los organofosforados trisustituídos y los diésteres utilizando la misma columna cromatográfica e idéntica fase móvil. La cuantificación de los primeros ha de hacerse mediante adición estándar en el caso de muestras de agua residual pero puede utilizarse la calibración externa para muestras menos complejas. En el caso de los organofosforados disustituídos puede utilizarse la calibración externa para todas las muestras.

Por último, resaltar que LC-MS/MS es, de las técnicas empleadas, la más potente para la determinación de estos OPs en muestras complejas por su sensibilidad, selectividad y capacidad para la separación/determinación de triésteres y diésteres sin necesidad de derivatización. La posibilidad de utilizar patrones marcados isotópicamente es una ventaja adicional de la técnica frente a GC-NPD o GC-ICP-MS.

CONCLUSIONS

The methodologies developed in this PhD dissertation are suitable for the determination of the selected organophosphorus compounds in environmental samples of variable complexity. Furthermore, they constitute an attractive alternative to the already available methods for these matrices. In addition to their usefulness, it is worth mentioning the novelty of some of the optimized procedures, since techniques such as MSPD, DLLME or HF-MMLE had not been previously applied to this group of analytes.

The proposed methods are, in general, simple and with low solvent consumption. Their analytical performance in terms of recoveries, precision and LOQs is better or at the same level than that from other available methods.

The application of the optimized methods to the analysis of real samples evidences their validity and confirms the ubiquity of the analyzed organophosphate compounds, mainly in water and indoor dust.

Next, some of the more relevant conclusions, that come out from the obtained results in each work, are outlined.

Microwave-assisted extraction of organophosphate flame retardants and plasticizers from indoor dust samples.

Recoveries obtained with MAE are similar to those achieved with Soxhlet, with the advantage of a lower solvent consumption, reduced extraction time and the possibility of carry out simultaneous extractions, up to 12 samples with the instrument employed in this work. Since target compounds are quantitatively extracted, samples can be quantified by external calibration.

Despite the NPD selectivity, the high complexity of MAE extracts requires the use of an exhaustive clean-up step to prolong the chromatographic column lifetime. In fact, this step is the longest, thus limiting the sample throughput. Therefore, further research was focused on shortening this stage. MSPD was a solution to overcome this limitation.

Optimisation of a matrix solid-phase dispersion method for the determination of organophosphate compounds in dust samples.

The proposed method is the first application of MSPD to the determination of the selected OPs. Compared with other available techniques for the analysis of dust samples, MSPD allows extraction and clean-up to be performed in a single step, thus entailing lower solvent consumption and greatly speeding-up sample throughput.

In spite of the fact that recoveries for three of the selected OPs (TCEP, TCPP and TPP) are slightly lower than those achieved with more exhaustive techniques such as MAE or Soxhlet, the use of the standard addition method is not required for quantitative purposes, thus obtaining quick results. Another inherent advantage of MSPD is the low cost per extraction. Achieved precision is good and LOQs are equal to those provided by the combination MAE-GC-NPD.

Determination of organophosphate flame retardants and plasticizers in sediment samples using microwave-assisted extraction and gas chromatography with inductively coupled plasma mass spectrometry.

This work constitutes the first application of GC-ICP-MS to the determination of four of the selected organophosphorus compounds (TiBP, TCPP, TDCP and TPPO). Furthermore, this is the first approach using direct solvent injection for OPs determination by means of GC-ICP-MS.

The developed method is a valuable alternative for the analysis of sediments. It presents low solvent consumption, requires a simple additional clean-up, provides LOQs comparable to those reported for other techniques such as GC-NPD or LC-MS/MS and much less complex chromatograms. These features point out to GC-ICP-MS as a suitable technique for the reliable determination of OPs in more complex matrices such as dust or sludge. Notwithstanding, analysis are expensive due to the high argon consumption, several liters per minute, the GC-ICP interface design is critical and the impossibility of using isotopically labeled standards for these compounds, in case of availability, is a limitation to take into account.

Pressurized liquid extraction of organophosphate triesters from sediment samples using aqueous solutions.

The proposed methodology requires low solvent consumption and is suitable for OPs extraction from sediment samples of variable complexity. Compared to previous PLE applications to OPs extraction, it provides a higher selectivity by the use of an aqueous-organic mixture with a high water content (75%). Recoveries of the method are above 77%, precision is good (RSD < 10%) and LOQs comparable to those achieved by other available methods for sediments analysis using GC-NPD or LC-MS/MS.

Evaluation of liquid-liquid microextraction using polypropylene microporous membranes for the determination of organophosphorus flame retardants and plasticizers in water samples.

The developed methodology is simple, requires minimal sample handling, entails reduced solvent consumption and its cost is affordable. Its main limitation is the slowness of the extraction, that can be attenuated by simultaneous unattended extraction of several samples in a multi-position magnetic stirrer. The disposable nature of the membranes avoids carryover problems associated to SPME. The technique provides good precision and LOQs for most of the studied OPs (except for TCEP and TEHP) and, in general, higher enrichment factors than DLLME and MASE. The extraction yield remained basically the same for ultrapure water, tap water, river water and effluent samples but underwent a reduction for raw wastewater. External calibration using standards subjected to the same extraction procedure as the sample is a suitable quantification approach for aqueous matrices of low to moderate complexity. The standard addition method is unavoidable for influent samples quantification.

Development of a dispersive liquid-liquid microextraction method for organophosphorus flame retardants and plasticizers determination in water samples.

The developed method is a valuable alternative to those existing methods for the determination of these compounds in water since it dramatically reduces solvent consumption, sample handling and sample intake. It provides a directly analyzable extract in a single step in which high enrichment factors are achieved. Another characteristic feature is its fast extraction kinetics. This work describes the first application of DLLME to the selected OPs determination and the use of 1,1,1-trichloroethane as extractant, which toxicity is lower than that of other employed solvents.

The precision of the method (RSD < 10%) is slightly better or similar to that of SPME or MASE. Direct comparison of obtained signals under optimized conditions for SPME and DLLME reveals that DLLME provides similar or higher responses for all species than SPME. Except for TEHP, external calibration using standards subjected to the same extraction procedure as the samples is a valid quantification method for aqueous matrices of low to moderate complexity. Again, the standard addition method for quantitative purposes is unavoidable in case of raw wastewater

Mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry for the determination of tri- and di-substituted organophosphorus species in water samples.

The developed procedure allows, using the mixed-mode Oasis MAX cartridges, the simultaneous concentration and sequential elution (using pure methanol followed by a 20 mM TBAS methanolic solution) of selected tri- and disubstituted organophosphorus compounds. The considered diesters are the only commercially available.

In comparison to other reversed-phase sorbents, the Oasis MAX polymer does not require nor sample pH adjustment neither the addition of ion pairing agents. Furthermore, it provides a higher selectivity in the sample preparation step, thus reducing matrix effects during ESI ionization.

The proposed method is the first that allows the determination of di- and trisubstituted OPs using the same chromatographic column and mobile phase. The quantification of the trisubstituted OPs should be done by the standard addition method in case of wastewater samples and external calibration can be used for less complex samples. In case of the diesters, external calibration can be used with all matrices.

Finally, it is worth outlining that LC-MS/MS is, among the employed techniques, the more powerful for these OPs determination in complex samples due to its sensitivity, selectivity and capability of separate/determine tri- and diesters with no need for derivatization. The possibility of using isotopically labeled standards is an additional advantage versus GC-NPD or GC-ICP-MS.

SIGLAS

| | | |
|---------|--|---|
| AAS | Atomic absorption spectrometry | Espectrometría de absorción atómica |
| AES | Atomic emission spectrometry | Espectrometría de emisión atómica |
| APCI | Atmospheric pressure chemical ionization | Ionización química a presión atmosférica |
| CID | Collision induced dissociation | Disociación inducida por colisión |
| DAD | Diode array detector | Detector de diodos en serie |
| DLLME | Dispersive liquid-liquid microextraction | Microextracción líquido-líquido dispersiva |
| EDAR | Sewage treatment plant | Estación depuradora de aguas residuales |
| EF | Enrichment factor | Factor de enriquecimiento |
| EI | Electron impact | Impacto electrónico |
| ESI | Electrospray ionization | Ionización por electronebulización |
| FID | Flame ionization detector | Detector de ionización en llama |
| FPD | Flame photometric detector | Detector fotométrico de llama |
| GC | Gas Chromatography | Cromatografía de gases |
| HF-MMLE | Hollow fiber-microporous membrane liquid-liquid extraction | Extracción con membranas microporosas huecas |
| HS | Headspace | Espacio de cabeza |
| HPLC | High performance liquid chromatography | Cromatografía líquida de alta resolución |
| ICP | Inductively coupled plasma | Plasma de acoplamiento inducido |
| IL | Ionic liquids | Líquidos iónicos |
| IS | Internal standard | Estándar interno |
| LC | Liquid chromatography | Cromatografía de líquidos |
| LLE | Liquid-liquid extraction | Extracción líquido-líquido |
| LOQs | Limits of quantification | Límites de cuantificación |
| LPME | Liquid-phase microextraction | Microextracción en fase líquida |
| MAE | Microwave-assisted extraction | Extracción asistida por microondas |
| MASE | Membrane-assisted solvent extraction | Extracción con disolventes asistida por membranas |
| MIPs | Molecularly imprinted polymers | Polímeros de huella molecular |
| MRM | Multiple reaction monitoring | Monitorización de reacciones múltiples |
| MS | Mass spectrometry | Espectrometría de masas |
| MSPD | Matrix solid-phase dispersion | Dispersión de matriz en fase sólida |
| NPD | Nitrogen-phosphorus detector | Detector de nitrógeno-fósforo |
| OPs | Organophosphorus compounds | Compuestos organofosforados |

| | | |
|-----------------|---|--|
| PAHs | Polycyclic aromatic hydrocarbons | Hidrocarburos aromáticos policíclicos |
| PBDEs | Polybrominated diphenyl ethers | Difenil éteres polibromados |
| PCBs | Polychlorinated biphenyls | Bifenilos policlorados |
| PCI | Positive chemical ionization | Ionización química positiva |
| PLE | Pressurized liquid extraction | Extracción con líquidos presurizados |
| PHWE | Pressurized hot water extraction | Extracción presurizada con agua caliente |
| PTV | Programmed temperature vaporizer | Vaporizador con temperatura programada |
| K _{ow} | Partition Constant octanol-water | Constante de partición octanol-agua |
| R | Recovery | Recuperación |
| RSD | Relative standard deviation | Desviación estándar relativa |
| SBSE | Stir bar sorptive extraction | Extracción con barras agitadoras |
| SD | Standard deviation | Desviación estándar |
| SDME | Single drop microextraction | Microextracción con gota suspendida |
| SFE | Supercritical fluid extraction | Extracción con fluidos supercríticos |
| SIM | Single ion monitoring | Monitorización de un solo ión |
| SPE | Solid-phase extraction | Extracción en fase sólida |
| SPME | Solid-phase microextraction | Microextracción en fase sólida |
| SWE | Subcritical water extraction | Extracción con agua subcrítica |
| TBAHS | Tetrabutylammonium hydrogen sulphate | Hidrógenosulfato de tetrabutilamonio |
| USAEME | Ultrasound-assisted emulsification-extraction | Emulsificación-microextracción asistida por ultrasonidos |
| USALLE | Ultrasound-assisted liquid-liquid extraction | Extracción liquid-líquido asistida por ultrasonidos |

ANEXOS: OTRAS PUBLICACIONES

Trends and recent applications of matrix solid-phase dispersion

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Abstract Matrix solid-phase dispersion (MSPD) is a sample-preparation technique with increasing acceptance in trace analysis of organic compounds using chromatographic and electro-driven separation techniques. It has been applied to the extraction and fractionation of a large number of substances from solid, semi-solid, and liquid matrices. Low sample and solvents consumption, straightforward application, and reduced cost, and its ability to simultaneously perform extraction and clean-up in a single step, are some of its major advantages. This review attempts to provide an updated, concise and critical overview on the latest trends and applications of MSPD, placing emphasis on comparison of its performance with that of other techniques, besides focusing on practical features to take into account depending on the nature of the sample and the properties of the analytes. Achievements, advantages, and limitations are discussed. The paper also highlights future challenges to be faced.

Keywords Review · Sample treatment · Matrix solid-phase dispersion · Accuracy evaluation · Environmental analysis

Introduction

Sample preparation, meaning extraction and purification of analytes, remains the bottleneck and key step determining the sample throughput and performance of most analytical

procedures, particularly when applied to the analysis of trace compounds. The success of a given approach to sample preparation for analysis of organic compounds in complex samples depends on a suitable balance between the yield and selectivity of the extraction process. Soxhlet and microwave-assisted extraction methods rely on the use of hard conditions to ensure quantitative recoveries at the expense of poor selectivity. Supercritical and pressurized fluids also apply rather drastic conditions; however, the complexity of the final extract can be reduced by use of an on-line clean-up step. On the other hand, matrix solid-phase dispersion (MSPD) has unique features as a sample-preparation technique. The use of mild extraction conditions (room temperature and atmospheric pressure) with a suitable combination of dispersant sorbent and elution solvent normally provides acceptable recoveries and medium selectivity. Additional advantages of MSPD are:

1. low cost per extraction,
2. no need for expensive instrumentation, and
3. moderate consumption of organic solvents.

The operating principles of MSPD, introduced about 20 years ago by Barker, are extremely simple [1–3]. In a first step, samples are ground with a solid sorbent in a mortar using a pestle in order to:

1. disrupt the structure of the raw material, and
2. achieve its homogeneous distribution around the sorbent particles.

The blend is then transferred to a column or cartridge, normally made of polypropylene, and analytes eluted with an appropriate organic solvent. Partition and/or adsorption equilibria, similar to those occurring in a chromatographic column, are responsible for analyte distribution between the dispersed sample and the elution solvent. The homoge-

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neous and thin layer of sample around the dispersant particles leads to highly efficient mass transference processes, which explains the high recoveries normally attained with a low volume of solvent.

Nowadays, although MSPD is not still a mainstream sample-preparation technique, around 40 new applications are developed every year. Most of these accord with the original idea developed by Barker and summarized in the previous paragraph; however, some relevant changes have been noticed in relation to its field of application, types of dispersant, elution solvents, and operating conditions. Moreover, increasing attention is being paid to the development of finely tuned on-line purification strategies, with the aim of obtaining a direct analysable extract from complex samples in a single step. All these trends are presented and discussed in the body of this review.

Extraction strategies

Operational steps in MSPD, and efficiency and selectivity of the extraction process, are conditioned by a number of factors, for example the physical state and the origin of the sample, the relative concentrations and properties (e.g. polarity and chemical stability) of analytes and interferences, and the suitable combination of dispersant, co-sorbent, and elution solvent.

The ease of achieving homogeneous blending with the dispersant basically depends on the physical state of the matrix considered. For low viscosity, liquid samples, e.g. juices, wine, melted fat, milk, etc., stirring normally suffices to achieve a thin layer of sample around sorbent particles. Sometimes liquid samples are merely deposited on top of a cartridge containing a small-amount of a solid sorbent [4–6]. Although these procedures have been referred to as MSPD applications, in our opinion they are more related to column purification or solid-phase extraction processes. In fact, most authors still recommend thorough blending of the biological liquid (or semisolid) samples and the dispersant sorbent in a mortar with a pestle to disrupt the cell membranes present in biological matrices [7, 8]. However, it is also true that, as far as we know, detailed studies evaluating the effect of grinding on the performance of MSPD extraction from liquid samples have not been published.

In addition to operational aspects, the most significant differences between application of MSPD to liquid and solid samples have to do with:

1. the stronger analyte–matrix interactions expected for the latter, and
2. the usually higher level of potential interferences in solid matrices.

Consequently, solid samples require a more careful selection of dispersant sorbents and elution solvents to enhance the yield of the extraction while maintaining a reasonable level of co-extracted interferences.

Sorbent and solvent selection

Classic applications of the MSPD technique employ reversed-phase sorbents as dispersants. Octadecyl-silica (C_{18}) is by far the most often used [2, 3, 8]. In addition, C_8 [8–10] and C_{30} materials [11] have also been considered. Theoretically, silica particles disrupt the gross architecture of biological samples whereas the bonded alkyl chains contribute to dissolving their components, providing relatively clean extracts from fatty matrices, such as muscle tissue [12–14], liver [15, 16], kidney [17] and fatty fish [18–20] when polar solvents (e.g. acetonitrile, methanol and combinations of these) are used as extractants. In general, organic species of medium polarity are efficiently extracted under these conditions. Recently, it has been proved that replacement of C_{18} by amino-propyl silica or primary and secondary amine (PSA) sorbents leads to cleaner extracts from complex fatty samples, e.g. olives [21–23]. It seems that the weak anion-exchange character of amino materials is responsible for this better selectivity, due to more effective retention of the fatty acids present in biological samples.

Normal-phase, non-bonded sorbents (Florisil, alumina, and silica) have also been proposed as dispersants in many MSPD applications. They interact with sample components solely by adsorption and, obviously, are not able to dissolve the sample matrix. The adsorption properties of these sorbents can be adjusted depending on their water content and acid or basic character. As an example, basic pesticides have been isolated from cereal samples using acidic silica as dispersant. Hydrogen bonds between amino groups in the structure of the analytes and silanol moieties in the acidified sorbent contributed to retention of target species in the MSPD cartridge. In a further step, a methanol–dichloromethane mixture was used to break these interactions, providing quantitative recoveries [24–26]. Some recent applications of MSPD using normal-phase materials as dispersants deal with environmental matrices. Some examples are the extraction of PAHs from freeze-dried sludge [27] and soils [28], halogenated phenols and bisphenols from sediments and sludge [29], and organophosphorus flame-retardants from indoor dust [30]. A summary of extraction conditions considered in the application of MSPD to environmental solid samples is shown in Table 1.

Selection of extraction solvents to be used in combination with normal-phase dispersants is function of analyte polarity. Non-polar substances can be recovered using

Table 1 Summary of experimental conditions in the application of MSPD to environmental solid samples

| Sample | Analytes | Dispersant | Co-sorbent | Rinsing solvent | Elution solvent | Detection technique | LOD (ng g ⁻¹ or ng mL ⁻¹) | Recovery (%) | Ref. |
|------------------------------|---|--------------------------|------------------|-------------------------|-------------------------------------|---------------------|--|--------------|------|
| Indoor dust (0.5 g) | Phenolic bactericides | C ₁₈ (1.25 g) | Florisil (2 g) | DCM (10 mL) | Acetonitrile (10 mL) | GC–MS–MS | 0.2–0.8 | 80–114 | [47] |
| | Organophosphate esters | Florisil (0.5 g) | Alumina (0.5 g) | Hexane (2 mL) | Acetone (2.5 mL) | GC–NPD | 12–15 | 80–116 | [30] |
| Soil (0.5 g) | Polycyclic aromatic hydrocarbons (PAHs) | Florisil (1 g) | | Methanolic KOH (0.5 mL) | Hexane–acetone (1:1), (6 mL) | LC–Fluorescence | 0.003–0.18 | 94–103 | [28] |
| Soil (10 g) | Pesticide residues | Florisil (10 g) | | | DCM (50 mL) | GC–NPD | 0.1–0.6 | 72–105 | [66] |
| Soil (4 g) | Phenthoate | Florisil (6 g) | | | Hexane–ethyl acetate (7:1), (30 mL) | LC–UV | | 79–94 | [88] |
| Sludge and sediments (0.2 g) | Halogenated phenols and bisphenols | Florisil (2 g) | Florisil (1–2 g) | | Acetonitrile (2.5 mL) | CE–UV | 91–336 | 81–105 | [29] |
| Sludge (2 g) | PAHs | Alumina (3 g) | | | DCM (12 mL) | GC–MS | 0.03–0.45 | 85–108 | [27] |

DCM, dichloromethane

apolar solvents, for example hexane [31–33], dichloromethane [27], or mixtures of both [34, 35]. When target compounds are of medium or high polarity, acetonitrile [29, 36], acetone [30], ethyl acetate [37] or mixtures of water with ethanol [38, 39] or methanol [17, 40–44] are the preferred choices.

Another trend reflected in the literature points to use of inert materials in MSPD processes. Replacement of reversed or normal-phase dispersants by sand, diatomaceous earth or Celite leads to cost-effective methods at the expense of limited selectivity, which is just controlled by the solubility of the different sample components in the elution solvent. However, in terms of the precision and recovery attained, several authors have proven that similar values can be obtained by using C₁₈ or sand as dispersants in the extraction of phenolic species and anthocyanins from vegetable and environmental samples [45–47].

Some MSPD applications involving the use of sand or diatomaceous earth as dispersants deal with vegetable samples, for example the determination of different types of pesticide in fruit juices [48, 49]. In addition, successful methods have also been proposed for other matrices, for example fatty foods [50] and tissues [51, 52]. Whatever the type of matrix, for polar analytes the combination of sand as dispersant with water as elution solvent has been reported as advantageous in order to obtain:

1. nearly quantitative recoveries, and
2. direct analysable extracts using LC-based techniques even from complex biological samples (Table 2).

On-line purification

Even after careful choice of dispersant sorbent and the elution solvent, MSPD often does not produce a ready-to-analyse extract. In these situations an on-line clean-up step can be integrated into the sample-preparation process. Purification of the raw extract can be achieved by placing a layer of co-sorbent at the bottom of the MSPD cartridge. Obviously, dispersant and co-sorbent should have different sorption behaviour. Normal-phase materials are usually employed as co-sorbents to retain polar interferences eluted from samples dispersed on C₁₈ or other reversed-phase functionalised silica sorbents. For example, silica has been used as co-sorbent in the extraction of fungicides, pesticides, and insecticides from fruit and vegetable samples [53–55]. Carbon is also useful for removing interfering pigments co-extracted from chilli powder and vegetables [36]. Other authors have combined the use of C₁₈ as dispersant with Florisil as co-sorbent [19, 47].

For analytes with high chemical stability, e.g. PCBs, PBDEs, and PBBs, a layer of acidified silica (in most cases impregnated with 44% sulfuric acid) is often placed at the bottom of the MSPD cartridge [56]. This alternative leads to lipid-free extracts, avoiding a further time and solvent consuming off-line clean-up step, normally using gel permeation chromatography. On the other hand, the major shortcomings of acidified silica are:

1. the risk of analyte retention in the layer of carbon produced by oxidation of the sample [19], and

Table 2 MSPD applications to complex biological samples with the combination of sand as inert dispersant and water as elution solvent, without additional clean-up

| Sample | Analytes | Elution solvent | Detection technique | LOD (ng g ⁻¹ or ng mL ⁻¹) | Recovery (%) (RSD) | Ref. |
|-----------------------------------|----------------------------|--|---------------------|--|--------------------|------|
| Vegetables and fruit | Carbamate insecticides | Water | LC-ESI-MS | 2–5 | 88–110 (<9) | [89] |
| Tomato | Bioactive amines | HClO ₄ (0.6 mol L ⁻¹) | LC-UV | 400–1200 | 95–102 (<3) | [90] |
| Muscle tissue | Sulfonamide antibacterials | Heated water | LC-MS | 1–5 | 75–98 (<8) | [51] |
| Kidney and Liver | Sulfonamide antibacterials | Heated water | LC-MS | 0.3–2.4 | 72–96 (<11) | [41] |
| Bovine muscle, kidney and liver | Antibiotics | Heated water | LC-MS | 0.1–2 | 74–95 (<9) | [91] |
| Fish | Microcystins | Heated acidified water | LC-MS | 0.5–1.2 | 61–92 (<10) | [67] |
| Bovine tissue | Quinolones | Heated water | LC-MS | 2–400 | 85–102 (<5) | [92] |
| Bovine, swine and poultry tissues | Tetracycline antibiotics | Heated water | LC-MS | 1–6 | 88–109 (<11) | [61] |
| Cheese | Sulfonamide antibacterials | Heated water | LC-MS | 0.1–0.3 | 75–105 (<11) | [93] |
| Cheese | Tetracycline antibiotics | Heated water | LC-MS | 0.3–0.6 | 90–107 (<12) | [94] |
| Milk | Aminoglycoside antibiotics | Heated water | LC-MS | 0.5–3.9 | 80–110 (<20) | [95] |
| Milk | Carbamate insecticides | Heated water | LC-MS | 1–4 | 85–105 (<9) | [96] |
| Milk | β-Lactam antibiotics | Heated water | LC-MS | 0.2–0.8 | 104–109 (<10) | [91] |
| Milk and yoghurt | Macrolide antibiotics | Modified heated water | LC-MS | 1 | 86–117 (<13) | [97] |
| Milk and eggs | Sulfonamide antibacterials | Heated water | LC-MS | 0.5–3 | 76–91 (<11) | [98] |
| Tea leaves and coffee | Caffeine | Heated water | LC-UV | – | – | [42] |

2. limitations regarding the type of elution solvents, because they should not react with sulfuric acid. In practice, this constrains the choice to low and medium-polarity solvents, e.g. alkanes, dichloromethane and chloroform.

A second possible way of increasing the selectivity of MSPD extractions involves rinsing the sample before analyte extraction. As an example, water is often used to remove sugars and polar compounds from fruit and vegetables dispersed on C₁₈. Valenzuela et al. [57, 58] applied this approach to the extraction of pesticides from citrus samples. Polar interferences were first removed with water then the analytes were extracted using dichloromethane. Tolls et al. [20, 59] analysed surfactants in fish samples dispersed on C₁₈ using the opposite combination of rinsing and elution solvents. Apolar interferences, such as lipids and fatty acids, were removed with hexane followed by ethyl acetate, before elution of target analytes with more polar solvent mixtures, e.g. ethyl acetate–methanol (1:1). Obviously, when rinsing and elution are performed with non-miscible solvents, the MSPD cartridge must be dried with a stream of nitrogen between these steps.

Other authors have considered both clean-up options to increase selectivity in the extraction of medium-polar analytes from complex environmental samples with organic carbon contents up to 30% [30, 47]. In this work the best balance between extraction yield and selectivity was achieved by using a low-polarity solvent to remove lipophilic interferences followed by a more polar solvent for analyte extraction (Fig. 1). Polar interferences released

by the latter were retained on the particles of the normal-phase co-sorbent. The closer the polarities of target species, the higher the effectiveness of this clean-up strategy.

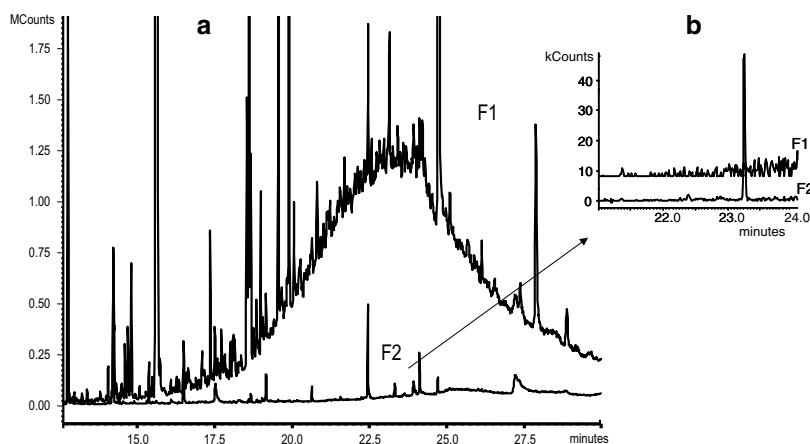
MSPD as an alternative extraction technique

Evaluation of the accuracy of MSPD methods

When mild conditions are used during sample preparation fewer interferences are co-extracted with the analytes [17, 18, 30, 47, 51, 60–62]; on the other hand, however, it could be expected that those soft conditions might impair the extraction efficiency. The best way of testing the accuracy, and thus the recoveries, of a given sample-preparation approach is based on the use of certified reference materials (CRMs). Because of their limited availability, it is understandable that only a few MSPD applications have been validated in this way. Most of these deal with analysis of PCBs, PBDEs, and PAHs in biological matrices (Table 3).

When CRMs are not available, many authors decide to evaluate the suitability of their optimized MSPD processes by comparison with an alternative, well-established protocol, using real-life polluted samples. Table 4 gathers some of these papers, detailing analytically interesting aspects such as recoveries (R%), precision (RSD%), limits of quantification (LOQ) and a brief description of sample-preparation conditions, which will be useful in next section in order to draw conclusions regarding the amounts of sample and solvent required, and length, degree of sample

Fig. 1 Overlay of GC–MS chromatograms obtained from determination of triclosan in an unspiked sample of indoor dust. *F1* represents the cleaning fraction with dichloromethane and *F2* the analysable extract in acetonitrile. **a**, Total ionic current chromatogram. **b**, Trace at 345+347 *m/z* units. For MSPD conditions see Ref. [47]



handling, and difficulty of this step. In view of Table 4, MSPD generally provides extraction yields and analytical figures of merit comparable to those achieved with widespread extraction techniques such as liquid–liquid extraction (LLE), Soxhlet extraction, microwave assisted extraction (MAE), or pressurized liquid extraction (PLE).

Nevertheless, very often MSPD accuracy is evaluated with spiked samples only. Two procedures can be considered in order to fortify a sample. The first consists in adding a few microlitres of a standard solution to a fraction of the sample (spot-spiking mode), which is normally processed after a few minutes or hours [13, 18, 20, 63–65]; the second involves preparing a slurry of the sample with a standard solution of the analytes in a volatile solvent. In this case, the mixture is thoroughly homogenized, the solvent allowed to evaporate, and the spiked sample stored for some days or weeks before being submitted to the analytical procedure [30, 34, 47]. In general, it is accepted that the latter approach constitutes a better simulation of analyte–matrix interactions occurring in real polluted solid samples. Shen and coworkers [66] evaluated the accuracy

and precision of a method based on the combination of MSPD and continuous liquid–solid extraction (LSE) for determination of organophosphorus pesticides in two spiked soils with different physicochemical properties, aged for 16 h or 15 days. For the sample with the higher organic matter content, slightly different results were obtained depending on the storage time after addition of the compounds, which highlights the non-negligible importance of the spiking procedure used. Notwithstanding, the main body of the MSPD literature refers to the use of spot-spiked samples for both optimization of extraction conditions and assessment of accuracy. In future works a more strictly accurate evaluation is recommended, restricting the use of spot-spiked samples to liquid, homogeneous matrices, e.g. wine and fruit juice.

Advantages and limitations of MSPD

Some attractive features of MSPD are clear from Table 4. MSPD is a simple process which markedly reduces solvent consumption [54, 60, 63, 67, 68], sample intake and

Table 3 Summary of MSPD procedures validated using CRMs

| Sample | Analytes | CRM | R (%) | RSD (%) | Ref. |
|-----------------------------|-------------------|-----------------------|---------|---------|------|
| Freeze-dried porcine tissue | Chloramphenicol | BCR-445 | 108 | <3 | [12] |
| Pork fat | PCBs | IRMM 446 | 90–100 | <7 | [34] |
| Freeze-dried fish | PCBs, PBDEs, PBBs | IAEA 406 ^a | 0–266 | <12 | [56] |
| Freeze-dried fish | PBDEs | WELL-WMF-01 | 81–106 | <23 | [32] |
| Pork fat | PCBs and BDE 47 | IRMM 446 | 82–96 | <13 | [32] |
| Freeze-dried mussel tissue | PAHs | NIST SRM 2977 | 79–102 | <18 | [19] |
| Sewage sludge | PAHs | – ^b | 85–108 | <13 | [27] |
| Soil | PAHs | BCR-524 | 100–111 | <5 | [28] |

R (%) denotes analyte contents expressed as a percentage of the certified values; PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; PBBs, polychlorinated biphenyls

^a Reference material, not certified

^b Commercial source and PAH levels in the reference material provided, but not for the CRM reference

Table 4 Comparison of MSPD performance with other techniques

| Ref. | Analyte | Matrix | Technique | Sample preparation | R (%) (RSD%) | LOQ | Detection technique |
|------|------------------------|--|---------------|---|------------------------------|--|---------------------|
| [67] | MCs and nodularin | Fish muscle | MSPD LLE | 1 g sample + 5 g sand. Extraction with 4 mL H ₂ O (80 °C, pH=2) pH adjustment and filtration LLE 2× MeOH, then washing 3× with hexane and finally to dryness and reconstitution with H ₂ O. Clean-up with C ₁₈ , washing with 30 mL MeOH 20%, elution with 50 mL MeOH. Extract to dryness and reconstitution with MeOH-H ₂ O (1:1) | 61–82 (<10) 51–59 (<8) | 1.6–4 ng g ⁻¹ – | LC-MS |
| [60] | PCBs | Biota (eggs of spoonbill, oysters, clams, fish, mussels) | MSPD NSPLE | 2 g sample + 4 g Florisil; 6 g Florisil as co-sorbent. Washing with 20 mL DCM-pentane (15:85). Extraction with 16 mL pentane-acetone (1:1). Extract to dryness and reconstitution with hexane 2 g sample + 1 g Celatom, 2×10 min-static extraction with pentane, 100 °C, 1600 psi. Flush volume 150%. Extract concentration, SPE clean-up with Florisil. Elution with 50 mL 3% DCM in pentane, dryness and reconstitution with hexane | RR: 93–115 (<12) | – | GC-ECD, GC-MS |
| | | | PLE | 2 g sample + 1 g Florisil, 6 g Florisil and dead volume with Celatom. 2×10 min-static extractions with DCM-pentane (15:85), 40 °C, 2000 psi. Flush volume 150%. Extract to dryness and reconstitution with hexane. | RR: 89–114 (<12) | – | |
| | | | Soxhlet | 2 g sample, 150 mL pentane-acetone (2:1, v/v), 13 h; extract concentration, SPE clean-up with Florisil. Elution with 50 mL 3% DCM in pentane, dryness and reconstitution with hexane | RR: 100 (<12) | – | |
| [12] | Chloramphenicol | Muscle tissue | MSPD | 2 g sample + 3 g C ₁₈ ; 0.5 g C ₁₈ co-column. Washing with 10 mL hexane and 12 mL ACN-H ₂ O (5:95). Elution with 10 mL ACN-H ₂ O (1:1). LLE clean-up with 2×5 mL H ₂ O saturated with EtOAc | 93–100 (<13) | 4 ng g ⁻¹ | GC-ECD |
| | | | LLE | 3 g tissue + 8 mL ACN-4% NaCl (1:1). LLE with 2×5 mL hexane. LLE with 2×5 mL H ₂ O saturated with EtOAc. SPE clean-up with C ₁₈ , washing with 5 mL ACN-H ₂ O (5:95); elution with 3 mL ACN-H ₂ O (1:1). LLE with 2×5 mL H ₂ O saturated with EtOAc | 99–105 (<4) | 2 ng g ⁻¹ | |
| [30] | Organophosphate esters | Dust | MSPD MAE | 0.5 g dust + 0.5 g Na ₂ SO ₄ anh. + 0.5 g Florisil; 0.5 g Al ₂ O ₃ co-sorbent. Washing with 2 mL hexane; elution with 3 mL acetone, addition 1 mL EtOAc and concentration. Solution filtration 0.5 g dust + 10 mL acetone, MAE 30 min at 130 °C. Extract Oasis HLB-SPE clean-up after dilution with 500 mL H ₂ O. Elution with 2 mL EtOAc; SiO ₂ -SPE, elution with 5 mL EtOAc and concentration | 80–116 (<17) 85–104 (<11) | 40–50 ng g ⁻¹ 40–50 ng g ⁻¹ | GC-NPD |

| | | | | | | | |
|-------|---|---|---------|---|-----------------|-------------------------------|--------------|
| [45] | Phenolic compounds | Leaves | MSPD | 0.5 g plant + 2 g C ₁₈ . Elution with 20 mL MeOH-H ₂ O (7:3, v/v). Dryness and reconstitution with 5 mL MeOH-H ₂ O (7:3, v/v) | RR: 48–92 (<9) | – | LC-UV |
| | | | SSDM | 0.5 g plant + 2 g sea sand. Elution with 20 mL MeOH-H ₂ O (7:3, v/v). Dryness and reconstitution with 5 mL MeOH-H ₂ O (7:3, v/v) | RR: 100 (<8) | 31–52 µg g ⁻¹ | |
| [69] | Isoflavonoids | Medicinal herb | SLE | 0.5 g plant + 20 mL MeOH-H ₂ O (7:3, v/v), agitation 24 h. Dryness and reconstitution with 5 mL MeOH-H ₂ O (7:3, v/v) | RR: 0–111 (<22) | – | |
| | | | MSPD | 0.5 g sample + 0.5 mL H ₂ O + 1 g C ₁₈ ; 1 g C ₁₈ as co-sorbent. Washing 10 mL H ₂ O, elution with MeOH-H ₂ O (90:10, v/v). Dryness and reconstitution with 1 mL MeOH | RR: 4–141 (–) | – | LC-UV, LC-MS |
| | | | US | 1 g sample + 10 mL MeOH-H ₂ O (90:10, v/v), US at 50 °C, 60 min. Centrifugation 3 min, volume adjustment and filtration | RR: 11–35 (–) | – | |
| [63] | Fungicides | Fruits and vegetables | Soxhlet | 5 g sample + 100 mL MeOH-H ₂ O (80:20, v/v), 8 h. Extract concentration and dilution. Solution filtration | RR: 100 (–) | – | LC-MS |
| | | | MSPD | 0.5 g sample + 0.5 g C ₁₈ . Elution with 10 mL EtOAc. Extract to dryness and reconstitution | 71–102 (<13) | 4–100 ng g ⁻¹ | |
| | | | SLE | 50 g sample + 50 mL FEP + 50 mL ACN + 6 g MgSO ₄ + 1.5 g NaCl and centrifugation. 1 mL of extract shaken with 50 mg PSA + 150 mg MgSO ₄ and centrifugation | 72–107 (<14) | | |
| [99] | Herbicides, insecticides and fungicides | Fruit, vegetables and cereal | MSPD | 5 g sample + 10 g silica gel. Elution with hexane-diethyl ether or MeOH-DCM. Extract to dryness and reconstitution | 81–96 (<4) | 0.02–0.25 µg mL ⁻¹ | LC-UV |
| | | | SLE-LLE | 10 g sample + 100 mL acetone, MeOH-HCl or acetone-HCl, shaking, filtration and evaporation. LLE with DCM or DCM-hexane. Extract to dryness and reconstitution | 72–75 (<6) | | |
| | | | SLE-SPE | 10 g sample homogenized with acetone or acetone-DCM-hexane (1:1:1), LLE with DCM and evaporation to dryness. Reconstitution and SPE clean-up. Elution with MeOH-DCM, hexane-diethyl ether or MeOH-DCM. Extract to dryness and redissolution | 79–82 (<5) | | |
| [54] | Fungicides | Fruit and vegetables | MSPD | 0.5 g sample + 0.5 g C ₁₈ ; 1 g silica as co-sorbent. Elution with 10 mL EtOAc and extract concentration | 62–102 (<15) | 0.05–0.1 µg g ⁻¹ | GC-MS |
| | | | SLE | 50 g sample + 50 g Na ₂ SO ₄ + 2 × 100 mL EtOAc. Solvent concentration | 45–11.3 (<12) | 0.002–0.03 µg g ⁻¹ | |
| [36] | Aflatoxins | Chilli powder, green bean, black sesame | MSPD | 1 g sample + 2 g Al ₂ O ₃ ; 70 mg graphitic carbon black as co-sorbent. Elution with 5 mL ACN and concentration | 88–95 (<6) | 0.1–0.25 ng g ⁻¹ | LC-FLD |
| | | | LLE-IAC | 5 g sample + 20 mL 80% MeOH, filtration and dilution with 20 mL H ₂ O. IAC purification. Washing with 10 mL H ₂ O, elution with 2 mL MeOH and concentration | 86–93 (<4) | 0.1–0.2 ng g ⁻¹ | |
| [100] | Pesticides | Fruit | MSPD | 0.5 g sample + 0.5 g C ₁₈ . Elution with 100 mL DCM-MeOH (1:1) and concentration | 52–108 (<35) | 0.05–2 µg g ⁻¹ | LC-MS |
| | | | SLE | 50 g sample + 50 g Na ₂ SO ₄ + 2 × 100 mL EtOAc. Extract evaporation | 14–101 (<25) | | |

Table 4 (continued)

| Ref. | Analyte | Matrix | Technique | Sample preparation | R (%) (RSD%) | LOQ | Detection technique |
|------------|--------------------------|-----------------|-----------|---|-----------------|-----------------------------|---------------------|
| [101, 102] | Benzoic acid derivatives | Medicinal plant | MSPD | 1 g sample + 2 g C ₁₈ + 1 mL hexane. Washing with 10 mL hexane and 10 mL DCM. Extraction with 10 mL MeOH-HCOOH (8:2) | 45–102 (<8) | – | LC-UV |
| | | | Soxhlet | 0.5 g sample + MeOH-H ₂ O, extraction 1 h. Dilution and clean-up. Washing with H ₂ O and MeOH; Elution with 5 mL MeOH-HAc (9:1). Extract to dryness and reconstitution | 17–82 (<6) | 0.010–0.040 µg/mL | |
| [83] | Parabens and triclosan | Dust | MSPD | 0.5 g dust + 0.5 g Na ₂ SO ₄ anh. + 1.25 g C ₁₈ ; 2 g Florisil co-sorbent. Washing with 10 mL DCM; elution with 10 mL acetonitrile. Concentration to 1 mL. Solution filtration | 80–114 (<10) | 0.6–2.6 ng g ⁻¹ | GC-MS |
| | | | PLE | 0.5 g dust + 1 g Na ₂ SO ₄ anh. + 3 g Florisil; 1 g Florisil co-sorbent and dead volume with Na ₂ SO ₄ anh. Washing with hexane 1 × 4 min-static 500 psi, with 50% flush volume. 3 × 1 min-static extractions with EtOAc, 103 °C, 2000 psi. Flush volume 100%. Concentration to 2 mL. | 76–98 (<11) | 1–4 ng g ⁻¹ | |
| [68] | PCBs | Foodstuffs | MSPD | 100 mg sample + 100 mg Na ₂ SO ₄ -SiO ₂ (1:1). Elution with 3 mL acetone-hexane (1:1). Concentration and purification with multilayer cartridge with 600 mg SiO ₂ and 750 mg SiO ₂ -H ₂ SO ₄ (44%). Elution with 3.5 mL hexane | 84–133 (<17) | 0.03–0.1 ng g ⁻¹ | GC-micro-ECD |
| | | | PLE | 100 mg sample + 100 mg Na ₂ SO ₄ + 100 mg SiO ₂ -H ₂ SO ₄ (44%); 900 mg SiO ₂ -H ₂ SO ₄ + 60 mg SiO ₂ on top of the dispersed sample. Extraction with 3.5 mL hexane, 2 static cycles of 7 min, 40 °C, 12 MPa | 83–127 (<23) | | |
| | | | Soxhlet | 300 mg sample + 35 mL hexane, extraction 8 h. Extract concentration and purification with multilayer cartridge with 600 mg SiO ₂ and 750 mg SiO ₂ -H ₂ SO ₄ (44%). Elution with 3.5 mL hexane | 83–127 (<26) | | |
| [28, 103] | PAHs | Soils | MSPD | 0.5 g lyophilized soil + 500 µL saturated KOH in MeOH + 1 g Florisil; 0.5 g SiO ₂ + 0.5 g Florisil as co-sorbents. Elution with 6 mL hexane-acetone (1:1), concentration, solvent exchange, and filtration | 94–104 (<19) | 0.01–0.6 ng g ⁻¹ | LC-FLD |
| | | | MAE | 0.5 g lyophilized soil + 0.8 mL H ₂ O + 4 mL saturated KOH in MeOH + 10 mL hexane, MW (129 °C, 17 min). Centrifugation, concentration, and SPE purification with SiO ₂ and Florisil. Elution with 8 mL hexane-DCM (1:1), concentration, solvent exchange, and filtration | RR: 68–119 (<9) | – | |

RR denotes the relative recovery to one of the techniques; – denotes not provided; MCs, microcystins; SSDM, sea sand disruption method; DCM, dichloromethane; NSPLE, non-selective pressurized liquid extraction; ACN, acetonitrile; EtOAc, ethyl acetate; SLE, solid-liquid extraction; MeOH, methanol; FEP, fluorinated ethyl propylene; PSA, primary secondary amine; IAC, immunoaffinity column; FLD, fluorescence detection

manipulation [26, 54, 63, 69], and total analysis time. Also remarkable is its ability to, sometimes, integrate extraction and clean-up in the same process [17, 28, 30, 38, 62].

Some proposed MSPD approaches are still rather complex, although they constitute a noticeable improvement on previously available methods. For instance, Kubala-Drinic et al. [12] developed a MSPD application for extraction of chloramphenicol from muscle tissue requiring a rather elaborated purification step. Nevertheless, the method they previously employed was tremendously time-consuming, involving six LLE and a SPE clean-up.

On the other hand, some articles have also been published where MSPD is not an appealing option as a result of either low recovery [70] or not being advantageous compared with the alternatives available. An example of the latter is the extraction of caffeine from tea leaves [42], for which infusion is frequently applied and recommended because of its efficiency and simplicity. For extracting caffeine from coffee, however, recoveries achieved are better when using MSPD. Bogusz et al. [71] compared the performance of MSPD with an SPE method to for determination of benzo(a)pyrene in olive oil samples, concluding that the latter methodology was simpler, faster, and more accurate and precise. Nevertheless, that MSPD protocol can certainly be used for solid or semi-solid fatty samples and is much simpler than standard methods requiring saponification, extraction, and further clean-up steps.

New trends

Alternative sorbents

Sorbent selection is of utmost importance since it is one of the variables controlling the selectivity of MSPD processes. Apart from the three main groups of dispersants discussed in a previous section, some recent works deal with unconventional MSPD sorbents, for instance, carbon or polymeric materials. Furusawa [72] extracted DDT from animal fats using Toyobo-KF, an activated carbon fibre, achieving recoveries higher than 81%. Torres and coworkers [73] assayed C₁₈ and carbon black as dispersants in the determination of fungicide and insecticide residues in fruit and vegetables. Although both sorbents were able to isolate target pesticides, C₁₈ provided better extraction yields. The non-ionic cross-linked aliphatic acrylic polymer XAD-7 HP has been successfully used for extraction of avoparcin and atrazine from kidney samples [74, 75]. Bajer et al. [70] compared various extraction techniques, including MSPD using Oasis HLB, a macroporous poly(divinylbenzene-co-*N*-vinylpyrrolidone) polymer, for determination of iso-flavonoids in plants; in general, they achieved better

recoveries with supercritical-fluid extraction or Soxhlet than with MSPD.

Molecularly imprinted polymers (MIPs) are affinity sorbents with a promising future due to their high selectivity and favourable characteristics compared with immunoaffinity materials. Crescenzi and coworkers [15] reported the on-line coupling of MSPD with a molecularly imprinted solid-phase-extraction (MISPE) sorbent to determine clenbuterol in bovine liver. This double-cartridge tandem system enabled isolation, in a single extraction/clean-up step, a clear extract with recoveries above 90%. Yan et al. [76] have published, very recently, the first application of MIPs as MSPD dispersant. They determined five fluoroquinolones in chicken eggs and swine tissues achieving better recoveries, from 86 to 105%, than with conventional sorbents such as C₁₈, silica, Florisil, or sand, with the further advantage of remarkably enhanced selectivity.

Water as elution solvent

As has been already noted, MSPD is advantageous in terms of solvent consumption, involving the use of moderate volumes of organic solvents. Key factors when selecting the elution solvent are its capability to selectively and quantitatively recover target analytes and its compatibility with the subsequent determination technique; harmlessness, low cost and environment-friendliness are also desirable attributes. Water fulfils some of these requirements and is compatible with reversed-phase liquid chromatography, which is one of the preferred techniques used in combination with MSPD.

Hot water has been successfully applied to the extraction of polar to moderate polar contaminants from several solid matrices (mainly foodstuffs), taking advantage of the drop of its polarity with increasing the temperature [77, 78]. High-temperature water extraction can be performed at either atmospheric or elevated pressures. The latter option is mandatory when temperatures over 100 °C are considered. Home made extractors, consisting of a LC pump propelling water through stainless steel tubing to the extraction cell (usually an empty LC column placed inside a GC oven), and commercially available pressurized liquid or supercritical-fluid extractors can be used to carry out MSPD extractions with water under subcritical conditions. In addition to those applications depicted in Table 2, where aqueous extracts are directly collected and injected in a LC system, more elaborated alternatives have been proposed.

Gentili and coworkers [79] extracted sulfonamides from meat and infant food, previously dispersed with C₁₈, using water at 160 °C and 100 atm. Achieved recoveries ranged from 70 to 101%. Curren and King [74, 75] also employed subcritical water as extractant and XAD-7 HP as dispersing material for analysis of kidney samples. They developed a

method which quantitatively extracted atrazine using 30% ethanol-modified subcritical water at 100 °C and 50 atm, in combination with SPME as further concentration technique and GC–MS analysis [74]. In other work [75], they carried out the subcritical water extraction of avoparcin at 75 °C and 50 atm, using ethanol (30%), also, as modifier. Aqueous extracts were then concentrated by SPE to achieve an overall recovery of 108%. Modifying hot water with organic co-solvents improved target compound recoveries but also increased the extraction of undesirable matrix components, and so further purification was needed. Addition of reagents to water in order to increase its extraction capability is not restricted to organic modifiers—use of surfactants, buffers, and derivatization reagents has also been reported [78], although not yet in combination with MSPD.

Temperature and pressure application

Some research groups have made profitable use of their PLE equipment to reach elevated temperatures and pressures in order to achieve faster, automatic, and more exhaustive MSPD extractions. In fact, it is usual in PLE applications to disperse the sample with sand or diatomaceous earth [80] and, lately, some applications with normal and reversed-phase materials have also been published; so it is clear that both techniques share some principles of operation. We will focus on some of those MSPD methods wherein the matrix is blended with non-inert sorbents.

Ramos and coworkers optimized a PLE method to extract PCBs from foodstuffs [68]—a small amount of sample (100 mg) was dispersed with Na₂SO₄ and acidic silica, with the latter efficiently contributing to fat removal. Achieved extraction efficiency was similar to that of two procedures, conventional MSPD and Soxhlet, used as reference methods. Gómez-Ariza et al. [60] extracted PCBs from biota tissues, blended with Florisil, at 40 °C and 2000 psi. The proposed procedure was successfully validated with a CRM and also tested versus other extraction methodologies applied to PCBs determination. As an example, in comparison with a PLE method, wherein Celaton was employed for sample dispersion, it avoided the need of further clean-up; compared with a conventional MSPD approach, it allowed automation of the sample-preparation process (Table 4). De la Cal et al. [81] ground sediment samples with alumina and copper and submitted them to PLE in order to determine PBDEs. The optimized method achieved an efficiency similar to that of conventional Soxhlet–SPE. Wang et al. [82] developed a PLE-based methodology for extraction of 16 PAHs from smoked food samples employing ODS (Supelclean LC-18) in conjunction with Na₂SO₄ to grind them. Extracts were then submitted to a sulfuric acid treatment and finally passed

through a Florisil-SPE cartridge to remove co-extracted lipid impurities. Obtained recoveries, above 72% except for 5 PAHs, were comparable with or better than those provided by Soxhlet, with significant reduction of extraction time and solvent consumption. Canosa [83] et al developed a PLE procedure for determination of parabens and triclosan in indoor dust in which samples ground with Florisil and sodium sulfate were extracted with ethyl acetate at 103 °C and 1500 psi, after a washing step with *n*-hexane. Precision and quantification limits achieved were similar to those of a previously published MSPD procedure; slightly greater amounts of methyl paraben (the most polar of the considered analytes) were extracted with the PLE approach, however. In addition, automation of the sample preparation step enabled quicker and unattended extractions to be performed.

Miniaturization and automation

Available amounts of sample for analysis are usually limited, particularly for biological samples, so mistakes in the sample-preparation process are inadmissible and protocols involving the use of small quantities of sample are really valuable. Some authors have optimized MSPD extractions for sample aliquots not higher than 50 mg [84–87]. In addition, in some of these applications the volume of eluting solvent needed was reduced to a few microlitres [85, 86]. Miniaturized MSPD approaches are expected to facilitate automation of the whole analytical procedure by on-line coupling with the subsequent separation–determination technique. For the moment, however, there are no published reports of this having been achieved.

Conclusions

MSPD is an attractive extraction technique which allows direct handling of solid, semisolid, and viscous samples, markedly reducing sample manipulation and, consequently, the likelihood of errors. Moreover, its wide acceptance is owed to its ease of implementation, reduced solvent consumption, low overall cost, and the possibility, in some cases, of integrating extraction and clean-up in a single step thus avoiding the need for additional manipulation of sample extracts. The usefulness of MSPD is clear in the light of the wide number of reported applications to extraction of an assortment of compounds in a broad variety of samples. The accuracy of this extraction technique has been successfully evaluated by means of certified reference materials, comparison of its performance with exhaustive well-established extraction methods and use of spiked samples. In relation to this last option, a reliable accuracy assessment, necessary to improve the

acceptance of the technique should be performed using homogenized and aged, instead of spot-spiked, samples.

MSPD shares some principles of operation with PLE, which sometimes makes it difficult to define a clear boundary between the techniques and, thus, to classify a given sample-preparation method as an MSPD or PLE application. Irrespective of this consideration, the combination of the principles of MSPD with PLE instrumentation enables automation of the process and precise control of temperature and pressure during extraction. The last possibility is of paramount importance when water is considered as elution solvent. The use of subcritical water as MSPD elution solvent is an outstanding advance which fulfils the requirement of more environmentally friendly sample-preparation procedures. In addition, the extracts obtained are usually directly analysable by liquid chromatography after filtration or pH adjustment only.

In general, MSPD applications show evidence of a steady broadening of employed extraction conditions, as regards the type of dispersant and elution solvent, and field of application, with some still scarce but promising results for soils, sediments, and sludge samples. Automation of the whole extraction process, miniaturization (with the consequent reduction of sample, sorbent, and solvent consumption), and the use of cost-effective and harmless solvents are also current trends, which will keep on going.

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Organophosphorus flame retardants and plasticizers in water and air

I. Occurrence and fate

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Organophosphate esters (OPEs), in particular triesters, are high-production-volume chemicals used as flame retardants and plasticizers to protect or to enhance the properties of plastics, textiles, furniture and many other materials. The widespread usage, which may even increase due to the ban of brominated diphenylethers as flame retardants, and the diffusion from host materials result in continuous release of OPEs and their distribution through water, especially wastewater, and air, particularly associated with airborne particulate matter. This work highlights the occurrence of OPEs in wastewater, surface water and groundwater as well as indoor and outdoor air and particulate material.

We discuss the major processes affecting the fate of OPEs in the environment, such as sorption, volatilization and biodegradation. Of the OPEs studied thus far, chlorinated tri(2-chloroethyl) phosphate (TCEP) and tri(chloropropyl) phosphate (TCPP) appear to be most recalcitrant and ubiquitous in water and air. We identified knowledge gaps concerning the fate of diesters and monoesters in the aqueous environment, the biodegradation of OPEs under less favorable conditions (sorbed to particles or under anoxic or anaerobic conditions) as well as the behavior of OPEs in the atmosphere and their potential for long-range transport.

A second part, addressing analytical methods will be published in the next issue [J.B. Quintana, R. Rodil, T. Reemtsma, M. García-López, I. Rodríguez, Trends Anal. Chem. (to be published in 27 (10) (2008))].

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Keywords: Air; Flame retardant; Indoor environment; Organophosphate ester; Organophosphorus pollutant; Plasticizer; Tri(2-chloroethyl) phosphate; Tri(chloropropyl) phosphate; Urban dust; Water

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1. Introduction

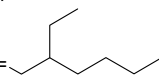
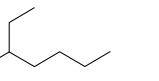
The so-called "emerging pollutants" have raised great concern in the past decade. In short, this term defines any chemical that was not previously included in national or international monitoring programs, but is continually being introduced into the environment due to anthropogenic activities. These chemicals do not necessarily need to be new, but their environmental fate and (eco)toxicological study have not, until recently, started to be evaluated. Obviously, the appropriate development of this recent research may lead to some of these emerging chemicals becoming included in monitoring programs or even restricted in the future.

The organophosphate-ester compounds (OPEs) that we consider in this review may be a good example. First reports date back to the late 1970s [2,3] and then, during

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Table 1. Structures and physico-chemical data of the most relevant OPEs covered in this review. Physico-chemical data obtained from [75] (experimental values), except for EHDPP (obtained from [76] (software-calculated values)) and MEHP (obtained from [17])

| Compound name | Substituents | CAS No. | Formulae | logK _{ow} | V _p (Torr) | |
|---------------|---|-------------|---|--------------------|-------------------------|--|
| TCEP | Tri(2-chloroethyl) phosphate $R_1=R_2=R_3=$ | 115-96-8 | C ₆ H ₁₂ Cl ₃ O ₄ P | 1.44 | 6.13 × 10 ⁻² | |
| TCPP | Tri(chloropropyl) phosphate $R_1=R_2=R_3=$ | 13674-84-5 | C ₉ H ₁₈ Cl ₃ O ₄ P | 2.59 | 2.02 × 10 ⁻⁵ | |
| TDCP | Tri(dichloropropyl) phosphate $R_1=R_2=R_3=$ | 13674-87-8 | C ₉ H ₁₅ Cl ₆ O ₄ P | 3.65 | 7.36 × 10 ⁻⁸ | |
| TMP | Trimethyl phosphate $R_1=R_2=R_3=$ -CH ₃ | 512-56-1 | C ₃ H ₉ O ₄ P | -0.65 | 8.50 × 10 ⁻¹ | |
| TEP | Triethyl phosphate $R_1=R_2=R_3=$ | 78-40-0 | C ₆ H ₁₅ O ₄ P | 0.80 | 3.93 × 10 ⁻¹ | |
| TPrP | Tripopyl phosphate $R_1=R_2=R_3=$ | 513-08-06 | C ₉ H ₂₁ O ₄ P | 1.87 | 4.33 × 10 ⁻³ | |
| TiBP | Tri-iso-butyl phosphate $R_1=R_2=R_3=$ | 126-71-6 | C ₁₂ H ₂₇ O ₄ P | 3.60 | 1.28 × 10 ⁻² | |
| TnBP | Tri-n-butyl phosphate $R_1=R_2=R_3=$ | 126-73-8 | C ₁₂ H ₂₇ O ₄ P | 4.00 | 1.13 × 10 ⁻³ | |
| TBEP | Tributoxyethyl phosphate $R_1=R_2=R_3=$ | 78-51-3 | C ₁₈ H ₃₉ O ₇ P | 3.75 | 2.50 × 10 ⁻⁸ | |
| TEHP | Tri(2-ethylhexyl) phosphate $R_1=R_2=R_3=$ | 78-42-2 | C ₂₄ H ₅₁ O ₄ P | 9.49 | 8.45 × 10 ⁻⁸ | |
| EHDPP | 2-Ethylhexyl diphenyl phosphate $R_1=$ $R_2=R_3=$ | 856800-52-7 | C ₂₀ H ₂₇ O ₄ P | 6.64 | 6.49 × 10 ⁻⁷ | |
| TPhP | Triphenyl phosphate $R_1=R_2=R_3=$ | 115-86-6 | C ₁₈ H ₁₅ O ₄ P | 4.59 | 6.28 × 10 ⁻⁶ | |
| TCrP | Tricresyl phosphate $R_1=R_2=R_3=$ | 1330-78-5 | C ₂₁ H ₂₁ O ₄ P | 5.11 | 6.00 × 10 ⁻⁷ | |
| DnBP | Di-n-butyl phosphate $R_1=$ -H $R_2=R_3=$ | 107-66-4 | C ₈ H ₁₉ O ₄ P | 2.29 | 4.26 × 10 ⁻⁹ | |

| Table 1 (continued) | | | | | | |
|---------------------|------------------------------|--|------------|--|--------------------|-------------------------|
| | Compound name | Substituents | CAS No. | Formulae | logK _{ow} | V _p (Torr) |
| DEHP | Di(2-ethylhexyl) phosphate | R ₁ =-H R ₂ =R ₃ =  | 298-07-7 | C ₁₆ H ₃₅ O ₄ P | 6.07 | 4.65 × 10 ⁻⁸ |
| MEHP | Mono(2-ethylhexyl) phosphate | R ₁ =R ₂ =-H R ₃ =  | 12645-31-7 | C ₈ H ₁₉ O ₄ P | 2.65 | 5.34 × 10 ⁻⁷ |

the 1980s, regarding their possible environmental (bio)accumulation and (bio)degradability, they were again a topic of research by Muir et al. [4,5]. These studies were then almost abandoned during the 1990s, as most aryl and alkyl phosphates initially considered were found to be degradable in the environment. However, research on these chemicals in the environment has re-emerged since many of them were found in indoor environments in 1997 by Carlsson et al. [6], and the chlorinated-alkyl phosphates were included in the 2nd (1995) and 4th (2000) European Union (EU) priority lists [7,8] for risk assessment and found to be rather persistent in the environment [9]. Thus, they may be classified as “re-emerging” rather than emerging pollutants.

The draft on the risk assessment of tri(2-chloroethyl) phosphate (TCEP) released in March 2006 concluded [10] that, at present, there is no need for further studies or reduction measures as regards environmental protection, while risk assessment with respect to human health has not yet been completed. Nevertheless, it recognized the carcinogenicity, high toxicity and environmental persistence of this compound. However, TCEP does not meet the PBT criteria (Persistence, Bioaccumulation and Toxicity [11]), as it is not bioaccumulative [10]. Risk assessment for the other two chlorinated compounds has not yet been completed, but tri(chloropropyl) phosphate (TCPP) is also a suspected carcinogen, while the carcinogenicity of tri(dichloropropyl) phosphate (TDCP) seems to have been proved more clearly [12]. These two compounds with higher log *K*_{ow} values (Table 1) may therefore eventually meet the PBT criteria and may then require regulatory measures to be taken.

In the case of non-chlorinated OPEs, trimethyl phosphate (TMP) was recognized as genotoxic [13] and neurotoxic effects were found for tri-*n*-butyl phosphate (TnBP) and triphenyl phosphate (TPhP) [14,15], whereas tributoxyethyl phosphate (TBEP) is also a suspected carcinogenic compound [16].

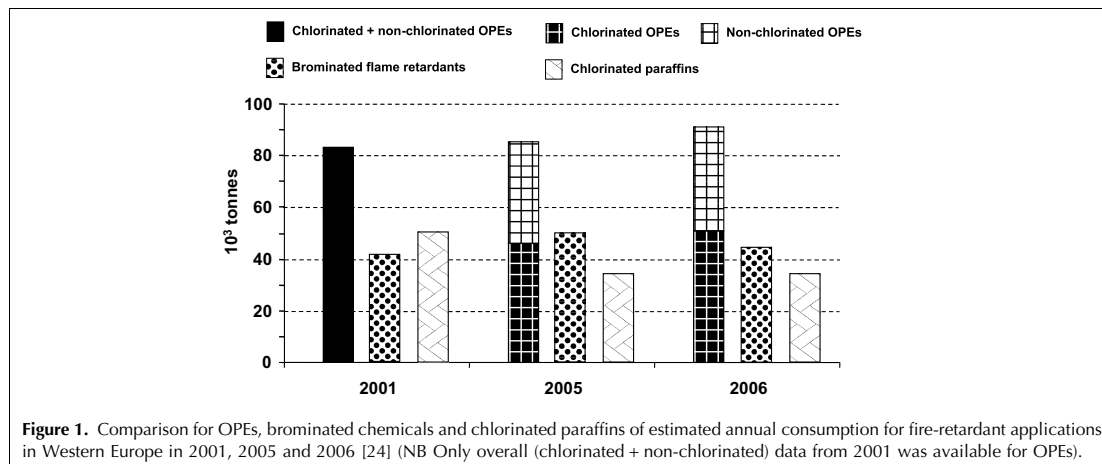
An extensive set of data on the toxicology and the ecotoxicology of OPEs is available within INCHEM – World Health Organization Health Criteria (e.g., [12,14,15]) and IUCLID Datasets (e.g., [17]), which can be accessed easily through the Web pages of INCHEM (<http://www.inchem.org/>) and the European Chemical Information System (<http://ecb.jrc.it/esis/index.php?PGM=ora>).

Considering the reports on OPEs occurring in domestic environment (air) and in nature (water), together with the toxic effects of some of these components, it seemed worthwhile to collect and to review the available information on these contaminants. Knowledge of the environmental behavior of trace pollutants strongly depends upon the availability and the further development of adequate analytical methods. We therefore divided this review into two parts. In this first, we summarize and discuss the literature on the occurrence and the behavior in (waste) water, indoor air and the atmospheric environment. To some extent, we could draw a consistent picture that is in agreement with the known physico-chemical properties of the OPEs. However, we identified significant knowledge gaps that merit future attention to complete our understanding of the fate of OPEs in the environment. We devote the second part to analytical methods for the determination of OPEs from environmental samples, water, air and particulate material [1].

2. Properties and usage

Table 1 summarizes the structures, names, abbreviations and physico-chemical properties of the most common organophosphorus flame retardants and plasticizers that are covered in this study. The well-known organophosphate pesticides, namely insecticides, are not covered here. Most of these compounds are triesters of phosphoric acid, with the exception of diesters DnBP and DEHP and monoester MEHP. Other organophosphorus flame retardants and plasticizers used to a minor extent (not included in Table 1) are bisphosphates, phosphonates and phosphinates.

Concerning usage, tri-chlororalkyl- and triaryl-phosphates are mostly employed as flame retardants in plastics, textiles, electronic equipment, as well as furniture and construction [18]. Non-chlorinated alkyl phosphates are mainly used as plasticizers [19], but also for anti-foaming, as additives to lacquers, in hydraulic fluids [20] or floor polishing, and as non-ionic



extractants in hydrometallurgy [21]. TnBP is an important extractant in nuclear fuel processing [22,23].

A good overview of the individual applications of several OPEs can be obtained from the European Flame Retardants Association (EFRA) [24] and from the work of Marklund et al. [25]. Most of these OPEs are regarded as high-production-volume chemicals and their use only as flame retardants in Western Europe accounted for ca. 83,000 tons/year in 2001, ca. 85,000 tons/year in 2005 and ca. 91,000 tons/year in 2006 [24], which represents increases of some 2.5% (2001–2005) and 7.1% (2005–2006), respectively.

Fig. 1 compares the consumption of OPEs, brominated flame retardants (BFRs) and chlorinated paraffins in this market. The increase trend may become even greater in the future due to the environmental concern about BFRs and the ban on usage of penta-BDEs and octa-BDEs (brominated diphenyl ethers) in the EU since August 2004 [26] and the very recent ban on the widely-used deca-BDE, effective on 1 July 2008 [27]. However, total consumption of OPEs must be considerably greater, because the data given above consider only their application as flame retardants.

The large tonnage and the mode in which OPEs are employed (mixed into but not chemically bonded to the product they are intended to protect) make diffusion into the surrounding environment very likely. Obviously, further distribution in the environment, (e.g., through water, air, particulate samples), possible accumulation in sediments and bioaccumulation strongly depend on their physico-chemical properties. As shown in Table 1, all triesters share the phosphate group's H-bonding basicity. However, their physico-chemical properties are rather variable and depend upon the alcohol moieties esterified to the phosphoric acid. In case of methyl (TMP), the triester is very polar and volatile ($\log K_{ow} -0.65$, $V_p 8 \times 10^{-1}$ torr), whereas, with the large ethylhexyl

groups (TEHP), it is very hydrophobic and non-volatile ($\log K_{ow} 9.5$, $V_p 8 \times 10^{-8}$ torr) (Table 1).

Most triesters are stable against hydrolysis at neutral pH. Hydrolysis half-lives at pH 7 for TMP, TEP and TPhP are in the range 1.2–5.5 years [28]. This contrasts with the properties of the triesters used as insecticides (cholinesterase inhibitors), which bear one alcohol group with electron-withdrawing substituents and good leaving-group properties (more acidic conjugated acid) and which hydrolyze more readily [28]. Hydrolysis can be significantly accelerated at basic pH [28] or by phosphoesterases [29,30].

Phosphoric acid monoesters and diesters, particularly DnBP, MEHP and DEHP (Table 1), are often employed as ionic extractants in hydrometallurgy [21], but also as plasticizers [19]. MEHP may also be used as an adjuvant in pesticide formulation. Moreover, diesters and monoesters may originate from the degradation of triesters as discussed later in this review. In contrast to triesters, monoesters and diesters are far less hydrophobic, non-volatile and highly acidic ($pK_a \leq 1$ [18]). Thus, under environmental conditions, monoesters and diesters occur in their dissociated state as dianions or monoanions, rendering them even more polar. Hence, sorption to particulate matter and partitioning into biota are weak [31]. Also, atmospheric transport is not expected to play a significant role on the environmental distribution of diesters and monoesters.

3. Occurrence and fate in water

Organophosphate triesters and diesters have been used for decades, so their occurrence in the environment is not a new issue. Since the 1980s, we find reports on their detection in surface waters [32,33], in groundwaters influenced by wastewater [34,35] and in

drinking water [36]. However, dedicated research on the occurrence of OPEs and their environmental fate that covers a larger number of OPEs has only started. Moreover, the use of gas chromatography (GC)-based analytical methods, without including a derivatization step within the sample-preparation scheme, has also prevented the determination and the study of diesters and monoesters (see Part II of this review [1]).

3.1. Wastewater

The number of studies devoted to the determination of OPEs in municipal wastewater and municipal wastewater-treatment plants (WWTPs) has been relatively limited (Table 2). Meyer and Bester [37] conducted a survey on seven triesters in two WWTPs from Germany involving secondary and tertiary treatment. Influent concentrations around or above 0.1 µg/L were found for six of the analytes, with TBEP showing the highest levels (Table 3). An average removal of 80–90% was estimated for TBEP and TiBP from the dissolved phase, together with moderate elimination of TPhP and TnBP. Chlorinated aliphatic esters TCEP, TCPP and TDCP showed no significant removal and, thus, were those triesters with the highest effluent concentrations (Table 3).

In a Swedish study, 12 analytes were monitored in 11 WWTPs [38], using one composite integrated sample, representing the average of one week. TBEP influent concentrations were about one order of magnitude higher than in the German study [37] (up to 35 µg/L). In agreement with the German study, the chlorinated aliphatic esters were also hardly removed in Swedish WWTPs. In this Swedish study, triester concentrations in the sludge were also determined. Here, EHDPP, TCPP and TBEP were the most abundant OPEs (up to a few

Table 3. Mean concentrations (ng/L) of phosphate triesters in the influents (In) and effluents (Out) and mean elimination (Elim. %) for two WWTPs in Germany. Data from [37], number of samples not given

| | WWTP A | | | WWTP B | | |
|------|--------|------|---------|--------|-----|---------|
| | In | Out | Elim. % | In | Out | Elim. % |
| TiBP | 1300 | 160 | 86 | 840 | 78 | 86 |
| TnBP | 1200 | 520 | 67 | 260 | 100 | 55 |
| TCEP | 290 | 350 | * | 180 | 370 | * |
| TCPP | 2000 | 3000 | * | 650 | 820 | * |
| TDCP | 100 | 130 | * | 110 | 150 | * |
| TBEP | 3700 | 440 | 88 | 4000 | 400 | 89 |
| TPhP | 130 | 70 | 57 | 81 | 20 | 75 |

*No elimination.

µg/g). TCPP sorption to activated sludge has been shown to be the major removal process in a WWTP, resulting in average concentrations of 5 µg/g in the sludge [39]. Unfortunately, due to the large difference in retention times of water and sludge in a WWTP, data were not sufficient to allow mass balances to be made.

Remarkably high influent concentrations of TBEP (12 µg/L) were recorded in another German WWTP [40]. Again, no removal was discernible for the chlorinated esters, which were, thus, prevalent in the WWTP effluent. Other chemicals covered in this study were bisphosphates resorcinol bis(diphenyl phosphate) (RDP) and biphenol A bis(diphenyl phosphate) (BDP) and less volatile triesters, such as TEHP. However, none of them was detected in wastewater, which was explained by the market share of the bisphosphates still being limited and the strong sorption tendency of TEHP [40].

A recent study investigating nine triesters in grab samples from the effluents of 16 WWTPs in Austria

Table 2. Studies on the occurrence of OPEs in wastewater

| Compounds studied | Type of matrix | Location | Year | Ref. |
|--|---|------------------------------|-----------|------|
| TCPP | Influent, effluent and sludge of one WWTP | Germany | 2002 | [39] |
| TPhP, TBEP, TnBP, TiBP, TCEP, TCPP, TDCP | Influent and effluent of two WWTPs | Germany | 2003 | [37] |
| TCEP, TCPP | Influent and effluent of eight WWTPs | Different European countries | 2003–2004 | [43] |
| TBEP, TPrP, TnBP, TCPP, TCEP, TDCP, TPhP, TMP, TEHP, DOPP | Influent and effluent of 11 WWTPs, sludge | Sweden | 2003–2004 | [38] |
| TCEP, TPPO, TCPP, TDCP, TPhP, TiBP, TnBP, TBEP, RDP, BDP, TEHP | Influent and effluent of one WWTP | Germany | 2004 | [40] |
| DPhP, DnBP, DiBP, MEHP, DBEP, DEHP | Influent and effluent of one WWTP | Germany | 2004 | [44] |
| TEP, TCEP, TCPP, TPhP, TDCP, TBP, TBEP, TCrP, TEHP | Effluents of 16 WWTPs (grab samples) | Austria | 2005 | [41] |

DOPP, Di-n-octylphenyl phosphate; TPPO, Triphenylphosphine oxide; RDP, Resorcinol bis(diphenyl phosphate); BDP, Bisphenol A bis(diphenyl phosphate).

confirmed this picture, as, again, TCPP was the most prominent phosphate ester [41].

Due to their persistence, chlorinated triesters were included in recent monitoring studies of municipal wastewater [42,43]. A survey of WWTPs in several European countries confirmed that TCEP and TCPP are routinely detected in their effluents, typically at concentrations of a few hundred ng/L. Nowadays, TCPP is more prominent in effluents than TCEP [43], reflecting the phasing out of the latter. Of a series of 29 polar trace pollutants, TCPP was among the 10 with no significant elimination in WWTP, whereas TCEP showed slight removal (only 20%) [43].

Removal of a trace pollutant in municipal wastewater provides a good estimate of its environmental behavior, because similar processes (biodegradation and sorption) affect its concentration in the dissolved phase. Based on WWTP-effluent concentrations and their degree of persistence, a so-called water-cycle spreading index (WCSI) has been calculated for a series of trace pollutants from municipal wastewater [43]. Based on this index, TCPP is almost as problematic a contaminant as carbamazepine in partially closed water cycles [43].

Data on the occurrence of diesters and monoesters in municipal wastewaters hardly exist. On the one hand, a suitable analytical method was missing and has only recently been published [44]. On the other hand, pure standards are commercially available for only three diesters. However, the few samples analyzed so far show that DEHP occurred in higher concentration than any of the triesters in the WWTP influent and was still present in the low $\mu\text{g/L}$ range in the WWTP effluent. Besides DEHP, four other diesters and MEHP could be detected [44]. This points to the need for a more detailed evaluation of the fate of these compounds in the environment, including the particulate matter and possible metabolites.

3.2. Biodegradation

For several reasons, the ecological profile of OPEs appears to be advantageous:

- 1) the phosphate-ester moiety is not xenobiotic but very common in living organisms, e.g., in ATP, and enzymes for the hydrolysis of phosphate esters are, thus, equally common;
- 2) alcohols released upon hydrolysis of the phosphate esters are generally readily degradable (e.g., methyl from TMP, ethanol from TEP or phenol from TPhP). Some of the OPEs (e.g., TBEP) exhibit further ether linkages in the alkyl chain that can also be attacked hydrolytically.

Indeed, investigations of WWTP influents and effluents have suggested that some of the triesters are biodegradable whereas others, especially the halogenated ones, are quite recalcitrant. However, biodegradation and sorption may occur in wastewater treatment and a

biological process may lead to primary degradation but not necessarily to mineralization.

Laboratory degradation studies have been performed to test whether biodegradation can occur at all and whether the triesters are mineralized or only hydrolyzed. Complete primary degradation in experiments carried out with high concentrations of OPEs was shown for TPhP, TCrP, TnBP and TiBP [3,9], but mineralization was not always complete. TEHP is known to be poorly biodegradable. Primary biodegradation of the aromatic triesters appears to be faster than for the aliphatic ones, while the halogenated triesters were not biodegradable [9].

In a recent study, in which LC-MS was used to monitor biodegradation intermediates, the microbial formation of the respective diesters from TPhP, TiBP and TBEP by bacteria was shown (Fig. 2). While the diphenyl

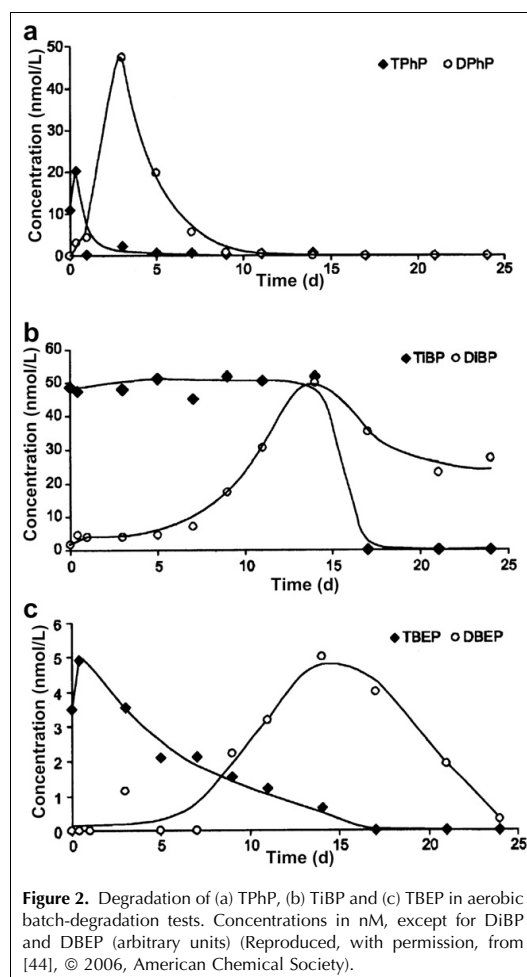


Figure 2. Degradation of (a) TPhP, (b) TiBP and (c) TBEP in aerobic batch-degradation tests. Concentrations in nM, except for DiBP and DBEP (arbitrary units) (Reproduced, with permission, from [44], © 2006, American Chemical Society).

phosphate (DPhP) and dibutoxyethyl phosphate (DBEP) formed were completely removed within 25 days, about 50% of di-iso-butyl phosphate (DiBP) remained after this period [44]. Bacteria capable of triester biodegradation have been isolated [45] as well as enzymes responsible for triester hydrolysis [29].

Several factors may affect the biodegradability of OPEs:

- 1) Bulky alcohol moieties (e.g., the isobutyl group in TiBP or the ethylhexyl group in TEHP) may sterically hinder the attack of hydrolases and slow down primary degradation at the level of the OPEs or mineralization at the level of the free alcohol. Indeed, measurable concentrations of ethylhexanol have been found in the environment, suggesting that, for this alcohol, biodegradation is slower than its release from precursor materials [46].
- 2) A similar steric effect may be responsible for the poor degradability of the chlorinated OPEs.
- 3) Low water solubility and strong sorption tendency can reduce the bioavailability of OPEs as for TEHP.
- 4) A further aspect that may be taken into consideration while evaluating the ecotoxicological profile of OPEs are potential biological effects of the alcohols released upon (enzymatic) hydrolysis (e.g., butoxyethanol, derived from TBEP, is a proven mutagen and is suspected of being an endocrine disruptor [47]).

3.3. Surface water

WWTPs are considered the major source of OPEs in surface waters [48]. In rural areas, ditches have become contaminated by TCrP due to leaching from large amounts of plastic films used for greenhouses [49]. Emission into groundwater may occur via landfill leachate [50] and release into the marine environment from dump sites [9]. Therefore organophosphate triesters are ubiquitous contaminants in the aqueous environment.

Table 4 compiles most relevant studies of OPEs in surface waters, drinking water and groundwater. However, the compounds found and the levels detected at certain locations in rivers and lakes strongly depend upon the local situation of emissions and dilution. Typical concentrations in surface water cannot therefore be given, and the OPEs encountered at a certain site may differ. However, some aliphatic and some chlorinated triesters appear to be omnipresent.

A long-term study covering various rivers in Japan in the period 1976–90 has been published [33], showing regional as well as temporal trends. A more recent study was performed for seven triesters along the River Ruhr in a densely populated region of Germany [51]. Most OPEs occurred at concentrations of 10–200 ng/L, with the highest levels being found downstream of WWTP discharges. Similar concentrations of TCEP, TCPP, TnBP and TBEP were recently reported for Austrian rivers [41].

In the River Rhine, the concentration of TCEP exceeded that of TCPP in the mid-1990s, with concentrations in the range 0.1–1 µg/L [52]. Probably as a response to the shift in usage from TCEP to TCPP, comparable concentrations of these two compounds were reported for various surface waters 10 years later, in Germany [51] and Austria [41].

3.4. Sub-surface water and bank filtration

Infiltration of surface water into the ground may be a significant source for OPEs in groundwater. Polar and poorly degradable compounds especially can pass a sub-surface or soil barrier and occur in groundwater, because neither sorption nor biodegradation will be effective for such compounds. For this reason, TCEP was detected regularly in bank filtrate of the River Rhine in the 1990s [52]. However, TnBP and TBEP were identified in bank filtrate of the River Oder with average concentrations of 0.2 µg/L [48], although these

Table 4. Studies on the occurrence of OPEs in surface waters and groundwater

| Compounds studied | Type of matrix | Location | Year | Ref. |
|---|---|------------------------|------------|------|
| Surface waters | | | | |
| TEP, TnBP, TCEP, TCPP, TDCP, TPhP, TBEP, TEHP, TCrP | Various rivers, Osaka Bay | Japan | 1976–1990 | [33] |
| TnBP, TCPP, TCEP, TPhP, TCrP | Rivers and seawater | Kitakyushu City, Japan | 1980 | [32] |
| TnBP, TPhP, TBEP | River Elbe, River Weser | Germany | 1983–1985 | [77] |
| TCEP, TCPP | River Rhine, one year | Germany | 1994, 1995 | [52] |
| TEP, TnBP, TCEP, TCPP, TDCP, TPhP, TBEP, TEHP, TCrP | Water and particles at marine waste-disposal site | Osaka, Japan | 1996, 1997 | [78] |
| TnBP, TCEP, TBEP | Six rivers | Germany | 2000 | [79] |
| TiBP, TnBP, TCEP, TCPP, TDCP, TPhP, TBEP | River Ruhr | Germany | 2002 | [51] |
| TEP, TCEP, TCPP, TPhP, TDCP, TnBP, TBEP, TCrP, TEHP | Four rivers (grab samples) | Austria | 2005 | [41] |
| Groundwater, Drinking water | | | | |
| TCEP | Bank filtrate, River Rhine | Germany | 1995 | [52] |
| TEP, TnBP, TPhP, TCEP, TCPP, TDCP, TBEP | Landfill leachate | Sweden | 1998 | [50] |
| TnBP, TCEP, TBEP | Bank filtrate, Oder River | Germany | – | [48] |
| TiBP, TnBP, TCEP, TCPP, TDCP, TBEP, | Drinking-water-treatment plant | Germany | – | [54] |
| TCEP, TnBP, TDCP, TPhP | Drinking waters | USA | 2001 | [53] |

compounds were shown to be biodegradable under aerobic conditions (see above). In this particular example, the authors assumed that anoxic conditions slowed down the degradation processes [48]. Besides infiltrating surface water, landfill leachates may also contribute to groundwater contamination, at least locally [50].

3.5. Removal during drinking-water treatment

With the occurrence of halogenated triesters in groundwaters and an even larger variety of triesters in surface waters used for drinking-water production, there is a general risk that some of these compounds may also occur in the drinking-water supply. Indeed, a study conducted in the USA in 2001 reported the occurrence of TBEP, TCEP, TDCP, and TnBP in raw waters and in finished drinking water [53], suggesting that drinking-water-treatment processes were inappropriate for the removal of OPEs in these cases.

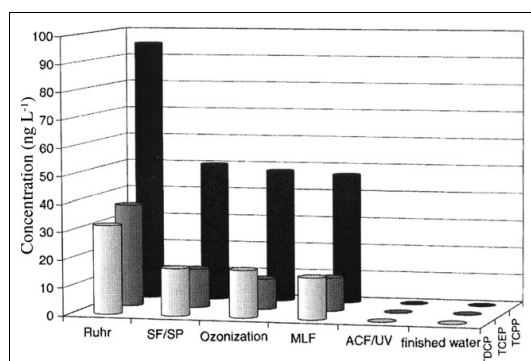


Figure 3. Concentration (ng/L) of chlorinated OPEs at different steps in a drinking-water-treatment plant for surface water of the River Ruhr (Germany) by sand filtration with soil passage (SF/SP), ozonation, rapid filtration (MLF) and activated carbon filtration with UV-treatment (ACF/UV) (Reprinted from [54], © 2006, with permission from Elsevier Science).

However, appropriate treatment technologies appear to be available: activated carbon filtration has been shown to be a very effective process for removal of chlorinated triesters (Fig. 3) [42,54]; whereas slow sand filtration was sufficient for the biodegradable aliphatic triesters [54]. Alternatively, membrane processes (e.g., nanofiltration or reversed osmosis) may also be used [42].

4. Occurrence and fate in air

4.1. Indoor air

OPEs have been added to building materials, electric appliances, upholstery and floor polishes. As a consequence, they are present at significantly higher concentrations in closed environments (e.g., offices, private houses, and cabins of vehicles) than in outdoor areas.

Table 5 summarizes the average values, as well as minimum and maximum concentrations, for a selected group of six OPEs in indoor air. Data were taken from works using active sampling and, in most cases, they represent the sum of concentrations in the gas phase and suspended particles. Only those works reporting data for at least four samples are reflected in Table 5; moreover, values under quantification limits were not considered to estimate average concentrations. The overall means for the most volatile species (TnBP, TCEP and TCPP) were clearly higher than those corresponding to TDCP, TBEP and TPhP. Moreover, the sum of mean concentrations for the halogenated alkyl phosphates (TCEP, TCPP and TDCP), which are considered the OPEs of most concern, exceeded 150 ng/m³. Assuming a daily respiratory volume of 20 m³ [55,56], their average intake in breath accounted for about 3 µg.

As observed in Table 5, there were considerable differences between minimum and maximum concentrations of OPEs for each series of samples. The types of building materials as well as furniture and electric appliances existing in the areas investigated were

Table 5. Average concentrations (ng/m³) of selected OPEs in indoor air. Minimum and maximum levels are reported within parenthesis

| Ref. | n ^a | TnBP | TCEP | TCPP | TDCP | TBEP | TPhP |
|--------------------|----------------|---------------|----------------|-----------------|---------------|---------------|----------------|
| [80] | 6 | 70 (<1.8–100) | 30 (<1.4–30) | n.d. | n.d. | 20 (<0.4–30) | 10 (<1.2–10) |
| [56] | 17 | 20 (<0.2–120) | 129 (<0.4–730) | 118 (5.1–570) | 36 (<0.2–150) | 7 (<0.2–55) | 7 (<0.1–23) |
| [6] | 5 | 22 (7–35) | 94 (11–250) | 31 (14–41) | n.d. | 3 (1.4–5.9) | 0.7 (0.5–0.8) |
| [81] | 6 | 14 (9–18) | 25 (15–36) | 21 (14–28) | n.d. | 29 (20–36) | 19 (12–40) |
| [55] | 16 | 11 (<0.1–29) | 17 (<0.2–56) | 110 (<0.1–260) | <0.1 | <3 | 2.8 (<1.5–5.7) |
| [60] | 30 | 20 (1–172) | 78 (1–870) | 273 (1–2300) | 6 (5–7) | 16 (<1–130) | 6 (<1–17) |
| [59] | 4 | 42 (4–138) | 44 (11–110) | 58 (10–112) | n.d. | 31 (0.8–46) | 12 (0.5–35) |
| [58] | 18 | 4 (<0.4–30.6) | 1.3 (<0.7–136) | 1.9 (<0.9–1260) | <0.6 | 1.8 (<0.6–14) | <5.4 |
| [61] | 50 | n.d. | n.d. | 52 | n.d. | n.d. | n.d. |
| Global mean | | 25 | 52 | 84 | 21 | 15 | 6 |

n.d., Not determined.
^aNumber of samples analyzed.

responsible for such a wide range of values. As an example, it is well known that computer screens and TV sets constitute important emission sources of TPHP [25], whereas floor polishes contribute greatly to the presence of TBEP in indoor environments [25]. Polyurethane-based insulations and wall coverings are other important sources of halogenated OPEs [57,58]. These compounds have also been detected, at extremely high values, in hospitals and prisons due to their intensive use as flame retardants in mattresses. Addition of TCPP to upholstery and plastic components has led to levels 10–100 times higher for this compound in cars and public transport vehicles than in private houses [59,60].

Unlike the plethora of data reported for air samples, less information is available in relation to the presence of OPEs in settled dust. Table 6 summarizes the most relevant data. All of them correspond to vacuum dust samples from confined areas. With the exception of only the relatively volatile TnBP, overall mean concentrations for the other OPEs remained over 5 µg/g level, with the highest levels corresponding to TBEP (Table 6). As regards the number of samples processed, Ingerowski et al. [61] carried out the most complete research; unfortunately, only TCEP and TCCP were considered in their

study. Their average concentrations were in the range 1–3 µg/g. Data provided in Table 6 show that concentrations are of the same order of magnitude, or even higher, as concentrations reported for the most abundant BDEs in reference materials of indoor dust, and Fig. 4 shows their mean values in 17 samples of dust collected from private houses in USA [62,63]. Another study carried out in Japan also showed concentrations of OPEs higher than those of BDEs in indoor-air samples [58].

On the basis of values shown in Table 6, oral ingestion of settled dust might represent a relevant contribution to exposure to OPEs indoors, at least in the case of crawling kids exhibiting the hand-to-mouth habit. Despite these high exposure levels, only TPHP has been detected in plasma and blood samples [64]. However, the reliability of this result is questionable since, in other work, it has been stated that bags used to store blood and plasma samples are an important source of TPHP [65].

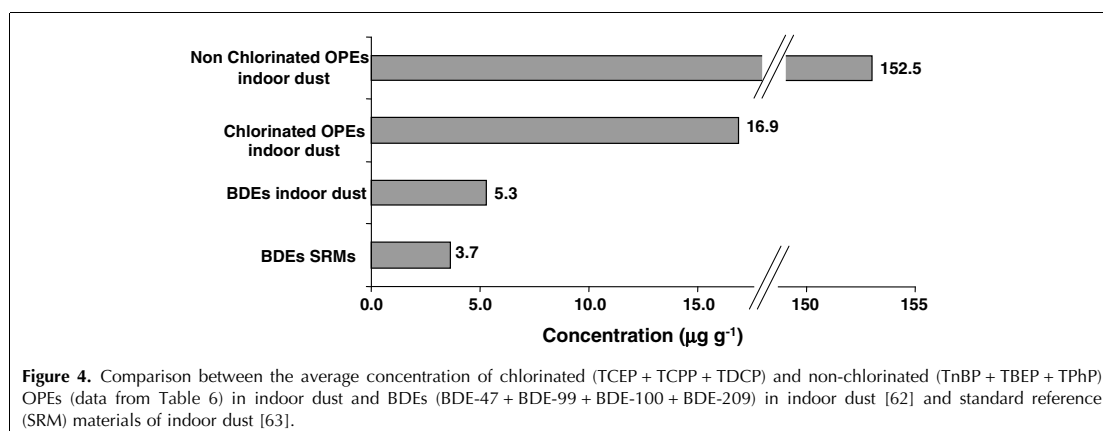
4.2. Outdoor environment

Although the concentration in outdoor environments is expected to be lower than that in indoor ones, several authors have reported direct or indirect measurements of

Table 6. Average concentrations (µg/g) of OPEs in settled indoor dust. Values within parenthesis correspond to minimum and maximum levels

| Ref. | n ^a | TnBP | TCEP | TCPP | TDCP | TBEP | TPhP |
|--------------------|----------------|------------------|----------------|-----------------|------------------|---------------|----------------|
| [25] | 15 | 0.54 (0.07–2.2) | 11.3 (0.2–94) | 11.0 (0.5–73) | 9.5 (0.2–67) | 419 (14–5300) | 11.4 (0.9–110) |
| [61] | > 400 | n.d. | 2.7 (<0.1–121) | 1.85(<0.1–375) | n.d. | n.d. | n.d. |
| [82] | 9 | 0.19 (0.04–0.9) | 5.6 (0.1–40) | 8.4 (1.2–40) | 6.1 (<0.05–11) | 11.4 (1.6–48) | 1.5 (0.4–4.9) |
| [83] | 8 | 0.25 (0.07–0.65) | 1.7 (0.25–9.8) | 3.9 (0.35–10.3) | 0.35 (<0.05–1.1) | 9.9 (1.2–19) | 2.6 (0.3–9.5) |
| Global mean | | 0.33 | 5.3 | 6.3 | 5.3 | 147 | 5.2 |

n.d., Not determined.
^aNumber of samples analyzed.



significant levels of OPEs in outdoor air and related matrices. Recently, they have been detected in suspended particulate matter and settled urban dust obtained in the vicinity of an urban avenue, at concentrations up to 2 $\mu\text{g/g}$ [66]. Similar levels were published recently for a reference material of urban dust (NIST 1649a) [67].

Snow analysis has also been proposed to confirm the presence of OPEs in outdoor atmospheres [68]. Diffusion from plastic materials and upholstery employed in vehicle cabins, added to the use of TPhP, TCrP and TnBP as additives in lubricants and hydraulic fluids, explains the presence of OPEs in air and deposition samples collected in outdoor areas with intense traffic and near to airports [69]. Detection of several OPEs in particulate matter and different types of environmental samples (e.g., pine needles, rain water and snow) from remote areas confirms the contribution of air transport to the ubiquitous distribution of these pollutants in the environment [70,71]. Ciccioli et al. also reported the presence of some alkylphosphates in airborne particulate matter from remote places in the Southern hemisphere [72].

In the past decade, atmospheric transport has been shown to be responsible for the distribution of environmental contaminants on a global scale [73] and for the occurrence of such compounds in Arctic biota. To date, OPEs have not been covered in monitoring studies in Arctic regions. However, very recently, more than 100,000 industrial chemicals have been screened for their similarity to known Arctic contaminants. Among the 822 compounds that provide physico-chemical and partitioning properties that would allow them to become Arctic contaminants, TCEP exhibited a so-called POP score of 4.1, which is comparable to that of pentachlorobiphenyl and higher than that of tribromodiphenyl-ethers, and comes close to the mean value of 4.4 that was found for known Arctic contaminants [74]. This POP-score calculation was based on the elemental composition and structural criteria of the compounds. Global atmospheric transport of some of the OPEs therefore deserves further attention.

5. Outlook and conclusions

The recent improvement in analytical methods (see Part II of this review [1]) has provided the tools necessary to improve our knowledge on the occurrence and the environmental fate of triesters in indoor particulate matter, air and the aqueous environment. It has been shown that some OPEs, namely the chlorinated ones, are almost ubiquitous. This is due to their widespread use, their volatility and their limited biodegradability. However, also organophosphate esters that are biodegradable are frequently detected due to their continual release from various sources into water and air.

This literature review revealed a number of significant knowledge gaps that merit future attention to complete our understanding of the fate of OPEs in the environment.

Concerning the fate of OPEs in water, the following aspects have not been answered sufficiently;

- occurrence and behavior of monoesters and diesters in the aqueous environment;
- fate of hydrophobic OPEs upon sorption to particulate matter;
- favorable conditions for biodegradation and mineralization, particularly for the chlorinated OPEs;
- stability in subsurface environment (bank filtration, groundwater); and,
- importance of abiotic transformation (hydrolysis, photolysis)

With respect to air and the atmosphere, we lack information on:

- stability and putative transformation products in air; and,
- relevance of long-range transport for OPE distribution

Finally, little is known about the occurrence of OPEs in biota. Work from the early 1980s [4,5] needs to be updated, as the market for OPEs has changed and better analytical methodologies are now available [1].

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Organophosphorus flame retardants and plasticizers in water and air

II. Analytical methodology

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Increasing usage of several organophosphate esters (OPEs), as well as their systematic presence indoors and in water samples, requires development of appropriate analytical procedures to understand their environmental distribution and to identify their transformation and degradation by-products. We review the latest analytical methods for the determination of OPEs in water, air and particulate material (e.g., sediments and dust), covering various extraction and clean-up techniques, as well as their determination by gas chromatography-nitrogen-phosphorus detection (GC-NPD), GC-mass spectrometry (GC-MS) and liquid chromatography-MS (LC-MS). Besides phosphoric acid triesters, we also consider diesters and monoesters. We discuss thoroughly the merits and the limitations of these methodologies. We also highlight some challenging issues in the determination of OPEs (e.g., the need of certified reference materials, isotopically-labeled surrogates of the congeners causing the greatest concern, and standards for the phosphoric acid diesters and monoesters. Finally, we point to potential future trends in the analytical determination of OPEs. A previous review covering the environmental occurrence and fate of OPEs was published in the last issue of this journal [T. Reemtsma, J.B. Quintana, R. Rodil, M. García-López, I. Rodríguez, Trends Anal. Chem. 27 (2008)].

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1. Introduction

Organophosphate esters (OPEs), namely triesters, are industrial chemicals widely used as flame retardants and plasticizers and in many other applications. We discussed the production, physicochemical-properties, environmental occurrence and fate of OPEs in the last issue of this journal [1]. Table 1 shows the abbreviations for the most common OPEs.

OPEs have been determined in environmental samples in water and air [1]. The oldest findings were from screening studies that covered a wide variety of trace pollutants rather than from studies devoted to targeted analysis of OPEs. However, there have been publications on the development of analytical methods primarily for the determination of organophosphate triesters but also of diesters and monoesters. We describe and discuss these methods in this review. Methods for trace analysis of organophosphate triesters have

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Table 1. Abbreviations of the most important OPEs covered in this review

| Abbrev. | Compound name | Abbrev. | Compound name | Abbrev. | Compound name | Abbrev. | Compound name |
|-------------|-------------------------------|-------------|-------------------------|--------------|---------------------------------|-------------|------------------------------|
| TCEP | Tri(2-chloroethyl) phosphate | TEP | Triethyl phosphate | TBEP | Tributoxyethyl phosphate | TCrP | Tricresyl phosphate |
| TCPP | Tri(chloropropyl) phosphate | TPrP | Tripropyl phosphate | TEHP | Tri(2-ethylhexyl) phosphate | DnBP | Di-n-butyl phosphate |
| TDCP | Tri(dichloropropyl) phosphate | TiBP | Tri-iso-butyl phosphate | EHDPP | 2-Ethylhexyl diphenyl phosphate | DEHP | Di(2-ethylhexyl) phosphate |
| TMP | Trimethyl phosphate | TnBP | Tri-n-butyl phosphate | TPhP | Triphenyl phosphate | MEHP | Mono(2-ethylhexyl) phosphate |

Further details on physico-chemical properties can be found in Part I [1].

to cope with the fact that this class, although chemically homogenous, covers analytes of quite diverse physico-chemical properties, depending on the respective ester moieties, from very polar and volatile (e.g., TMP) to very hydrophobic and non-volatile (e.g., TEHP) [1].

Determination of OPEs in the environment is normally carried out by combining an enrichment or extraction step, sometimes followed by a clean-up procedure, with a final determination using gas chromatography (GC) or liquid chromatography-mass spectrometry (LC-MS). However, before discussing into detail the different extraction, clean-up and determination approaches for each particular matrix, we need to consider two issues: choice of internal standard and blank contamination.

Regarding internal standards, some authors have employed different alkyl OPEs, which were not expected to occur in real samples {e.g., TPrP [2], TPeP (tripentyl phosphate) [3,4] or THxP (trihexyl phosphate) [4]}. However, Bacaloni et al. recently found that TPrP can be present in real samples [5], while there is no data about the possible occurrence of TPeP and THxP. Thus, some authors decided to work without an internal standard or to synthesize perdeuterated TPhP [5]. Fortunately, perdeuterated TEP, TnBP and TPhP have recently been made commercially available by well-known chemical standard suppliers, so that this difficulty has been resolved when GC-MS and LC-MS are employed, but remains for other detection techniques. However, as far as we know, labeled standards for chlorinated alkyl organophosphates are still not commercially available. Considering the large differences in polarities and volatilities of OPEs, the use of a single surrogate is unsuitable to compensate for non-quantitative extractions and/or analyte losses during sample preparation.

Concerning blanks, there have been reports of contamination of cellulose and quartz-fiber filters and Soxhlet cellulose thimbles with TiBP and TnBP [3,6] and of some headspace vial caps with TBEP [7]. Contamination of quartz-fiber and cellulose materials can easily be avoided by thermal or solvent pre-cleaning, respectively. Cellulose Soxhlet thimbles can also be replaced by glass thimbles, while vial-cap contamination can be overcome in SPME by selecting other types of cap or, for

the less volatile species, simply performing the extraction in open vessels.

2. Sample preparation

2.1. Water samples

Procedures for the determination of OPEs in water samples involve an enrichment step followed by chromatographic analysis, as summarized in Table 2. Traditionally, liquid-liquid extraction (LLE) [8–12] and solid-phase extraction (SPE) [2,5,7,13–15] have been the most often employed extraction methods. Normally, for surface-water samples, additional extract purification is not performed. However, some authors recommend clean-up on silica columns of the SPE extracts from samples to remove interferences, especially in the case of sewage samples [7,14].

LLE has been used for the extraction of wastewater, drinking-water and surface-water samples (typically 0.5–2 L) with dichloromethane or toluene. Although it allows filtered and unfiltered samples to be processed, the recovery for polar analytes (e.g., TCEP) by LLE with toluene is low (ca. 39%) [8,9]. In addition, LLE has well-known disadvantages (e.g., the requirement for large volumes of organic solvents, foam formation and difficulties in automation). Thus, SPE is preferred for water extraction.

Several SPE sorbents have been used for the analysis of OPEs in water, including disks {e.g., DVB-hydrophobic Speedisks [14]} and cartridges {e.g., C18 [15] and divinylbenzene (DVB) polymers [2,5,7,13] (Bakerbond Speedisk Hydrophilic DVB polymer, Oasis HLB and Bond Elut PPL)}. Most of these sorbents provide satisfactory recoveries from filtered surface-water and wastewater samples (0.1–5 L), the only exception being very polar analytes (e.g., TMP and TEP). For these latter compounds, only hydrophilic DVB polymers (Oasis HLB and Bakerbond Hydrophilic DVB) have provided quantitative extraction yields [2,5]; anyhow, their breakthrough volumes remained at the 0.5-L level [5], whereas samples larger than 2 L can be concentrated for the most lipophilic OPEs [7]. Typical sorbent-bead masses range

Table 2. Extraction conditions for the determination of OPEs in water samples

| Extraction technique | Matrix | Extraction conditions | Detection | LOQs (ng/L) | Recoveries (%) | Ref. |
|-----------------------|--|--|---------------------------------------|--|-------------------|---------|
| LLE | Wastewater, surface and drinking water | Sample volume: 1 L, solvent: toluene (10 mL), time: 30 min | PTV-GC-EI-MS | 5–20 | 89–107 (TCEP: 31) | [8,9] |
| LLE | Wastewater and snow | Sample volume: 2 L, solvent: dichloromethane (100 + 40 + 25 mL), time: 5 min | GC-NPD | 3–10 | 81–100 (TMP: 47) | [10,11] |
| LLE | Wastewater and river water | Sample volume: 0.5–0.8 L, solvent: dichloromethane (25 mL + 2x5 mL) | LC-ESI-MS/MS | 3–8 (river water) 4–13 (wastewater) | 63–94 | [12] |
| SPE | Wastewater | Sorbent: DVB-hydrophobic Speedisks 45 mm, elution solvent: MTBE and toluene | PTV-GC-MS | 1–7 | 75–90 | [14] |
| SPE | River water and groundwater | Sorbent: Bond Elut PPL 100 mg, sample volume: 5 L, elution solvent: ACN:MeOH (1:1) | GC-MS | 1 | 83–89 | [13] |
| SPE | Drinking water | Sorbent: C18, sample volume: 1 L, elution solvent: acetone | GC-MS (chloroalkyl-) LC-DAD (TPPO) | Not available | Not available | [15] |
| SPE | Wastewater | Sorbent: Oasis HLB 60 mg, sample volume: 100 mL, elution solvent: MeOH | LC-ESI- MS/MS | 3–81 | 28–90 | [2] |
| SPE | Drinking and surface water | Sorbent: Bakerbond Speedisk Hydrophilic DVB 200 mg, sample volume: 500 mL, elution solvent: MeOH | LC-ESI-MS/MS | 0.3–4 | 20–103 | [5] |
| SPE | River and wastewater | Sorbent: Oasis HLB 60 mg, sample volume: 1 L, elution solvent: ethyl acetate | GC-NPD | 5–10 | 24–109 | [7] |
| SPE of mono-/diesters | Wastewater | Sorbent: C-18 500 mg, sample volume: 100 mL + 11 mL TrBA buffer, elution solvent: MeOH | LC-ESI- MS/MS | 7–14 | 71–112 | [16] |
| SPME | River and wastewater | Fiber: PDMS-DVB, direct sampling, sample volume: 22 mL, time: 40 min, 300 mg/mL NaCl, room temperature | GC-NPD | 10–25 | Not available | [7] |
| MASE | Wastewater | Solvent: cyclohexane (1 mL), sample volume: 100 mL, time: 3 h, 300 mg/mL NaCl, 60 °C | LC-ESI- MS/MS | 1–25 | 5–98 | [18] |
| DLLME | Wastewater | Solvent (1 mL): acetone: trichloroethane (98:2), sample volume: 10 mL, 200 mg/mL NaCl | GC-NPD | 10–80 | 23–109 | [42] |

LLE, Liquid-liquid extraction; SPE, Solid-phase extraction; SPME, Solid-phase microextraction; MASE, Membrane-assisted solvent extraction; DLLME, Dispersive liquid-liquid microextraction; DVB, Divinylbenzene; MTBE, Methyl-*tert*-butyl ether; TrBA, Tri-*n*-butyl amine (ion-pairing agent); PDMS, Polydimethylsiloxane.

between 60 mg (hydrophilic DVB polymers) and 500 mg (C18), providing LOD values at the low-ng/L level, depending on the volume of sample extracted and determination technique employed (Table 2). Elution can be accomplished with different solvents, depending on the further determination technique and the target species, including MTBE/toluene, acetonitrile, acetone, ethyl acetate and methanol. In contrast to triesters, study of the extraction of diesters and monoesters is limited in part by the lack of pure standards. However, ion-pair SPE on C18 cartridges was shown to be suitable for the extraction of three diesters from water samples [16].

One disadvantage of SPE procedures is that samples with suspended matter cannot be extracted without filtration because of sorbent clogging. However, filtration may result in losses of the most hydrophobic OPEs associated with particles being trapped on the filter. Marklund et al. [10,11] resolved this inconvenience by passing the first portion of solvent through the filter to release OPEs, which was then used for LLE. Also, in general, low recoveries are obtained for the most hydrophobic compounds (e.g., TEHP), due to sorption on the glass material used for SPE and association with dissolved organic matter [7]. Rodil et al. [2] minimized this problem by rinsing sample bottles and SPE tubing with methanol. These methanol solutions were subsequently passed through the SPE cartridge and used for elution of the analytes.

A further difficulty in OPE analysis arises from losses of the most volatile compounds (TMP and TEP) during concentration of sample extracts [5,12]. Bacaloni et al. considered different evaporation temperatures and final volumes, and they showed that, as expected, these losses increased as the temperature increased and the final volume decreased [5]. Under controlled conditions (37°C and final volume > 0.7 mL), they measured a loss of ca. 20% TMP during the evaporation step. However, these conditions make extract concentration time-consuming, and, anyway, for this compound, overall recoveries were still quite low (20–47%). Thus, all reported procedures [5,10,11] are suitable for screening TMP but not for its quantification until isotopically-labeled TMP becomes commercially available.

Besides SPE and LLE, microextraction techniques are playing an important role in the determination of OPEs. Miniaturization, reduction of organic solvents and improvement in extraction selectivity are some of their potential advantages [17]. Solid-phase microextraction (SPME) [7], membrane-assisted solvent extraction (MASE) [18] and dispersive liquid-liquid microextraction (DLLME) [19] have been successfully applied to the determination of OPEs in water samples.

Rodríguez et al. [7] developed an SPME method for the determination of OPEs. Among the SPME fibers tested, maximum efficiencies were achieved using PDMS-DVB and PDMS-CAR-DVB coatings. However, carry-over

problems were observed for TPhP with the PDMS-CAR-DVB coating, so Rodríguez et al. preferred PDMS-DVB. Extraction was carried out in the direct-sampling (DS) mode, at room temperature with the addition of NaCl to increase the yield of extraction for the most polar OPEs. Again, sorption problems for TEHP, which produced extremely poor correlation, were observed using SPME [7], rendering this technique unsuitable for TEHP determination. Nevertheless, limits of quantification obtained by SPME (10–25 ng/L) from a 22-mL sample for eight OPEs were only a factor of two higher than those obtained by SPE (5–10 ng/L) from 1-L samples [7].

MASE can be performed on a commercial device comprising a thin-layer polypropylene bag made to fit into conventional headspace extraction vials. This procedure improves the selectivity of LLE, avoiding most of its drawbacks, as it prevents formation of emulsion and uses a much lower volume of solvent (ca. 0.5–1 mL). Quintana and Reemtsma evaluated several parameters that affect the extraction efficiency of OPEs by MASE [18]. MASE was performed with 1 mL of cyclohexane at elevated temperature (60°C) in order to improve diffusion through the membrane. As in the case of SPME, NaCl was added to enhance efficiency in extracting the most polar analytes. In combination with LC tandem MS (LC-MS/MS), limits of detection (LODs) achieved were similar to those obtained by SPE for moderately polar and hydrophobic OPEs. Due to the higher selectivity of MASE, signal-suppression problems were not observed for wastewater samples. However, MASE yielded low extraction efficiencies for polar compounds ($\log K_{ow} < 2.5$), 5% for TCEP. In addition, because of the manual sample and extract handling of the extraction bags, relative standard deviations were comparatively high (13–27%) for samples spiked at low concentration levels [18].

Finally, DLLME employs an extraction mixture comprising a high-density solvent (extractant) and a water-miscible polar solvent (disperser). When this mixture is added to the water sample, a cloudy state is formed, comprising fine droplets of the extractant dispersed in the aqueous solution [19]. In the case of OPEs, 10 mL of sample were extracted with 1 mL of acetone/trichloroethane (98/2). As with SPME and MASE, the ionic strength of the sample is increased by adding NaCl in order to improve the extraction yield of polar analytes. Finally, 12 μ L of trichloroethane were collected after centrifugation and injected into a GC-nitrogen-phosphorus detection (NPD) system. The large contact surface between the sample and the droplets of the extractant speeded up mass-transfer processes. Thus, DLLME provided responses similar to or higher than SPME, with the additional advantages of being much faster (5 min vs. 40 min) and cheaper. However, as for MASE, low extraction efficiencies were obtained for TCEP (23%), and automation of sample preparation was not straightforward.

Table 3. Summary of extraction and desorption conditions for the determination of OPEs in indoor areas using active sampling devices

| Sorbent | Flow rate (L/min) | Air volume (m ³) | Desorption solvent | LODs (ng/m ³) | Ref. |
|---|-------------------|------------------------------|---|---------------------------|---------|
| Charcoal, 100 + 50 mg | 1 | 4.3 | Toluene (US, 1 mL) | 2–8 | [21] |
| Aminopropyl silica, 25 mg | 3 | 1.5 | MTBE (5 mL) | 0.1–0.3 | [4] |
| Aminopropyl silica, 25 mg | 2.5 | 1.7 | Dichloromethane (10 mL) | 0.1–3.9 | [28] |
| Quartz fiber filter + C18 membrane, 47 mm | 5 | 7.2 | Acetone (US, 8 mL) | 0.1–0.6 | [25] |
| Styrene-divinylbenzene, 300 mg | 2 | 3 | Acetone (5 mL) | 0.7–7 ^a | [23] |
| Glass-fiber filter plus PUF (15 mm OD × 15 mm length) | 3 | 1.5, 2.1 | Dichloromethane (Soxhlet extraction, 50 mL or US, 2 × 5 mL) | 0.17 ^a | [20,27] |
| PUF (22 mm OD × 76 mm length) | 4 | 1.9 | Dichloromethane (US, 2 × 37 mL) | 0.73–4.1 | [26] |
| Quartz fiber plus cellulose filter | 3 | 1.4 | Dichloromethane (US, 2 × 20 mL) | 0.1–1.4 | [24] |
| PUF (22 mm OD × 76 mm length) | 5 | 1 | Not available | 1 | [22] |
| Glass-fiber filter | 2.5 | 0.06 | Methanol (0.5 mL/min) ^b | 0.06–0.2 | [30] |
| Glass-fiber filter plus PUF (15 mm OD × 15 mm length) | 3 | 0.18 | Hexane-MTBE (0.2 mL/min) ^b | 0.03–0.18 | [29] |
| C8 membrane, 47 mm | 10 | 14 | Methanol (0.2 mL/min) ^b | 0.001–0.019 | [31] |

PUF, polyurethane foam; OD, outer diameter; MTBE, Methyl-*tert*-butyl ether; US, Ultrasonication.
^aCalculated from reported quantification limits divided by 3.
^bOn-line desorption.

2.2. Air samples

Levels of OPEs in air are the result of two contributions: compounds in gas phase; and, those associated with airborne particles. Most procedures dealing with the determination of OPEs in indoor atmospheres estimate their overall concentration in both particulate and volatile fractions. In addition, some authors have considered the analysis of settled dust, normally obtained from vacuum cleaner bags, as an indicator of OPE concentrations in closed areas. From an analytical perspective, bulk dust requires an approach to sample preparation completely different from air analysis, so we discuss it in Section 2.3, which is devoted to solid samples.

Sampling is the first step in sample preparation for determination of OPEs, particularly in indoor atmospheres. Active sampling is used most often [4,20–28]. Normally, air is pumped through a device comprising a glass-fiber or quartz-fiber filter followed by a solid sorbent [20,25,27,29]. The filter retains the fraction of OPEs associated with debris and particulate matter and the solid sorbent those compounds in the gas phase. It has been demonstrated that OPEs are mainly particle-associated rather than in the gas phase [27], so fiber filters sufficed to retain them quantitatively [24,30]. However, many authors have used solid sorbents, without a previous filter, to trap OPEs associated with suspended particles as well as those in gas phase [4,22,23,26,28,31]. Table 3 summarizes trapping sorbents, desorption solvents and typical air-flow rates for monitoring OPEs indoors. The relatively low sampling rates (1–15 L/min) and the moderate total volumes (0.1–14 m³), added to the semi-volatile character of most OPEs, explain their effective retention using very simple devices.

Taking into account the above considerations, SPE cartridges, containing just a few mg of an appropriate sorbent, are preferred to polyurethane-foam plugs (PUFs) and SPE membranes, since the two latter configurations require large volumes of organic solvents in the desorption step. This leads to diluted extracts and potential procedural blank problems associated with the evaporative concentration of primary extracts. An additional advantage of SPE cartridges vs. PUFs is the possibility of rinsing the sorbent with a non-polar solvent, before eluting OPEs, thus reducing the complexity of the final extract without introducing an additional step in sample preparation [4]. However, in practice, the use of selective detectors [e.g., NPD, flame-photometric detection (FPD) and MS in selected-ion monitoring (SIM) mode] guarantees the selective determination of OPEs without any need for a clean-up step, at least when sampling moderate air volumes from clean indoor environments (e.g., private houses and office buildings). However, the main drawback of SPE cartridges is their relatively high resistance to air pumping in comparison with PUFs and reversed-phase sorbents (C18 and C8) in membranes 0.5 mm thick [4].

Automation of OPE desorption, using on-line coupling between the trapping sorbent and the chromatographic system, has been proposed to increase the sample throughput, to improve the precision of the method and to prevent contamination of extracts with OPEs from the laboratory atmosphere [29–31]. In LC analysis, the trapping sorbent is packed into a small-volume stainless-steel cell, connected between two ports of a six-way valve, and OPEs are eluted using a solvent miscible with the mobile phase. For example, Tollbäck et al. employed a methanol flow-rate of 0.2 mL/min for 4 min for on-line

desorption of several OPEs from a C8 membrane, previously cut and packed in a stainless-steel cell [31]. Broadening of the injection band was avoided by diluting the methanol stream with water. Under these conditions, the desorbed OPEs were strongly retained, and thus focused on the head of the reversed-phase LC column [31]. Using GC as separation technique, the coupling was also rather straightforward, requiring, in terms of hardware, just a six-port valve and a GC instrument equipped with a large-volume injector [29].

Table 3 shows LODs achieved for OPEs in indoor air, using active sampling methodologies. In general, they remained at the low-ng/m³ level, depending on sample and extract volumes as well as the detection technique chosen. When similar air volumes were considered, lower values were attained with desorption on-line rather than off-line (Table 3).

Alternative sampling devices for the determination of OPEs in gas phase are SPME fibers. In published works, analyte concentration is performed under dynamic conditions forcing a constant, linear air flow over the fiber [32–34]. Operating under well-controlled conditions, particularly for sample temperature and humidity, the amount of each compound incorporated into the fiber is proportional to: (1) its concentration in air; and, (2) the exposure period. If the latter is longer than the equilibrium time, then the distribution constants of OPEs between the SPME coating and air samples (K_{fs}) can be used directly to estimate their gas-phase concentrations. Isetun et al. obtained K_{fs} values for TnBP, TiBP, TCEP and TCPP between polydimethylsiloxane (PDMS) fibers and air at room temperature [33]. These constants were then used to calculate the concentration of these substances in indoor-air samples. Differences between values provided by this approach and those obtained with active sampling were below 10% [32,33]. Although the

extraction capacity of SPME fibers is very low in comparison with SPE sorbents, trapped compounds are transferred quantitatively to the GC column during the thermal desorption step in a splitless injector. Consequently, LODs obtained were 0.01–2 ng/m³, which were similar to those reported for active sampling [32–34].

We have not found any references describing the use of passive sampling devices to estimate indoor levels of OPEs. However, this methodology has been proposed to assess the diffusion of TCPP from indoor materials (e.g., wallpaper). Ni et al. described a passive sampler comprising a C18 membrane housed in a glass cylinder, with a variable height and an internal diameter of 47 mm, which can be placed over the surfaces under investigation [35]. The system is relatively simple when compared with the use of emission chambers and active sampling [36], and, moreover, it more realistically estimates the diffusion rates of OPEs from polluted materials. Migration of OPEs from interior materials (floor, ceiling and walls) and electric appliances (computer screens and TV sets) has been also evaluated by placing the C18 membrane in direct contact with the surfaces under investigation [37].

2.3. Solid samples

This section reviews sample-preparation methodologies for the determination of OPEs in solid matrices from different sources, as well as significant amounts of particulate matter retained on quartz-fiber or glass-fiber filters. Normally, analytes are extracted with medium-polarity solvents (e.g., dichloromethane, ethyl acetate and acetone), and the efficiency of the process is enhanced by using high temperatures, high pressures or a large number of extraction cycles (Table 4). Although it has been reported that selective detection techniques (e.g., GC-NPD and GC-MS in SIM mode) can deal with raw extracts from medium-complexity sam-

Table 4. Extraction and clean-up conditions for the determination of OPEs in solid matrices

| Matrix | Extraction technique | Extraction solvent | Clean-up | Detection | Recoveries (%) | Ref. |
|------------------------------------|----------------------|--|--|----------------------------|----------------|------|
| Indoor dust | Sonication | Dichloromethane (2 × 25 mL) | – | GC-NPD | 97 | [38] |
| Indoor dust | Soxhlet | Hexane: acetone, 8:2 | – | GC-EI-MS | Not available | [22] |
| Sludge | Soxhlet | Ethyl acetate | SPE (silica sorbent) plus GPC | GC-EI-MS | 110 | [40] |
| Sludge | PLE | Ethyl acetate (50 mL) | GPC plus SPE (silica sorbent) | GC-EI-MS | 93–117 | [10] |
| Indoor dust | MAE | Acetone (10 mL) | SPE (Oasis HLB plus silica) | GC-NPD | 85–104 | [6] |
| Wet sediment | Solvent shaking | Acetone plus methanol (100 + 50 mL) | LLE with dichloromethane | GC-FPD | Not available | [41] |
| Urban dust and outdoor particulate | PLE | Ethyl acetate (approx. 8 mL) | On-line clean-up with alumina | GC-NPD and GC-PCI-MS/MS | 67–110 | [3] |
| Indoor dust | MSPD | Acetone (3 mL) | On-line clean-up with n-hexane and alumina | GC-NPD | 80–116 | [42] |

PLE, Pressurized liquid extraction; MAE, Microwave-assisted extraction; MSPD, Matrix solid-phase dispersion.

ples (e.g., settled dust [22,38]), a clean-up step is recommended to prolong the GC-column lifetime. In the case of freeze-dried sludge, which may contain up to a 40% organic carbon [39], it is essential to purify the extracts.

The combination of SPE, using normal-phase sorbents, with gel-permeation chromatography (GPC) is recommended for clean-up of Soxhlet or pressurized liquid extracts from sludge and dust samples [10,40] (Table 4). Obviously, this methodology is time-consuming and solvent-consuming, particularly the GPC technique. Dilution of acetone extracts, from sediments and indoor dust, with water and further liquid-liquid partitioning into dichloromethane or SPE have also been proposed to decrease the complexity of raw extracts from dust and sediment samples [6,41]. However, in practice, this option is also cumbersome. By far the most attractive strategy to decrease the level of co-extracted interferences is based on combining mild extraction conditions with on-line purification of the extract. With this approach, Quintana et al. reported selective isolation of OPEs from urban dust using pressurized liquid extraction (PLE) [3]. Ethyl acetate, at a relatively low temperature (50°C), was used as the extraction solvent and a layer of alumina, placed at the bottom of the cell, was the retainer of co-extracted polar interferences. Recoveries of the method for spiked urban dust were 67–110%. This approach provided visually clean extracts from outdoor particulate matter retained on quartz filters [3]. García et al. have also optimized a fast, selective procedure for the extraction of seven OPEs from indoor dust with organic-carbon content up to 25% [42]. Samples were dispersed with florasil and transferred to a polypropylene cartridge containing 0.5 g of activated alumina. Non-polar interferences were first rinsed with n-hexane and then OPEs were extracted from the matrix solid-phase dispersion cartridge with just 3 mL of acetone, whereas more polar organic substances, released from the dispersed sample with acetone, remained in the alumina layer. Extraction yields for spiked dust samples were 80–116%. Direct comparison with exhaustive extraction techniques reported relative recoveries of 72–102% for several real-life polluted dust samples.

From the information in Table 4, it is evident that the most challenging issues relating to extraction of OPEs from solid matrices are:

- (1) reduction in the consumption of organic solvents; and,
- (2) simplification of the clean-up step in order to improve sample throughput.

Analytical performance of these new sample preparation procedures should be assessed using real-life polluted samples. In this sense, the availability of certified reference materials, particularly for indoor dust, due to the ubiquitous presence of OPEs in this matrix and its toxicological concern, would be of interest for estimating

the accuracy of data obtained with different sample preparation strategies.

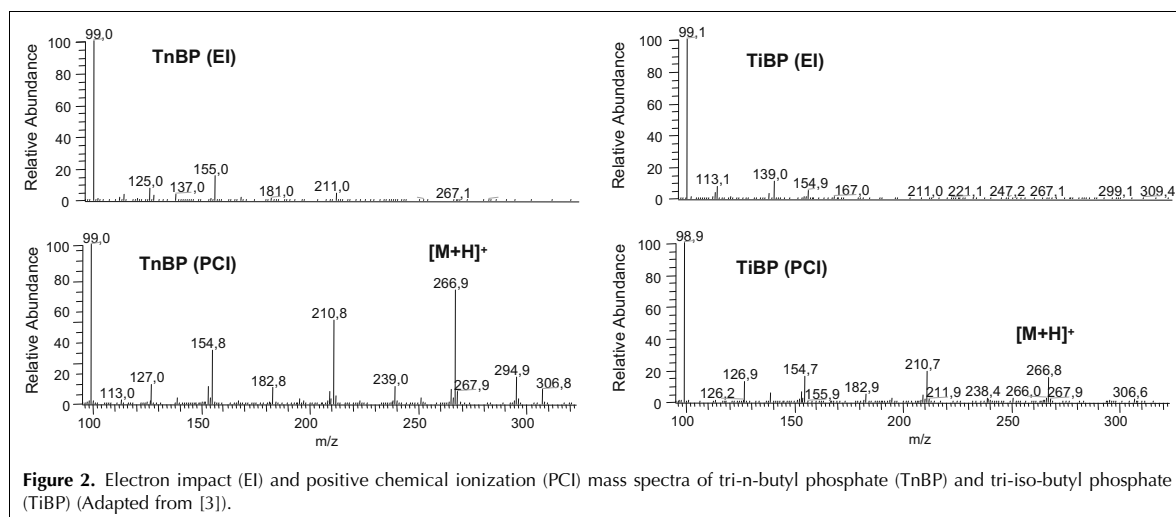
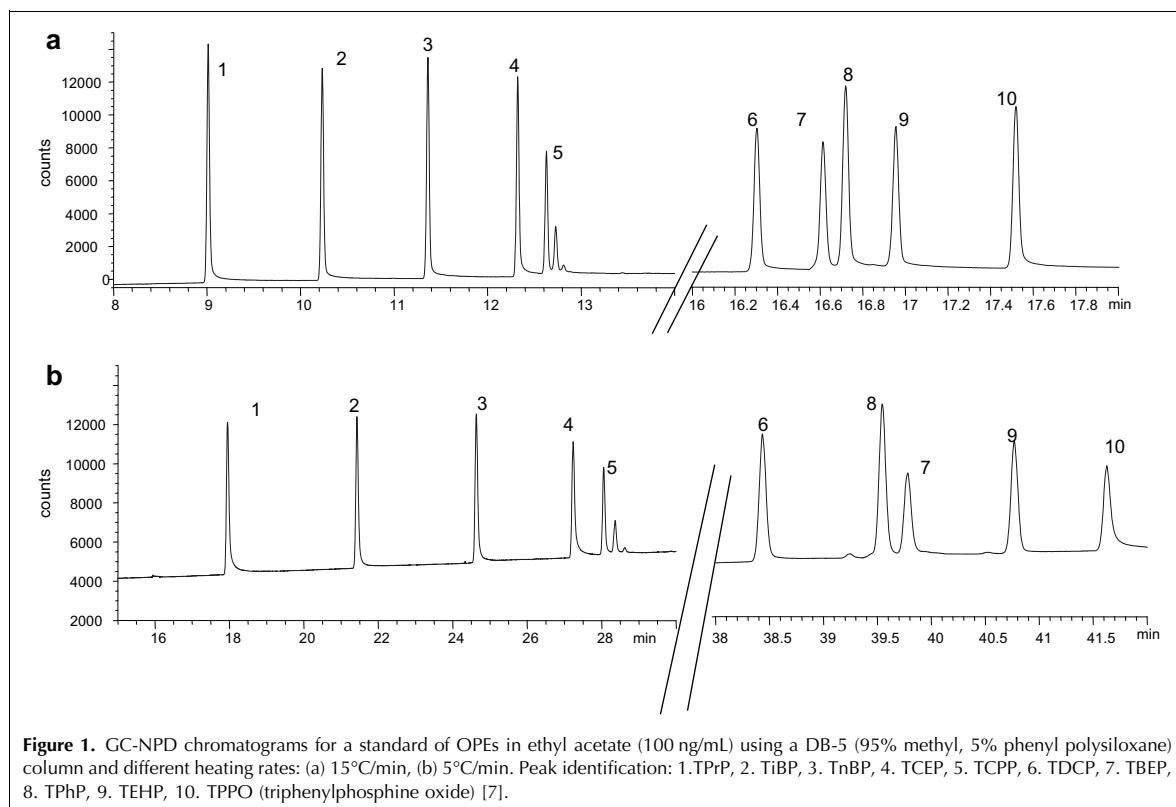
3. Determination

3.1. Gas chromatography

To date, GC is the most common technique in determination of triesters. This is because most OPEs are sufficiently volatile, and GC, especially if used with inexpensive NPD, provides good selectivity and sensitivity. Moreover, LODs can be improved by large-volume injection, as programmed-temperature vaporizer (PTV) injectors are quite popular, provided that the detector or the sample preparation scheme guarantees appropriate selectivity [3,8]. However, we need to bear in mind that the injector liner, and GC column head, for example, should be kept clean to minimize tailing problems, particularly for TBEP and triphenylphosphine oxide (TPPO).

Baseline separation of OPEs can be achieved on a DB-5 (95% methyl, 5% phenyl polysiloxane) capillary column, with the exception of TBEP and TPhP, where an R_s value of approximately 1.4 is obtained [7]. Interestingly, for this pair of compounds, the elution order can be exchanged, depending on the heating rate of the column. Thus, TPhP elutes first at a heating rate of 5°C/min, but TBEP elutes first for a heating rate of 15°C/min (Fig. 1). This is useful, as the concentration of TBEP in some samples may greatly exceed that of TPhP, so that it can still be quantified by decreasing the heating rate [7]. More polar columns [e.g., SPB-1701 (14% cyanophenyl, 86% dimethyl polysiloxane)], provide good separation of TBEP and TPhP, but TCEP and one of the TCPP isomers coelute [7].

Mostly, detection is performed with NPD [4,6,7,10,11,14,19,27,28,42,43] and MS (by electron impact ionization; EI) [8,10,11,14,26–28]. GC-EI-MS, although available in most analytical laboratories, suffers from unfavorable fragmentations, particularly for the aliphatic triesters. These OPEs undergo three consecutive McLafferty rearrangements, leading to EI-MS spectra that are rather poor in signals and have their base peak at m/z 99 (corresponding to protonated phosphoric acid). This hampers quantitative analysis, as matrix constituents often interfere with low-mass ions [3,24]. Fig. 2 shows EI-MS spectra of TiBP and TnBP. For this reason, GC-EI-MS is normally used as a confirmation technique only, while GC-NPD is employed for routine operation [4,10,11,28]. NPD is more sensitive than, and as selective as, EI-MS [24], and also quite common in analytical laboratories. Indeed, the major drawback of this detector is its low stability, as the Rb-active element continually degrades during use and needs to be replaced quite often. As an alternative to NPD, some authors have employed FPD, which provides sensitivity and selectivity similar to NPD [21,44,45].



In some cases, the complexity of real environmental samples requires selectivity over and above that provided by NPD and EI-MS. Hence, positive chemical ionization (PCI), with methanol or methane as reagent gases, combined to ion trap-MS/MS has been suggested [3,24].

GC-PCI-MS/MS can be performed at a cost comparable to GC-EI-MS, providing LODs some 50-fold lower [24]. When compared to NPD, PCI-MS/MS provides much better selectivity with only slightly higher LODs {i.e. ca. 5 pg (NPD) versus ca. 2–50 pg (PCI-MS/MS) [3,24]}.

However, even with PCI, it is still difficult to obtain the molecular ions for branched alkyl phosphates, as TiBP (Fig. 2) or TEHP [3]. Other options reported in the literature for selective detection of these OPEs are atomic emission detection (AED) [27] and inductively coupled plasma (ICP)-MS [46]. Although selectivity is gained by both options, AED sensitivity for P detection is low (ca. one order of magnitude lower than NPD), whereas ICP-MS sensitivity for P detection is good, but a collision reaction cell is required in order to avoid isobaric interferences at 31 m/z units from polyatomic species [46], and that substantially increases the cost of ICP-MS equipment.

3.2. Liquid chromatography-mass spectrometry

Methods for the determination of OPEs by LC-MS are comparatively rare because most of the phosphoric-acid triesters are very amenable to GC-MS analysis. Anyhow, LC-MS analysis offers some options that are also attractive for analyzing OPEs:

- the selectivity and the sensitivity of multiple reaction monitoring (MRM)-detection with triple-quadrupole (QqQ)-MS is not provided by GC-SIM detection on single-quadrupole instruments or by GC-MS/MS on ion traps, due to their lower sensitivity and the difficulty of producing molecular ions by EI, and even PCI (see previous section);
- aqueous samples can be injected directly without the need for analyte transfer into an organic solvent;
- there is potential to include other, more polar compounds (e.g., novel polyphosphates [47] and organophosphate monoesters and diesters [16]).

An LC-MS method was first developed for the determination of nine trialkyl and triaryl phosphates in human blood samples [48]. In that work, atmospheric pressure chemical ionization (APCI) in positive mode was favored over electrospray ionization (ESI) because matrix effects were reduced. Then, the first method for the determination of triesters by LC-MS/MS from aqueous samples was published in 2005 [2]. Besides nine trimesters, two bisarylphosphate flame retardants and TPPO were included in that study, which used ESI in positive mode. Direct determination from aqueous samples was possible for concentrations around 1 $\mu\text{g/L}$, thus providing a fast screening procedure for many wastewater samples. This sensitivity is sufficient for the most widely used triesters in untreated municipal wastewaters. Even in wastewater-treatment-plant effluents, some triesters were detectable by direct injection, especially the stable chlorinated TCEP and TCPP (Fig. 3). With SPE as an enrichment technique, limits of quantification (LOQs) in the moderate ng/L range were obtained for 100-mL samples [2]. The same analytical approach was later extended towards surface waters and drinking waters, increasing the sample intake up to

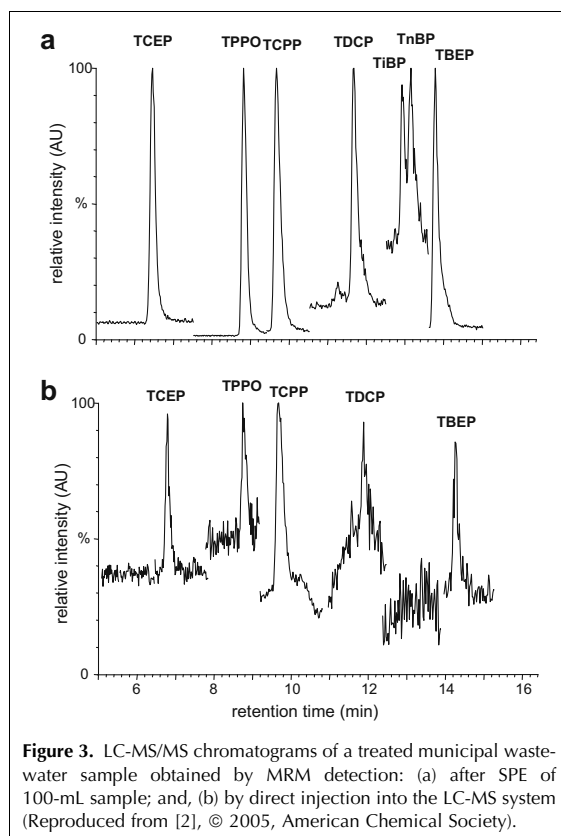
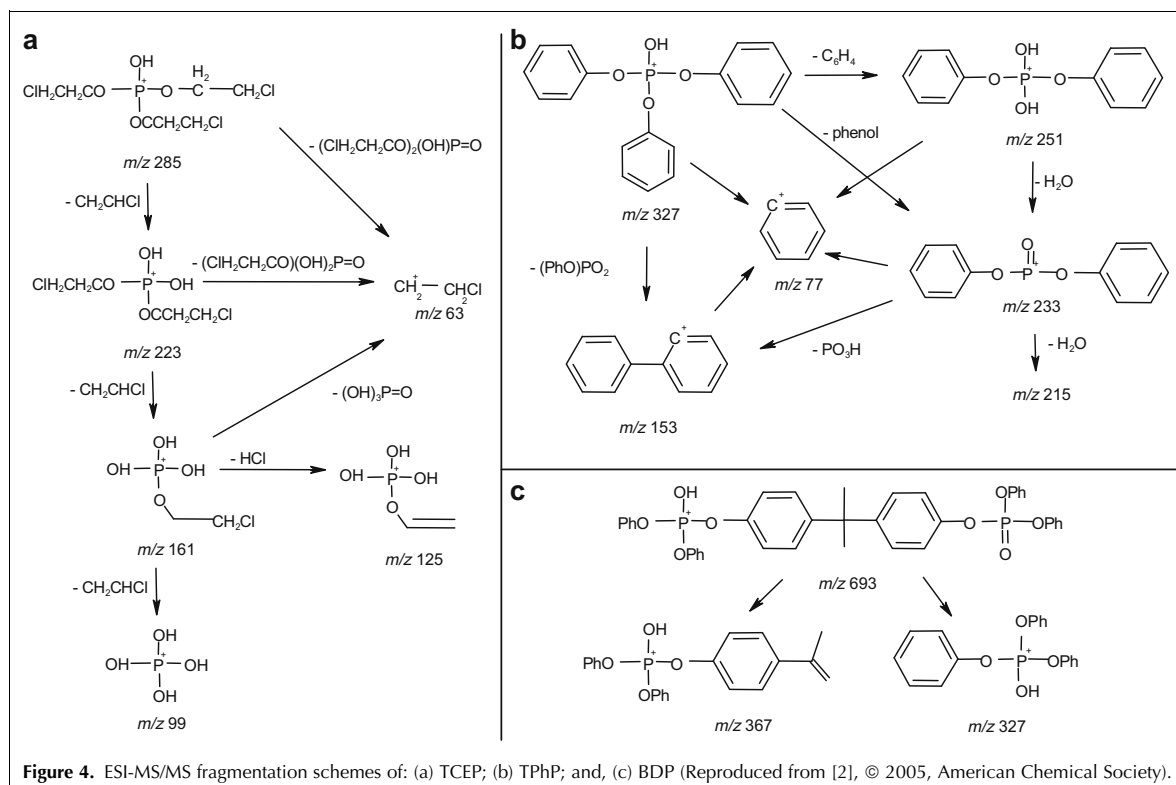


Figure 3. LC-MS/MS chromatograms of a treated municipal wastewater sample obtained by MRM detection: (a) after SPE of 100-mL sample; and, (b) by direct injection into the LC-MS system (Reproduced from [2], © 2005, American Chemical Society).

500 mL [5]. Instead of SPE, one may also use MASE for extraction from water, and that provides better selectivity and thus reduced matrix effects (Section 2.1) [18].

Both APCI and ESI operated in positive mode are efficient in yielding the protonated molecular ion of triesters. Subsequent collision-induced dissociation (CID) fragmentation of triesters has been shown to depend upon the alcohols used for esterification [2,48]. For the trialkyl esters, there occur three successive McLafferty rearrangements, by which the alkyl substituents are expelled as neutral alkenes and protonated phosphoric acid (m/z 99) is formed as a prominent fragment ion (Fig. 4a), resembling EI-MS spectra. Some triesters undergo charge migration onto one of the alkyl groups. In that case, phosphoric acid, or its monoesters or diesters, split off as a neutral molecule and an alkyl cation is formed. This fragmentation appeared to be more prominent for the chlorinated esters (TCEP) and for those with branched alkyl chains (TEHP, TiBP). For the triaryl esters, a McLafferty rearrangement is less favorable and occurs only once. Here, the positive charge may migrate into the phenyl rings and the phenyl as well as the biphenyl cation were detected in the product-ion spectrum of TPhP [2] (Fig. 4b). Aromatic bisphosphates

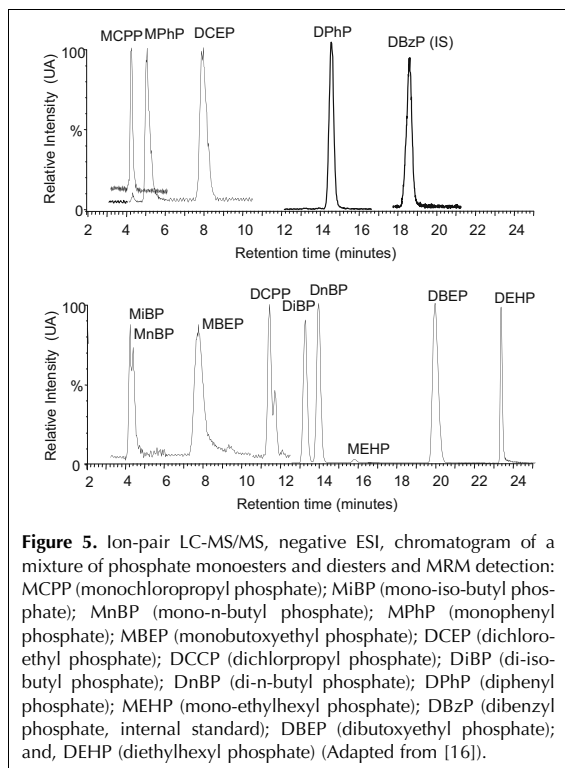


are more stable than the respective monophosphates: bisphenol A bis(diphenyl phosphate) (BDP) shows two clear fragments that are formed by a fission of the bond between the central quaternary carbon to yield two aromatic fragments (Fig. 4c).

Phosphoric acid diesters and monoesters, especially DEHP and MEHP, are used as plasticizers and extractants and can also be formed from triesters by microbial degradation and chemical hydrolysis (see Part I of this review [1]). However, their occurrence in environmental samples has so far received only little attention. LC-MS analysis would appear very attractive for these compounds and, indeed, their determination as anions with ESI in the negative-ion mode poses no problem [16,49]. Chromatographic separation is more difficult to achieve, since routine reversed-phase chromatography does not offer sufficient retention for these strongly acidic compounds. Ion-pair chromatography involving triethylamine (TEA, 0.5 mM) as ion-pairing agent was therefore used to determine diphenyl phosphate (DPhP) and DEHP from biological fluids [49]. As an alternative, a carbon column may be used [50]. For the more polar diesters with small ester moieties and for monoesters, the use of a larger, more hydrophobic cationic counterion is advisable because it provides stronger retention [51]. Tributylammonium (TrBA) acetate was therefore used

and was also able to retain very polar monoesters [e.g., mono-iso-butyl phosphate (MiBP) or mono(chloropropyl) phosphate (MCP)], together with 11 other monoesters and diesters (Fig. 5) [16]. Ion-pairing was also shown to be suitable for improving the extractability of three diesters from aqueous samples by SPE, resulting in LOQs in the 10-ng/L range [16]. An extension of this approach to a larger number of diesters and to monoesters was hampered by the lack of commercially-available reference materials and still awaits verification.

Like the cations of trialkyl esters, the anions of dialkyl esters undergo a McLafferty rearrangement upon CID, by which the anion of the monoester is formed. Then the alcohol is expelled as neutral molecule, yielding the phosphite anion ($[\text{PO}_3]^-$; m/z 79) as a major fragment [16,52]. Correspondingly, the monoesters produce only one intense fragment (m/z 79) [16,49], which hampers confirmation of positive detection in real samples by a second MRM transition [16]. The butoxyethyl esters (DBEP, MBEP) allow an additional expulsion of butanol (-74 Da) by fragmentation of the ether bond [16]. The chloroalkyl esters exhibit a very different fragmentation pattern, which is dominated by the chlorine, that is either eliminated as neutral HCl ($[\text{M}-\text{H}-36]^-$) or as chloride anion $[\text{Cl}]^-$ (m/z 35). Fragmentation of the



anion of aromatic diester DPhP is governed by the ability of the aromatic system to stabilize the negative charge, thus yielding phenolate (m/z 93) as the major fragment ion, and also some m/z 79. The latter remains the only fragment ion found in the product-ion spectrum of monophenyl phosphate (MPhP).

4. Outlook and conclusions

Research on analytical chemistry of OPEs has advanced in the past few years. The progress in analytical methods has been fostered by improvements in GC-MS and LC-MS instrumentation and by development of novel sample preparation strategies that increased throughput at reduced costs. These sample preparation methodologies combine extraction and clean-up, and also reduce sample intake and solvent wastage. Some perdeuterated internal standards, that are available commercially, enhance accuracy and precision. Nevertheless, commercialization of other isotopically-labeled internal standards (e.g., for TMP and mono-/diesters) and even pure reference standards for monoesters and diesters is necessary in order to advance analytical and environmental research. Also, there need to be certified reference materials available, particularly for dust.

On the basis of the information summarized in this review, we highlight the following trends in the determination of OPEs in water and air samples:

- The on-line combination of extraction and determination has simplified and improved the reliability of OPE analysis in atmospheric samples. The next challenge is to integrate air sampling in these automated devices.
- Use of microextraction approaches, based on equilibrium processes, has improved selectivity in extracting OPEs from water samples. For particulate samples (e.g., dust and sludge), we also expect sub-critical water to provide cleaner extracts than other conventional approaches with organic solvents, at least for the most polar OPEs.
- GC-NPD and GC-FPD will remain as routine workhorse techniques for the determination of phosphoric acid triesters in water and air samples; however, we expect a considerable increase in the number of applications of LC-MS/MS in this field. Lower LODs, higher selectivity and feasibility to determine tri-alkyl organophosphates, as well as diesters and monoesters, are the major advantages of LC-MS/MS versus GC-based techniques. Nowadays, the lack of deuterated or ^{13}C -labeled standards is a serious handicap for extensive application of MS-detection techniques, especially for LC-MS/MS due to ionization-suppression problems, to the analysis of OPEs in complex matrices (e.g., sewage water, sludge and dust).

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